

AN INVESTIGATION OF THE HYDRAULIC CALCIUM SILICATE CEMENT, BIODENTINE, AS A VITAL PULP TREATMENT MATERIAL

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fulfilment of the requirements for the degree of Doctor of Philosophy**

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work. I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement. I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

A handwritten signature in black ink, appearing to read 'Rob', is written over a horizontal line.

Roberto Careddu

Summary

In recent years, a drive towards minimally invasive dentistry has promoted the need to preserve pulp vitality, when possible. Vital pulp treatments (VPTs) are not a new concept, as previously they were regarded as temporary measures used prior to root canal treatment. Recent advancements in understanding pulpal reparative mechanisms and the advent of new hydraulic calcium silicate cements (HCSCs) have brought new enthusiasm towards VPTs even in those clinical situations where a root canal treatment was previously considered the only option. Unfortunately, there remains a considerable lack of knowledge in many aspects in this field, from the mechanisms of pulp repair, to the best materials to use, a lack of robust diagnostic tools and an absence of clinical protocols. The overarching aim of this thesis was to address some of these clinical and diagnostic questions while studying a popular HCSCs material Biodentine.

Chapter One represents a thorough literature review Biodentine compared with the current gold standard MTA as a VPT material, assessing both clinical and laboratory features. An analysis of the characteristics of the two materials compared with the ideal characteristics was carried out in order to point out the strengths and drawbacks of the materials. After analysis, while an ideal material does not yet exist, both MTA and Biodentine perform similarly well in the clinic and the lab differing principally in features such as setting time and radiopacity.

Previous research has shown wide divergence in opinion with regards to the implementation of VPT in practice; however, less is known about how endodontists manage deep carious lesions. An international survey involving two European endodontic societies was carried out in Chapter Two. This questionnaire-based study, based around vignettes, allowed the analysis of both patient-related and dentist-related influencing factors and explored the reasons behind the decision-making process in managing pulp exposures and deep caries. No consensus was shown amongst individuals from the two societies and even among different groups of the same society, indicating a lack of structured training, postgraduate education and practical information.

Developing biological understanding about the *in vivo* behaviour of Biodentine, compared with MTA, is critical when evaluating the materials. The third chapter describes a histological study analysing Biodentine and MTA's ability to induce pulpal reparative processes at 6 months. Both materials produced a range of responses from necrosis, through low quality mineralisation to tubular dentine indicating not only the influence of technical procedure and asepsis on the success of VPT, but also that regeneration of dentine was possible but not predictable even with HCSCs.

In Chapter four the effectiveness of Biodentine as a partial pulpotomy capping material procedure after carious exposures presenting with a range of symptoms was investigated prospectively over 1-year. Pulpal symptoms as dichotomised into reversible and irreversible pulpitis was not an accurate predictor of VPT outcome, while the further subdivision evident in Wolters classification was prognostically useful. Notably, other prognostic markers such as tenderness to percussion preoperatively and pulpal haemorrhage intraoperatively were not effective in predicting outcome.

Diagnosis of pulp inflammatory status currently is subjective and unpredictable. Objective accurate tests that can identify the threshold between reversible and irreversible damage are urgently required. Molecular diagnostics of blood or other tissue fluid offer hope in this regard. Chapter five reports the results of a preliminary study using a point-of-care CRP test as a diagnostic tool to investigate the severity of pulp inflammation. Accepting the limitations, including the non-specific nature of the test and the small sample size, the initial results are encouraging and indicate that this test may represent a useful diagnostic tool in the future.

In conclusion, VPT appears to be a viable alternative to traditional root canal treatment even in case of the so called "irreversible" pulpitis. Biodentine is an evidenced-based and valid alternative to MTA having practical advantages in terms of setting time and not inducing discolouration

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*Gratus animus virtus est
non solum maxima sed etiam mater virtutum omnium¹*

Reflecting on these past years, I can clearly see that I was never alone on this journey that brought me here. There have been numerous individuals who helped, counselled, and supported me. Without them, none of this would have been possible.

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Nunc est bibendum²

¹ "Gratitude is not only the greatest virtue, but also the mother of all virtues". Cicero

² "Now is the time for drinking". Horace

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Abbreviations

%	Percentage
°C	Degree Celsius
10x	Ten Times Magnification
AAE	American Association of Endodontists
AIE	Accademia Italiana di Endodonzia
ALP	Alkaline Phosphatase
AMTA	Angelus-MTA
BD	Biodentine
BECs	Bioactive Endodontic Cements
BSP	Bone Sialoprotein
CBCT	Cone-Beam Computed Tomography
CEM	Calcium-Enriched Mixture Cement
CG	Control Group
CH	Calcium Hydroxide
CRP	C-Reactive Protein
CSCR	Resin-based Calcium Silicate Cement
DDUH	Dublin Dental University Hospital
DMP1	Dentin matrix acidic phosphoprotein
DPSC	Dental Pulp Stem Cell
DSPP	Dentin Sialophosphoprotein
ELISA	Enzyme-Linked Immunoassay
EMD	Enamel Matrix Derivative
EPT	Electric Pulp Test
ESE	European Society of Endodontology
FDI	World Dental Federation
G0.	Group 0
G1	Group 1
G2	Group 2
G3	Group 3
G4	Group 4
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GIC	Glass Ionomer Cement
H&E	Haematoxylin and Eosin
HCSCs	Hydraulic Calcium Silicate Cements
HCSMs	Hydraulic Calcium Silicate Materials
HDPCs	Human Dental Pulp Cells
HO-1	Heme Oxygenase
Hrs	Hours
HSCH	Hard Setting Calcium Hydroxide
IES	Irish Endodontic Society
IL	Interleukin
IRM	Intermediate Restorative Material
ISO	International Organization for Standardization

JREC	Research Ethics Committee at St James's Hospital
LDF	Laser Doppler Flowmetry
LED	Light Emitting Diode
MDPC	Murine Pulpal Cell-Line
MeSH	Medical Subject Headings
mg/L	milligrams/Litre
MM-MTA	MicroMega MTA
Mm	Millimetre
MMP9	<i>Matrix Metalloproteinase 9</i>
MTA-HP	MTA Repair HP
MTA	Mineral Trioxide Aggregate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NaOCl	Sodium Hypochlorite
NMTA	Neo MTA Plus
NO	Nitrous Oxide
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
NSCH	Non Setting Calcium Hydroxide
OC	Osteocalcin
OMTA	Ortho-MTA
ON	Osteonectin
OP	Osteopontin
P	Positive
PBS	Phosphate Buffered Saline
PGE2	Prostaglandin E2
PO	Pulse Oximetry
PrMTA	ProRoot MTA
RBC	Resin-Based Composite
RCT	Randomized Clinical Trials
RCTx	Root Canal Treatment
rpm	Revolutions Per Minute
RPS18	Ribosomal Protein S18
Runx2	Runt-Related Transcription Factor 2
SEM	Scanning Electron Microscopy
TCal	TheraCal
TGF	Transforming Growth Factor
TNF α	Tumour Necrosis Factor Alpha
TTP	Tenderness to Percussion
U	Unclear
v/v	Volume per Volume
VPT	Vital Pulp Treatment
w/v	Weight per Volume
ZOE	Zinc Oxide Eugenol-Based Cement
μ l	Microlitres
μ m	Micrometre

Chapter 1.

An Analysis of the Hydraulic Calcium Silicate Cements Biodentine and Mineral Trioxide Aggregate in Vital Pulp Treatment: A Review of the Literature

(Adapted from Careddu R, Duncan HF (2018). How does the pulpal response to Biodentine and ProRoot mineral trioxide aggregate compare in the laboratory and clinic? *British Dental Journal* **225**, 743-9)

1.1 Introduction

Exposure of the pulp is a common clinical occurrence due to trauma, caries or iatrogenic reasons. In health, the pulp is protected by an outer shell of enamel and dentine; however, when this protection is breached the pulp is colonised by microbes with the subsequent simulation of a pulpal inflammatory response (Tronstad & Mjör 1972). If the pulpitis process goes untreated it will increase in intensity ultimately resulting in pulp necrosis (Farges *et al.* 2015). Notably, if pulp vitality can be maintained, the irritant stimulus removed and the tooth adequately restored then regeneration or repair of the pulp is possible (Tronstad & Mjör 1972). Recently the importance of retaining all or part the pulp has been highlighted as it is more conservative, potentially less technically-demanding and more biologically-based compared with pulpectomy procedures (Özyürek & Demiryürek 2016, Smith *et al.* 2016, ESE 2019).

The aim of the vital pulp treatment (VPT) is to preserve the vitality and function of the pulp, while stimulating hard tissue repair processes (ESE 2006, Witherspoon 2008). VPT encompasses procedures with no pulp tissue removal like pulp capping (indirect and direct) as well as techniques with varying degrees of pulp excision such as pulpotomy (partial or complete) (ESE 2019). In the past, the recommendations for direct pulp capping or pulpotomy of mature teeth were limited to traumatic/iatrogenic exposures without bacterial contamination of the pulp tissue (ESE 2006). Several factors influence the success of VPT procedures including the level of pre-operative pulp inflammation (Mejäre & Cvek 1993), the post-operative prevention of further microbial insult (Cox *et al.* 1987, AAE 2021), the use of an antimicrobial lavage (Ballal *et al.* 2021) and the material used to interface with the biological tissue (Nair *et al.* 2008). As a result, an 'enhanced

protocol' including the use of magnification, hydraulic calcium silicate cements (HCSCs) as the pulp capping material and sodium hypochlorite as the disinfecting agent was proposed in a recent position statement (ESE 2019). Historically, due to an incomplete understanding of pulp defensive mechanisms (Smith *et al.* 2016) and the dental materials available, VPT was considered as an unpredictable procedure, however pulp vitality preservation is now proposed as treatment of choice even in cases where the pulp has been contaminated by bacteria but maintains vitality (Barthel *et al.* 2000, ESE 2019). When carrying out VPT procedures calcium hydroxide has been the material of choice for many years (Zander & Glass 1949, Pitt Ford & Roberts 1991), but it is limited by poor mechanical properties, unspecific mechanism of action (Sangwan *et al.* 2013), absence of sealing properties (Browne *et al.* 1983) and incomplete hard tissue formation over the pulp wound (Cox *et al.* 1987, Nair *et al.* 2008). Developments in our understanding of pulp defence mechanisms (Smith *et al.* 2016) and the advent of HCSCs such as mineral trioxide aggregate (MTA) have created new opportunities and enthusiasm for VPT (Simon *et al.* 2014). Notably, MTA has demonstrated superior histological (Aeinehchi *et al.* 2003, Nair *et al.* 2008) and clinical outcome compared with calcium hydroxide in VPT procedures (Hilton *et al.* 2013, Taha & Kahzali 2017).

MTA though is not an ideal material with several disadvantages highlighted including a long-setting time, tooth discolouration (Bortoluzzi *et al.* 2007, Lenherr *et al.* 2012), high-cost (Hilton *et al.*, 2013) and an unspecific mechanism of action (Duncan *et al.* 2011). Over the last few years new generation calcium silicate-based cements have been introduced, which attempt to address many of the short-comings of MTA. Biodentine (Septodont, Saint-Maur-des-Fosses, France) is a HCSC marketed specifically for its use in VPT procedures. Several publications have highlighted its positive effects on pulp mineralisation processes (Zanini *et al.*, 2012), improved shorter setting-time, better sealing properties, improved mechanical resistance and ease of use compared MTA (Tran *et al.* 2012, Nowicka *et al.* 2013, Kim *et al.* 2015, Careddu&Duncan 2021) concluding that Biodentine may be a more suitable material for use in VPT procedures.

MTA cements are chemically composed of Portland cement with bismuth oxide added as radiopacifier (Camilleri 2008). There are a range of commercial

MTA products available, including the original ProRoot MTA (Dentsply Sirona, Ballaigues, Switzerland) as well as Angelus MTA (The Angelus, Londrina, Brazil), MM-MTA (Micro-Mega, Besancon, France), Ortho MTA (BioMTA, Seoul, Republic of Korea) and MTA Plus (Avalaon Biomed, Houston, USA), which share the presence of bismuth oxide, calcium silicates and calcium aluminates (Ha *et al.* 2015). In addition to these common elements, minor differences between MTA products may be the presence of calcium sulphate, calcium carbonate and phyllosilicates (Ha *et al.* 2015). As the broad compositions of the various MTAs are similar the endodontic literature considers all MTA products as one material (Asgary *et al.* 2009). Biodentine is also chemically similar to MTA, but unlike the MTA products does not contain calcium aluminate, calcium sulphate or bismuth oxide using zirconium oxide as a radiopacifier rather than bismuth oxide (Ha *et al.* 2015). The absence of bismuth oxide in Biodentine is potentially significant not only for radiopacity, but also for other properties such as staining potential (Marciano *et al.* 2014), setting time and micro-hardness (Grazziotin-Soares *et al.* 2014). Due to differences in chemical formulation a recent review has suggested that these materials could be grouped as ‘bioactive endodontic cements (BECs)’ rather than calcium silicate materials (Parirokh *et al.* 2017)

The aim of this review was to comprehensively analyse the laboratory and clinical studies, which compared MTA and Biodentine specifically in relation to the dental pulp. The evidence-base was analysed with a view to considering whether Biodentine could be justified as a more suitable material than MTA for pulpal applications.

1.2 Search Strategy

A comprehensive MEDLINE search up to December 2021 was conducted using various medical subject headings (MeSH) in combination with “and” or “or”. The major MeSH terms searched were “Endodontics”, “Dental Pulp Disease”, “Dental Pulp Test”, “Pulpotomy” and “Dental Pulp Capping” in combination with a series of related subheadings. In addition, the following terms were added “Biodentine”, “Mineral Trioxide Aggregate”, “MTA”, “calcium silicate cements”, “calcium silicate materials” and “calcium silicates”. Bibliographies of all relevant papers and previous review articles were hand-searched for further studies of relevance. Any relevant work published in the English language and presenting pertinent

information related to this review was considered for inclusion. Titles were generally excluded if they were conference reports or if aspects of calcium silicate cements were discussed that were not the subject of the current review. Laboratory (biocompatibility, biological, material aspects) studies were considered if Biodentine and MTA were compared within the same study, while clinical studies focused on comparative responses in permanent teeth.

1.3 How does the pulp repair after exposure and what is the influence of the material?

The odontoblast cell forms the primary dentine during development, secondary dentine throughout the life of the tooth and when challenged the deposition of tertiary dentine in an attempt to 'wall off' the irritation (Simon *et al.* 2009). Depending on the severity of the stimulus tertiary dentine deposition can be reactionary or reparative (Lesot *et al.* 1994); reactionary dentine being formed by an upregulation of surviving post-mitotic odontoblasts exposed to the influence of relatively mild stimuli, while reparative dentine is formed generally after a stronger stimuli, which has led to death of the odontoblast cell (Lesot *et al.* 1994, Smith *et al.* 1995). At a cellular level the new reparative dentine is generally believed to be produced following cyto-differentiation of pulpal progenitor cells and the formation of a new generation of odontoblast-like cells (Lesot *et al.* 1994, Smith *et al.* 1995); however, alternative theories suggest that other cells such as fibroblasts may cyto-differentiate to produce the mineralised tissue (Ricucci *et al.* 2014). The cellular differentiation is likely to be guided by the influence of growth factors and bioactive molecules derived from both the dentine and the pulp matrix (Cassidy *et al.* 1997, Smith 2003). Although reactionary and reparative dentinogenesis are for didactic purposes considered separately it is likely that in the event of pulp exposure reparative dentine will form the mineralised bridge, while at the periphery of the cavity reactionary dentinogenesis will occur simultaneously (Smith *et al.* 2016).

A degree of inflammation is also an important stimulus to drive the reparative process (Cooper *et al.* 2010) with odontoblasts involved in initial sensory stimulus transmission from the dentine and also an immunocompetent role in cellular defence (Couve *et al.* 2013). Indeed the low level release of inflammatory mediators such as interleukins (e.g. IL-1 α , -1 β , -2, -6) in

mineralising cells in contact with MTA supports the need for a degree of inflammation in promoting regenerative processes (Huang *et al.* 2005, Galler *et al.* 2021). In the dentine there are a wide range of bioactive dentine matrix components, which although “fossilised” in the mineralised tissue are released into pulp during caries or trauma (Rutherford *et al.* 1993, Nakashima 1994, Smith *et al.* 2016). Demineralisation of dentine and indeed contact with materials such as MTA (Tomson *et al.* 2007), calcium hydroxide (Graham *et al.* 2006) and other agents (Duncan *et al.* 2017) has been shown to release a plethora of bioactive molecules including members of the TGF- β superfamily which can stimulate a complex cascade of molecular events that promote pulp repair (Cassidy *et al.* 1997, Graham *et al.* 2006). Notably, there is no available evidence to suggest that Biodentine is capable of sequestering dentine matrix components so comparative bioactivity analysis with MTA is not possible.

Using biologically-based dental materials that promote the healing process are paramount in VPT (Ferracane *et al.* 2010) and other irrigation strategies are being developed which enhance the release of bioactive molecules from dentine to improve the wound repair (Widbiller *et al.* 2017). Over the last 10 years, calcium silicate materials, including ProRoot MTA and Biodentine, have demonstrated superior histological response compared with the traditional gold standard material calcium hydroxide in VPT (Nair *et al.* 2008, Tran *et al.* 2012). Although it is uncertain how different calcium silicate materials exert their dentinogenic effect, it is assumed that they work in a non-specific manner which is more efficient than calcium hydroxide (Sangwan *et al.* 2013) and in a similar way to each other (Tran *et al.* 2012) (**Figure 1.1**). MTA and Biodentine have shown the capacity to promote the early synthesis of reparative dentine, perhaps due to enhanced regulation of TGF- β 1 (Nowicka *et al.* 2013), Osteopontin (Tran *et al.* 2012) and other growth factors.

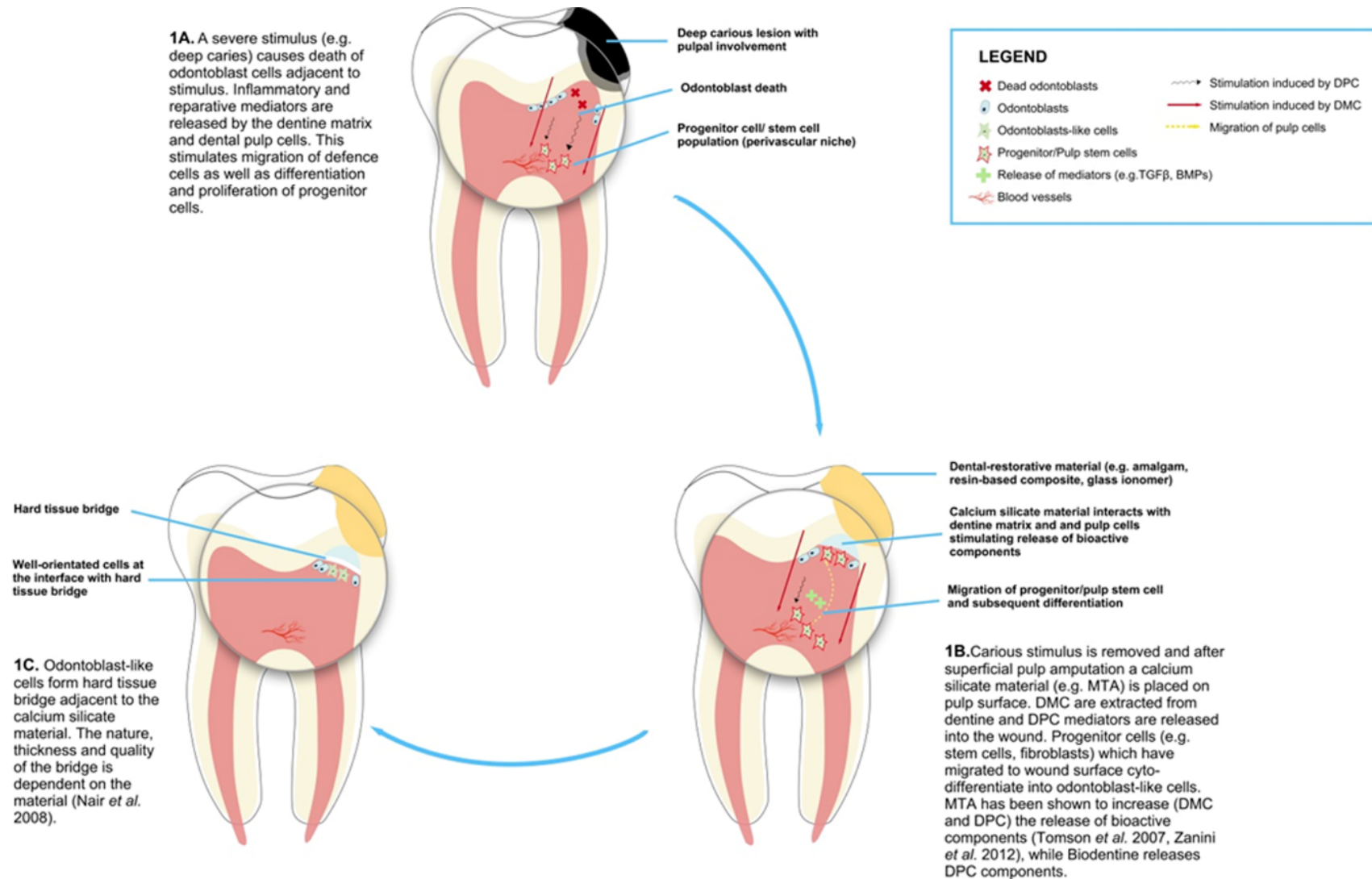


Figure 1.1 Schematic theoretical representation of the process of reparative dentinogenesis after a VPT procedure using calcium silicate cements

1.4 How are the success of MTA and Biodentine in VPT procedures compared clinically?

The aim of VPT is to maintain the vitality of the tooth in the absence of an apical radiolucency and, in case of immature teeth, achieve apexogenesis (Witherspoon 2008). Therefore outcome should be assessed from both a clinical and radiographic perspective (Haskell *et al.* 1978). Clinically, the treated tooth should respond positively to pulp sensibility testing (if it is possible to assess) and not present any sign or symptoms such as pain, tenderness to percussion or presence of a sinus tract (Mass & Zilberman 1993, ESE 2019). The radiographic examination should demonstrate no signs of apical periodontitis and no evidence of internal resorption and continued root development in immature teeth (Fuks *et al.* 1982, ESE 2006, Mente *et al.* 2010). In terms of follow up an appointment no longer than 6 months postoperatively is recommended as well as a minimum recall of one year; however long-term follow up (ideally four years) is also required to assess the success of the procedure (ESE 2019).

VPT materials should ideally be compared in adequately powered, randomized clinical trials (RCT), which are free from bias (Malekafzali *et al.* 2011, Hilton *et al.* 2013, Grewal *et al.* 2016, Katge & Patil 2017); however, the clinical performance of new materials can also be investigated, but not compared, in case series (Asgary & Ehsani, 2009) and case reports (Patel & Cohenca 2006, Borkar & Ataide 2015). Clinical trials comparing MTA and Biodentine pulpotomy procedures in carious deciduous molars, have demonstrated that the two materials have equally effective outcomes in young patients (Cuadros-Fernández *et al.* 2015, Kusum *et al.* 2015). Unfortunately, there is still a paucity of clinical studies comparing the outcome of Biodentine and MTA in VPT procedures in permanent teeth. Two RCTs compared Biodentine and MTA as pulp capping materials in asymptomatic cariously-exposed permanent molar teeth and reported no difference between the materials after one year recall (Brizuela *et al.* 2017, Katge & Patil 2017). In one of those studies, 169 patients with carious exposures of one permanent molar were treated with calcium hydroxide, MTA or Biodentine with no significant differences between the materials and very few failures reported in any of the experimental groups at one year; however, for practical reasons it was concluded that Biodentine and MTA offered advantages over calcium hydroxide (Brizuela *et al.* 2017). Brizuela *et al.*

study (2017) as well as Katge & Patil's (2017) investigated calcium silicate pulp capping on carious exposures in permanent teeth of a young patients (7 to 9 years and 7 to 16 years old respectively), which by the authors admission contained significant numbers of teeth with open apices. In an attempt to homogenise the patient sample other studies elected to investigate VPT procedures in older patients, 15 to 30 years (Hegde *et al.* 2017) and 18 to 40 years (Linu *et al.* 2017). In one of these studies, a small clinical trial of 24 patients with cariously exposed molars were pulp capped with either Biodentine or MTA and reviewed up to six months; MTA was reported to perform better but the results were not significantly different (Hegde *et al.* 2017). Another small retrospective pulp capping study investigating the two materials in carious molars concluded after 18 months that both materials performed predictably and equally (Linu *et al.* 2017); however, care must be taken in extrapolating the results of these studies as the size of the sample in both studies is inadequate to potentially recognise any statistical difference between the materials.

A non-inferiority randomised trial compared the outcome of partial pulpotomy using ProRoot MTA or Biodentine on young permanent teeth that displayed deep carious lesions radiographically and symptoms indicative of irreversible pulpitis (Uesrichai *et al.* 2019). The patients were aged 6 to 18 years with 69 cases treated and reviewed clinically and radiographically every 6 months for a range of review periods. The results showed that Biodentine was not inferior to ProRoot MTA in terms of clinical and radiographic outcome, with Biodentine inducing less coronal discolouration (Uesrichai *et al.* 2019). Awawdeh *et al.* reported comparable favourable results when either Biodentine or MTA were used for pulpotomy and pulp capping. This prospective randomised controlled trial compared the results for three years and stressed the importance of correct coronal restoration for a long-term success regardless of the pulp capping material used (Awawdeh *et al.* 2018). In a study of traumatised anterior teeth carried out over 18 months, discolouration occurred in almost all samples treated with MTA, but not when Biodentine was used (Abuelniel *et al.* 2020).

It would appear from the limited evidence available that the success rate for VPT procedures is high with both materials; however, existing studies remain preliminary, limited by small numbers and short follow-up period.

1.5 How are Biodentine and MTA compared in the laboratory with respect to the pulp?

The majority of research comparing the histological response to VPT materials is carried out in sound teeth (Fransson *et al.* 2016), which limits confounding factors and enables comparison, but decreases the clinical relevance of the study (Ricucci *et al.* 2014). Recruiting human subjects who are prepared to consent to a VPT procedure on a sound tooth prior to later scheduled extraction is challenging and as a result the majority *ex vivo* studies are carried out using animal models (Sazak *et al.* 1996, Murray *et al.* 2008, Shayegan *et al.* 2012, Youssef *et al.* 2019), which has its own limitations. Several qualitative and quantitative human studies comparing different VPT materials have been published (Aeinehchi *et al.* 2003, Accorinte *et al.* 2008, Nair *et al.* 2008, Nowicka *et al.* 2013 & 2015). These studies should carefully evaluate histologically with serial sections the soft tissue response - inflammation, necrosis, odontoblast-like cell presence, bacterial infiltration, pulpal calcifications - as well as hard tissue bridge formation, recording its thickness, homogeneity and the cellular interface with the materials (Nair *et al.* 2008, Nowicka *et al.* 2013). Cone-beam computed tomography (CBCT) has been used to assess the hard tissue bridge prior to the histological examination (Nowicka *et al.* 2015). One histological study compared Biodentine and MTA (as well as calcium hydroxide and composite resin) and concluded that both MTA and Biodentine materials induced a similar mineralised bridge formation at the pulp-material interface. The bridge from BD and MTA were homogeneous and with minimal tunnel defects (Nowicka *et al.* 2015).

In comparison to the small number of *ex vivo* studies a large number of *in vitro* biological studies have compared Biodentine with MTA (**Table 1.1**). There have been cell culture studies using primary cells (rodent or human dental pulp cells) or odontogenic cell lines (e.g. MDPC-23), which analyse cellular processes such as cytotoxicity, migration, viability, differentiation, inflammation and mineralisation (Zanini *et al.* 2012, Pérard *et al.* 2013, Chang *et al.* 2014, Margunato *et al.* 2015, Poggio *et al.* 2015, Widbiller *et al.* 2016, Collado-Gonzalez *et al.* 2017, Araujo *et al.* 2018, Dahake *et al.* 2020) (**Table 1.1**). Although these laboratory studies represent the bulk of research on this subject, unfortunately it is not possible to conclude from these biological studies comparing Biodentine and MTA that one material is consistently better than the

other (**Table 1.1**). If additional evidence is collated from clinical, *ex vivo* and *in vitro* studies and analysed with respect to Ingles' criteria (**Table 1.2**) it is evident, that Biodentine compares equally to MTA for reparative dentine formation, maintaining pulp vitality, anti-bacterial properties , long-term insolubility and preventing coronal leakage (Bogen & Chandler 2008, Nowicka *et al.* 2013, Nowicka *et al.* 2015, Bhavana *et al.* 2015, Kaup *et al.* 2015, Özyürek T & Demiryürek 2016, Katge & Patil 2017, Bakhtiar *et al.* 2017, Tsesis *et al.* 2018) . Notably, Biodentine performed consistently better than MTA in adherence to dentine measured by push-out testing (Guneser *et al.* 2013, Nagas *et al.* 2016, De-Deus *et al.* 2016); however, MTA was more radiopaque than Biodentine, which consistently fell below ISO standards (Tanalp *et al.* 2013, Lucas *et al.* 2017) (**Table 1.2**). Certainly, within these studies Biodentine compares favourably to MTA and both calcium silicate materials are generally superior to other materials tested including glass ionomer cement (GIC), Dycal (calcium hydroxide), Thercal (resin-modified calcium silicate), Calcimol (calcium hydroxide), Calcicur (calcium hydroxide) and zinc-oxide eugenol cements such as IRM.

REFERENCE	METHODOLOGICAL APPROACH AND SPECIES	MATERIALS INVESTIGATED	VARIABLES ANALYSED	PRINCIPAL RESULTS
Pérard <i>et al.</i> 2013	- <i>In vitro</i> - Two murine pulpal cell-lines (MDPC-23, OD-21)	- BD - PrMTA	- Cell viability (acid phosphatase assay) - Gene expression (qRT-PCR) OP, ALP, Runx2, Collagen-1 - Phenotypic analysis (SEM)	- MDPC-23 cells cultured with PrMTA superior viability to BD at 7d - MTA and BD reduced cell proliferation compared with control - Colla1 expression reduced in PrMTA compared with BD
Chang <i>et al.</i> 2014	- <i>In vitro</i> - Immortalised human pulp cell line used as HDPCs	- BD - OMTA - AMTA - IRM	- Cell viability (MTT assay) - ALP activity - Mineralisation (Alizarin Red) - Gene expression (qRT-PCR) Mineralisation-associated (OP, BSP, Runx2, DSPP, DMP1) Inflammatory-associated (NO, PGE ₂ , TNF α , IL6, IL8, MMPs) - Protein expression (NO, PGE ₂ , HO-1) - ROS production	- BD and MTA groups similar cell viability, IRM less viability - Mineralised nodules formation: best results OMTA at 14d. BD and AMTA induced higher formation of mineralised nodules than IRM. - OMTA, AMTA and BD higher mRNA expression (OP, BSP, DSPP, DMP1) than IRM - BD and both MTA showed similar inflammatory response, lower than IRM
Corral Nunez <i>et al.</i> 2014	- <i>In vitro</i> - Murine embryo fibroblast cell line (3T3)	- BD - PrMTA - GIC	- Cell viability (Alamar blue assay) and proliferation - Cell morphology in response to dental material (SEM) - Cytokine expression (Semi Quantitative RT-PCR) for IL1 β , IL6	- Similar cell viability for BD and PrMTA at 72h, reduced for GIC. - BD showed increased cell growth and development (extended cellular processes) in comparison with PrMTA and GIC - No difference in IL-1 β expression, all groups. PrMTA showed increased IL6 expression at 3 hrs compared with BD, but not at 24 hrs
Widbiller <i>et al.</i> 2015	- <i>In vitro</i> - Human DPSCs	- BD - PrMTA - GIC	- Cell viability (MTT assay) - ALP activity - Gene expression (Quantitative RT-PCR) for ALP, Runx2, Col1a1, DSPP, RPS18	- BD showed higher cell viability than control. PrMTA had reduced viability at 7d; however, at 10d and 14d the difference not significant, instead GIC significantly lower cell viability

				- Reduction of ALP activity with PrMTA and BD compared to control - Gene expression of Col1a1 initially increased with PrMTA before decline. DSPP expression increased in BD and MTA. Expression of Runx2 down-regulated.
Poggio <i>et al.</i> 2015	- <i>In vitro</i> - Murine pulpal cell-line (MDPC-23)	- Dycal - Calcicur - Calcimol - TheraCal - PrMTA - AMTA - BD	- Cytotoxicity - Cell viability (MTT, Alamar blue assay) - Cell damage/apoptosis (Confocal Laser Scanning Microscope)	- BD, AMTA, PrMTA not cytotoxic; Calcimol, Calcicur, TheraCal slightly more cytotoxic than control; Dycal high cytotoxicity. - BD cell viability remains stable, PrMTA and AMTA showed slightly decreased viability at 72h, Calcicur maintained discrete cell viability while Calcimol and TheraCal have important reduction at 72h. Dycal less cell viability
Gomes-Cornelio <i>et al.</i> 2015	- <i>In vitro</i> - Human odontoblastic cells (Saos 2)	- MTA Plus - BD - Calcium silicate cement (CSCR)+ 30% Zr₂O₅ - CSCR + 30% Nb₂O₅	- Cell viability (MTT assay) - Proliferation and migration (wound healing assay) - ALP activity - Mineralisation (Alizarin Red) - Gene expression (Quantitative RT-PCR) for ALP, GAPDH	- MTA and BD demonstrated a dose-dependent increase in cytotoxicity and decrease in cell viability. CSCR Zr₂O₅ maintained cell viability similar to control in all experimental dilutions. CSCR Nb₂O₅ cell viability lower than control - MTA greatest cell proliferation - ALP expression is higher or similar to control for all groups but CSCR Nb₂O₅ that showed overexpression. - All groups produced comparable mineralised nodules but CSCR Nb₂O₅ showed greatest response
Bortoluzzi <i>et al.</i> 2015	- <i>In vitro</i> - Human pulp stem cell (hDPSCs)	- BD - AMTA - TheraCal - IRM(control)	- Cell viability (XTT assay) - ALP activity - Mineralisation (Alizarin Red) - Apoptosis (Flow Cytometry) - Gene expression (qRT-PCR) for ALP, GAPDH, OC, BSP, Runx2, DSPP, DMP1	- MTA and BD > cell viability than TheraCal - TheraCal more cytotoxic than MTA and BD - BD and MTA up-regulated expression of all genes. - BD produced more mineralised nodules than other materials, MTA better than TheraCal

Margunato <i>et al.</i> 2015	- <i>In vitro</i> - Stem cells isolated from human mandibular bone marrow (hBMSCs)	- BD - PrMTA - MM-MTA	- Cell viability (ELISA) - ALP activity - Mineralisation (Alizarin Red) - Gene expression (qRT-PCR) for Col1a, ON, Runx2	- Similar cell viability in all groups - ALP activity increased in both MTA species in relation to control group, BD decreased - Increased gene expression of Runx2 and ON compared to control group. - No significant differences in mineralisation between materials
Collado-Gonzalez <i>et al.</i> 2017	- <i>In vitro</i> - Stem cells isolated from human exfoliated deciduous teeth (SHEDs)	- AMTA - BD - Theracal - IRM	- Cell viability (MTT assay) - Apoptosis (Flow Cytometry) - Cell migration (Scratch assay) - Cell morphology (SEM) - Mineralisation (Alizarin Red)	- Cell viability and apoptosis higher for BD than MTA. Theracal and IRM low viability. - Cell phenotype unchanged - Cell migration greatest BD - BD increased mineralisation
Tomás-Catalá <i>et al.</i> 2017	- <i>In vitro</i> - Human dental pulp stem cell (hDPSCs)	- BD - NMTA - MTA-HP	- Cell viability (MTT assay) - Cell migration (wound healing assay) - Cell morphology (SEM/EDX)	-All materials good biocompatibility, but BD showed highest cell viability - Higher cell migration with BD, MTA showed values close to control group - More and well organised cells covered BD's surface compared to MTA
Araújo <i>et al.</i> 2018	- <i>In vitro</i> - Stem cells isolated from human exfoliated deciduous teeth (SHEDs)	-BD -AMTA -CH	-Cell viability (MTT assay) -Cell proliferation (SRB assay) -Cell migration (Transwell assay) -Gene expression (RT-PCR) for GAPDH, DMP1	- Quicker migration for BD and MTA compared to CH -CH showed higher proliferation - All materials induced expression of DMP1
Dahake <i>et al.</i> 2020	- <i>In vitro</i> - Stem cells isolated from human exfoliated deciduous teeth (SHEDs)	-EMD -BD -AMTA	- Cell viability (MTT assay) - Mineralisation (Alizarin Red)	- All samples showed cytodifferentiation potential, however EMD gave the highest number of cells functionally differentiated. - EMD appears to have the highest calcification potential, followed by BD and then MTA

Table 1.1 Biological laboratory *in vitro* studies comparing the efficacy of MTA and Biodentine (BD) within the same publication.

Abbreviations: ALP=Alkaline phosphatase, AMTA=Angelus-MTA, BSP=Bone sialoprotein, CH=Calcium hydroxide, DSPP=Dentin sialophosphoprotein, DMP1=Dentine matrix protein-1, EMD=Enamel Matrix Derivative, GIC=Glass ionomer, HO-1=heme oxygenase, IL=Interleukin, IRM=Intermediate Restorative Material, MM-MTA=MicroMega MTA, NMTA=Neo MTA Plus, MTA-HP=MTA Repair HP(Angelus), PrMTA=ProRoot MTA, NO=Nitrous Oxide, OC=Osteocalcin,

ON=Osteonectin, OP=Osteopontin, OMTA=Ortho-MTA, PGE2=Prostaglandin E2, Runx2= Runt-related transcription factor 2, RPS18=Ribosomal protein S18, TNF α =Tumour necrosis factor alpha

IDEAL MATERIAL CHARACTERISTIC	REFERENCE	COMPARATIVE RESULTS
Stimulate reparative dentine formation	Nowicka <i>et al.</i> 2015 Katge & Patil 2017 Bakhtiar <i>et al.</i> 2017 Brizuela <i>et al.</i> 2017	Clinical trial, histological <i>ex vivo</i> study. Tested CH, single bond universal RBC, MTA, BD. Both MTA and BD materials induced a similar hard tissue bridge. The bridge (BD and MTA) was homogeneous with no tunnel defects. MTA, BD and CH, showed a thicker dentine bridge than RBC. Randomised controlled clinical outcome trial. Both BD and MTA induced a hard tissue bridge formation with no differences at 12 months at radiographic analysis. At 6 months MTA shows a better bridge formation on radiograph. Clinical trial, histological <i>ex vivo</i> study. Tested BD, PrMTA and TCal. Teeth vital at 8 weeks with all materials. Significant increase inflammation, tissue disorganisation and incomplete hard tissue bridge formation with TCal when compared to BD and PrMTA. Similar results for BD and PrMTA (lack of inflammation and good tissue organisation); however, BD group showed complete dentinal bridge formation in all teeth with TCal (11%) and PrMTA (67%), respectively. Randomised clinical trial. Tested CH, BD and PrMTA. No significant difference between the materials, selected failures in PrMTA and CH groups, but BD had success rate of 100%.
Maintain pulpal vitality	Nowicka <i>et al.</i> 2013 & 2015 Katge & Patil 2017 Brizuela <i>et al.</i> 2017	Both MTA and BD maintain pulp vitality in 'sound' teeth over the duration of the study (6 weeks). Same duration in both studies. Both MTA and BD maintain pulpal vitality in all treated teeth (caries permanent molars) after pulp capping at 12 months. Both MTA and BD maintain pulpal vitality. Even if no statistically significant MTA showed one case of failure
Release fluoride		No fluoride release from BD or MTA commercial materials
Bactericidal or bacteriostatic	Bhavana <i>et al.</i> 2015 Ceci <i>et al.</i> 2015	MTA, BD and GIC tested against: <i>S. mutans</i> , <i>E. faecalis</i> , <i>E. coli</i> and <i>C. albicans</i> . BD showed best antimicrobial activity. Both MTA and BD are more effective than GIC. PrMTA, AMTA, BD and IRM were checked against <i>S. mutans</i> , <i>S. salivarius</i> and <i>S. sanguis</i> . PrMTA AMTA and IRM showed better results against <i>S. mutans</i> and <i>S. salivarius</i> . BD showed better results against <i>S. sanguis</i> , but was not active against <i>S. mutans</i> .

	Özyürek T & Demiryürek 2016	MTA better antimicrobial activity against <i>P. aureus</i> and <i>E. faecium</i> ; BD better against <i>E. coli</i> and <i>S. aureus</i> .
Adhere to dentine	Guneser <i>et al.</i> 2013	Tested by push out strength test BD, PrMTA, IRM, Amalgam, Dyract AP. BD demonstrated greater adherence to dentine than MTA.
	Nagas <i>et al.</i> 2016	Compared PrMTA and BD by push out strength testing. BD better adhesion to dentine.
	De-Deus <i>et al.</i> 2016	Tested BD and AMTA by push out testing. BD better resistance than AMTA.
	Lucas <i>et al.</i> 2017	Tested BD, AMTA and ZOE by push out strength testing. BD higher bonding strength to dentine than AMTA.
Adhere to restorative material	Altunsoy <i>et al.</i> 2015	Compared MTA, CEM, BD. BD showed the weakest adhesion to flowable composites.
Resist forces during subsequent restoration placement	De Paula <i>et al.</i> 2017	Tested BD, MTA and ZOE. BD and MTA best compressive strength, materials were similar after 24h but BD higher compressive strength after 21 days.
Long-term insolubility restoration	Kaup <i>et al.</i> 2015	Both materials fulfil the ISO standards of solubility but BD is significantly more soluble than PrMTA.
Sterile		Not mentioned in the manufacturer's instructions for either material
Radiopaque	Tanalp <i>et al.</i> 2013	Tested BD, AMTA, MM-MTA. Both MTA significant higher radiopacity, BD under ISO standards.
	Kaup <i>et al.</i> 2015	Tested PrMTA and BD. MTA higher radiopacity, BD below ISO standards.
	Lucas <i>et al.</i> 2017	Tested BD, AMTA and ZOE. MTA higher radiopacity, BD below ISO standards.
Provide seal against bacterial ingress	Nowicka <i>et al.</i> 2013	Both materials did not allow bacterial infiltration.
	Tsisis <i>et al.</i> 2017	Tested BD, PrMTA, IRM. All materials allow bacterial colonization at the interface material/dentine, but bacteria showed the less viability in samples treated with BD/IRM compared with MTA.

Table 1.2 Laboratory and clinical comparison of MTA and Biodentine as an ideal material for vital pulp treatment according to criteria listed in Ingles's Endodontics (Bogen & Chandler 2008).

Abbreviations: AMTA=Angelus MTA, BD=Biodentine, CEM=Calcium-enriched mixture cement, CH= Ca(OH)_2 , GIC=Glass ionomer cement, IRM=Intermediate restorative material, MM-MTA= MicroMega MTA, PrMTA=ProRoot MTA, RBC=Resin-based composite, TCal=TheraCal, ZOE=Zinc Oxide eugenol-based cement

1.6 How are the practical aspects of Biodentine and MTA compared in the laboratory and clinic?

Crown discolouration as a result of VPT does not signify a failure of the procedure (ESE 2006); however, patients are understandably concerned about potential shade change and staining issues as a result of any dental treatment. Comparative studies carried out extracted teeth (animal and human) have concluded that Biodentine has less staining potential compared with MTA (Vall & Duran-Sindreu 2013, Marconyak *et al.* 2016, Shokouhinejad *et al.* 2016, Mozyńska *et al.* 2017, Uesrichai *et al.* 2019, Abuelniel *et al.* 2020) (**Table 1.3**).

Initially MTA was only available in a grey formulation, which was later superseded by a white formula for use in aesthetic areas. White ProRoot MTA has also been demonstrated to discolour teeth (Lenherr *et al.* 2012), particularly in the presence of sodium hypochlorite (Camilleri 2014) and blood (Felman & Parashos 2013). This discolouration has been attributed to the radiopacifier bismuth oxide, which forms a precipitate in the presence of collagen and sodium hypochlorite creating stains (Marciano *et al.* 2014). The anaerobic environment of the root canal system and light irradiation have been reported to enhance formation of stains, while presence of oxygen reduces discolouration (Vall *et al.* 2013). Biodentine contains zirconium oxide rather than bismuth oxide, which has been attributed to reduced colour change in Biodentine treated teeth (Camilleri 2015, Kohli *et al.* 2015, Vallés *et al.* 2015). While, it is acknowledged that tooth discolouration remains possible with all calcium silicate materials, particularly in the presence of blood products, in general Biodentine induced-staining significantly less than MTA (Keskin *et al.* 2015, Yoldas *et al.* 2016). Indeed, only one study reported that Biodentine induced a higher incidence of stains than ProRoot MTA (Beatty & Svec 2015) (**Table 1.2**). Although, the absence of the radiopacifier bismuth oxide in Biodentine has been attributed to the lack of discolouration observed with this material it is also a practical drawback. The radiopacity of Biodentine is consistently reported as being below ISO standards (Tanalp *et al.* 2013, Kaup *et al.* 2015, Lucas *et al.* 2017) and indeed cannot be clearly visualised as distinct to dentine by the operator.

An important practical limitation of MTA is the time taken to set (Kogan *et al.* 2006; Lee *et al.* 2014). This process can take several hours, which limits the practicality of the material to be used in a single visit. As a result, new calcium

silicates such as Biodentine were developed to reduce this time with a setting time of 12 minutes claimed by the manufacturers. This shortened setting time is not predictable; however, with times of up to 100 minutes reported (Grechet *et al.* 2013, Kaup *et al.* 2015, Careddu & Duncan 2021). Direct comparison of setting times between studies is problematic as different ISO value are often employed as standards; however, overall it appears that Biodentine may not set as quickly as the manufacturer claims it does set faster than MTA products (Torabinejad *et al.* 1995, Kogan *et al.* 2006, Wiltbank *et al.* 2007, Kaup *et al.* 2015) with only one study stating a similar setting time between that MTA Angelus and Biodentine (Butt *et al.* 2014).

Traditionally, MTA has been reported to be difficult to handle because of its consistency and formulation (Kogan *et al.* 2006, Wiltbank *et al.* 2007, Ma *et al.* 2011), while Biodentine is considered easier to mix and use (Butt *et al.* 2014), but this may though be a matter of operator preference or training. Questionnaire-based studies have recently investigated Biodentine and MTA use in general (Ha *et al.* 2016) and specifically in vital pulp treatment (Chin *et al.*, 2016). It was highlighted that many dentists avoided using any calcium silicate materials in VPT citing reasons of cost, lack of training and difficulties in material handling (Chin *et al.* 2016, Careddu *et al.* 2021).

ISSUE	MTA	BIODENTINE	REFERENCES
Discolouration	- MTA reported to cause crown discolouration. - Related to the presence of bismuth oxide	- No or less discolouration reported. - Generally less compared with MTA. - Only one study reports more discolouration than MTA (Beatty & Svec 2015)	Bortoluzzi <i>et al.</i> 2007, Lenherr <i>et al.</i> 2012, Felman & Parashos, 2013, Marciano <i>et al.</i> 2014, Camilleri 2015, Kohli <i>et al.</i> 2015, Vallés <i>et al.</i> 2015, Beatty & Svec 2015, Keskin <i>et al.</i> 2015, Yoldas <i>et al.</i> 2016, Uesrichai <i>et al.</i> 2019, Abuelniel <i>et al.</i> 2020
Setting time	- Long setting time between 15 min (MTA Angelus) and > 2 hours (ProRoot MTA)	- BD manufacturer setting time of 12 minutes - Reported up to to sevenfold longer - No significant difference between BD and MTA Angelus reported	Torabinejad <i>et al.</i> 1995, Kogan <i>et al.</i> 2006, Wiltbank <i>et al.</i> 2007, Grech <i>et al.</i> 2013, Kaup <i>et al.</i> 2015
Handling	- Handling is considered difficult because of formulation and difficulties in compacting - Can mix to desired consistency	- BD is generally considered easier to use and mix - Cannot mix to desired or bespoke consistency	Kogan <i>et al.</i> 2006, Wiltbank <i>et al.</i> 2007, Ma <i>et al.</i> 2011, Butt <i>et al.</i> 2014
Cost	- High cost	- High cost	Chin <i>et al.</i> 2016
Radiopacity	- Good radiopacity above ISO radiopacity standards	- BD difficult to visualise as radiopacity similar to dentine - Below ISO standards for radiopacity of dental materials	Tanalp <i>et al.</i> 2013, Kaup <i>et al.</i> 2015, Lucas <i>et al.</i> 2017

Table 1.3 Comparison of practical issues and drawbacks of Biodentine (BD) and MTA investigated *in vivo* and *in vitro*

1.4 Conclusions

Concerns over the cost and destructive nature of dental treatment have led to the promotion of minimally invasive, biologically-based dental restorative solutions. This has resulted in a shift towards more conservative dental procedures aimed to protect the pulp and harness its natural regenerative capacity. The choice of material for pulpal application is critical with MTA establishing itself as the gold standard; however, Biodentine has recently emerged as an alternative as it potentially overcomes many of MTA's limitations. Comparative analysis of the materials within this review revealed a plethora of biological *in vitro* studies, with a range of methodologies and conflicting findings; however, both materials pulpal interaction is consistently better than other materials investigated. There are a smaller number of clinical studies comparing the pulpal response to the materials in permanent teeth. Of the available evidence, histological evaluation highlights a similar response, while VPT outcome studies although preliminary and often under-powered, show equally high material-efficacy. Currently, practical issues consistently separate the materials, with MTA attributed to more tooth staining but greater radiopacity than Biodentine. In the future there is an urgent need for well-designed, adequately-powered, clinical trials in this area as well as improved functional understanding of the interaction of Biodentine with the dentine-matrix.

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Chapter 2.

The Management of Deep Carious Lesions by Members of Two European Endodontic Societies: A Questionnaire-based Study

2.1 Introduction

Maintaining the health and vitality of the dental pulp is a principle goal of Operative Dentistry and Endodontics (Duncan *et al.* 2019). Although, the management of deep caries and the exposed pulp has been carried out for many years, it has traditionally been considered to have a poor outcome (Barthel *et al.* 2000, Bjørndal *et al.* 2010), which limited its indication to the treatment of teeth with immature apices or after traumatic pulp exposures (ESE 2006). Recently, vital pulp treatments (VPTs) have undergone a renaissance with good success reported after one and two-visit selective removal techniques to avoid pulp exposure, as well as carious exposures treated by pulp capping (Bogen *et al.* 2008, Marques *et al.* 2015), partial pulpotomy (Taha & Khazali 2017) and full/coronal pulpotomy (Simon *et al.* 2013), even in case with signs and symptoms indicative of irreversible pulpitis (Taha *et al.* 2017, Cushley *et al.* 2019). Notably, the recent increase in the success of VPT in teeth with deep carious lesions, has largely been in studies carried out by endodontic researchers, employing enhanced protocols (Bjørndal *et al.* 2019), including disinfectant lavage of the pulpal wound, magnification and application of hydraulic calcium silicate cements (HCSCs) (Bogen *et al.* 2008, Simon *et al.* 2013, Marques *et al.* 2015, Taha *et al.* 2017). Ironically, endodontists may not be the principal group treating the bulk of carious exposures due to the nature of the referral system or their scope of practice (Bjørndal *et al.* 2019).

For many years calcium hydroxide [Ca(OH)₂], has been considered the material of choice for management of the exposed pulp (Glass & Zander 1949, Stanley & Lundy 1972, Tronstad 1974), however, it is limited by incomplete hard tissue bridge formation and poor sealing qualities and has been superseded by HCSCs such as mineral trioxide aggregate (MTA) or Biodentine as the material of choice (Nair *et al.* 2008, Hilton *et al.* 2013, Duncan *et al.* 2019). Questionnaire-based surveys in which dentists examine radiographs of carious lesions and describe treatment options, have highlighted the dilemma of whether a tooth

should be treated conservatively by VPT or whether a more invasive approach is required (Oen *et al.* 2007, Stangvaltaite *et al.* 2017). The results have demonstrated a huge variance between respondents and highlighted that there is no uniform decision-making for the management of deep carious lesions (Chin *et al.* 2016). Currently, the majority of dentists adopt an invasive approach choosing to expose the pulp or perform a pulpectomy and root canal treatment (RCTx), rather than avoiding pulp exposure (Stangvaltaite *et al.* 2017, Schwendicke *et al.* 2017). However, at present the bulk of these questionnaire-based studies are carried out in general practice and it is unclear the effect of further education in endodontics or geographical location has on decision-making for the management of deep carious lesions.

2.2 Study aims and objectives

As there appears to be no consistency amongst general dental practitioners in Europe on the most appropriate way for management of deep carious lesions, the aim of this study was to investigate whether being a member of an Endodontic society resulted in more standardised treatment protocols in this area.

- Objective 1: To investigate and compare how the management of deep carious lesions and the exposed pulp is carried out by members of the Irish (IES) and Italian (AIE) Endodontic Societies.
- Objective 2: To investigate the influence of patient-related factors, including age and symptoms on the decision-making process for the management of deep carious lesions.
- Objective 3: To investigate the influence of dentist-related factors, including material choice between members of endodontic national societies of two different countries use to manage the exposed pulp.

2.3 Materials and methods

2.3.1 Ethical Approval

Ethical approval was granted for the study from St James's Hospital Research Ethics Committee (REC/2017/04/1).

2.3.2 Questionnaire

A structured online questionnaire-based study was sent to all registered members of two endodontic societies (IES and AIE) as an e-mail link. The questionnaire was designed by the principal researcher (RCD) and principal investigator (HD) and was based around two radiographs highlighting deep carious lesions with two scenarios for both radiographs (**Figure 2.1a & b**). In order to confirm that the questionnaire could be completed within 10 minutes and that the questions and flow of the survey were coherent and logical, a small pilot was organised and evaluated using three endodontists in Dublin. This was carried out in paper format, with the average time to complete the survey five minutes. The evaluators were encouraged to be critical; however, the feedback was generally positive. In order to avoid possible misunderstandings and longer time to complete the survey by AIE members, it was translated in Italian by the principal researcher (RCD). There was no difference in contents between the two versions of the questionnaire.

After completion of the pilot study, and subsequent minor alterations an online version was created using "Google Docs" (<https://docs.google.com/forms>). The investigators elected to use this platform as it was familiar and data could be collected without asking the participants to download software or required other actions. This online version was created using RCD's account and verified through a "test link". After confirming that the survey displayed correctly and that the sample answers were coherent, the survey was considered ready for distribution.

Initially, the principle researcher contacted the secretaries of the two endodontic societies, the AIE in Italy, and the IES in Ireland in order to confirm that they were willing to participate in an online research study, which asked their members to discuss management of deep carious lesions. After both societies agreed to engage in the study, a final copy of the questionnaire was sent to both

the AIE and IES for their formal approval. The questionnaire was appraised by the committee of the AIE and IES who agreed with the format of the questionnaire. Finally, they distributed the survey via email to all members of the AIE (197 members) and the IES (50 members), who had provided their email address to the society with a deadline of completion of three weeks. Subsequently, two invitations/reminders to complete the survey were sent to the secretaries of the Societies.



Figure 2.1a Case 1: An 18-year-old patient presenting with a deep carious lesion on 36. Tooth remained vital and the pulp was accidentally exposed during non-selective carious tissue excavation. There were two scenarios; in scenario one there were no symptoms, while in scenario two there were mild symptoms triggered by cold/sweet and tenderness when biting



Figure 2.1b Case 2: A 45-year-old patient presenting with a deep carious lesion on 46. The tooth remained vital and the pulp was accidentally exposed during non-selective carious-tissue excavation. There were two scenarios; in scenario one there were no symptoms, while in scenario two there were mild symptoms triggered by cold/sweet and tenderness when biting.

2.3.3 Demographic component of questionnaire

Within the questionnaire format, a series of general demographic questions were asked in order to collect as much information as possible, related to age, experience and training (**Table 2.1**). Qualifications and numbers of years since obtaining the primary degree were recorded, as well as the nation where the participant obtained the primary degree and was currently based their practice. Finally, in order to quantify the experience of the participant, the number of VPT procedures carried out every month was asked (**Table 2.1**).

DEMOGRAPHIC DATA COLLECTED
- Age - Country where you work - Country of primary qualification - Number of years after obtaining primary dental qualification - Qualifications - List any university-accredited post-graduate qualifications you have in endodontics (e.g. certificate, diploma, masters, PhD, Royal College of Surgeons examination) - How many vital pulp treatments would you carry out per month? (0, 0-5, 5-10, 10+)

Table 2.1 Questions related to demographic information and volume of VPT carried out per month.

2.3.4 Clinical component of questionnaire

Periapical radiographs from two separate patients demonstrating deep carious lesions were used as the basis of the scenarios (Case 1 and 2). For the purposes of this study deep caries was defined as ‘Caries reaching the inner quarter of dentine, but with a zone of hard or firm dentine between the caries and the pulp, which is radiographically detectable when located on an interproximal or occlusal surface. There is a risk of pulp exposure during operative treatment’ (Duncan *et al.* 2019). Case 1 was based on a radiograph demonstrating a radiolucency indicative of a deep carious lesion in the 36 (lower left molar) in an 18-year-old patient (**Figure 2.1a**), while case two (**Figure 2.1b**) was based on a radiograph showing a similar radiolucency of the 46 (lower right first molar) in a 45-year-old patient. In both case 1 and 2, the clinical examination described the pulp as being “exposed during complete carious tissue excavation”. Within both case 1 and 2 there were two additional scenarios, which differed in their description of the presence or the absence of symptoms. In scenario 1, there was a complete absence of any pulpal symptoms, while in scenario 2, the patient reported mild symptoms triggered by cold and sweet substances and on examination the tooth was tender to percussion (TTP).

Four further multiple-choice questions were asked about each of the scenarios (**Figure 2.2**). The first question related to the treatment of choice for each scenario, with a range of answers from minimally invasive techniques to RCTx (patient-related question). The second question related to the material of choice for VPT, while the third question asked for the specific reason behind the choice of material choice, again selecting from a list of options (operator-related). A fourth and final question was asked whether the use of an antibiotic, either topical or systemic would be considered (operator-related) (**Figure 2**).

1. HOW WOULD YOU TREAT THIS TOOTH?

- ☐ Pulp Capping
- ☐ Partial Pulpotomy (or Cvek pulpotomy)
- ☐ Full coronal pulpotomy
- ☐ Pulpectomy and root canal treatment

2. IF PULPOTOMY IS PERFORMED WHAT MATERIAL WOULD YOU CHOOSE?

- ☐ Hard setting calcium hydroxide
- ☐ MTA
- ☐ Biodentine
- ☐ Non-setting calcium hydroxide
- ☐ Ledermix/ Odontopaste
- ☐ Other _____

3. WHY?

- ☐ It gives me predictable outcomes
- ☐ I was trained to use it at college
- ☐ I am not familiar with the others
- ☐ It is easiest to handle
- ☐ Expense
- Other _____

4. WOULD YOU USE ANTIBIOTICS (EITHER TOPICAL [E.G. LEDERMIX] OR SYSTEMIC)

☐ Y

☐ N

Figure 2.2 Multiple choice questions asked about each of 4 scenarios in the questionnaire. The same template was used for both cases and scenarios.

2.3.5 Data collection and statistical analysis

The participants received the survey as a link, specific to the AIE (<https://forms.gle/qDVkUD1NEeQuiUj28>) and specific to the IES (<https://forms.gle/tzZov6Etzzfw7Mow8>). After accessing the link, the participant was redirected to a webpage to complete the questionnaire. At the end of the questionnaire the information was automatically and anonymously forwarded to a 'Google Doc' account of the principal researcher (RCD). The responses were individually analysed initially for completeness and then transferred and collated into an electronic database for analysis. The Chi-square and Fisher's exact test were used for evaluation of the results. The *P*-value <0.05 was considered significant.

2.4 Results

2.4.1 Demographic results

From a total of 197 AIE members, 96 responded and from a total of 50 IES members, 24 responded (total 120). This represents a response rate of 48.7% for the AIE and 48% for the IES. Demographic data revealed a broadly similar age distribution between the two societies (**Table 2.2a**). In terms of further education in Endodontics, the majority of AIE members had no further qualifications (62.5%), while the majority of IES respondents had a masters in endodontics (70.83%). Five AIE, but no IES respondents had a PhD (**Table 2.2b**). The reported volume of VPT carried out per month differed between the two societies. AIE respondents reported a range of VPTs per month, with 1–5 cases completed in 31.5%, 5-10 in 32.5% and over 10 completed in 36%, while in comparison IES respondents reported completing less VPT with 1-5 cases in 61%, 5-10 in 13%, over 10 in 8.5%, with 17.5% not carrying out any VPT.

AGE (years)	25-35	35-45	45-55	55+
AIE	26.3%	30.5%	22.1%	21.1%
IES	25.1%	20.8%	33.3%	20.8%

Table 2a Age distribution between the two societies.

	PhD	MSc	Diploma	None
AIE (96)	5 (5.21%)	20 (20.83%)	11 (11.46%)	60 (62.50%)
IES (24)	0	17 (70.83%)	2 (8.33%)	5 (20.83%)

Table 2.2b Post-Graduate Endodontic education reported by participants. Figure in brackets = number of participants.

2.4.2 Patient-related results

2.4.2.1 Do patient symptoms alter the decision-making process?

After pooling the results of both societies, the presence or absence of symptoms significantly altered treatment planning decisions ($p < 0.001$). The younger asymptomatic patient described in Case 1 Scenario 1 resulted in more conservative-treatment options, while there was a significant increase in the selection of RCTx when symptoms were added to the history (Case 1 Scenario 2) (**Table 2.3**). Symptoms had a similar impact on older patients, with the absence of symptoms (Case 2 Scenario 1) resulting in more VPT than if symptoms were present (Case 2 Scenario 2) ($P < 0.0001$) (**Table 2.3**). The presence of symptoms led to more invasive treatment choices, with a 27% increase in RCTs in the young (Case 1) and 32% in the older patient (Case 2). Pulpotomies were selected more often in both societies for young symptomatic patients, compared with asymptomatic cases (partial pulpotomy 24% versus 18%; full pulpotomy 12% versus 8%); however, this pattern was not evident in the older patient. Pulp capping was commonly selected treatment modality for asymptomatic carious exposure regardless of age (**Table 2.3**).

	Pulp Capping	Partial Pulpotomy	Full Pulpotomy	RCTx
C1 – S1	64 (53%)	22 (18%)	9 (8%)	25 (21%)
C1 – S2	19 (16%)	29 (24%)	14 (12%)	58 (48%)
C2 – S1	52 (43.5%)	22 (18.5%)	4 (3%)	42 (35%)
C2 – S2	17 (14%)	18 (15%)	5 (4%)	80 (67%)

Table 2.3 Pooled results of AIE and IES (total 120) with regard to symptoms. Difference in treatment planning in absence (Scenario 1 – S1) or presence (Scenario 2 – S2) of symptoms for younger (Case 1 - C1) and older patient (Case 2 – C2).

2.4.2.2 Does the patient's age alter the decision-making process?

Patient age significantly influenced the treatment choice. There was a significant difference between younger (Case 1) and older patient's (Case 2) treatment plans in both the absence ($p=0.043$) or presence ($p=0.012$) of symptoms when the results were pooled (**Table 2.3**). In the older patient, participants tended to carry out less VPT and be more invasive selecting RCTx 14% more often for asymptomatic older patients and 19% for the older symptomatic cases compared with younger cohorts (**Table 2.3**).

If the overall responses of the AIE and IES are compared, there were no significant differences in the treatment of a young patient (Case 1) in the presence ($p=0.302$) or absence of symptoms ($p=0.297$). Treatment decisions for an older patient (Case 2), demonstrated a significant difference between the two Societies for both symptomatic ($p=0.041$) and asymptomatic scenarios ($p=0.044$) with significantly more RCTs carried out in the AIE than the IES (**Table 2.4**).

Case 2	Society	Pulp Capping	Partial Pulpotomy	Full Pulpotomy	RCTx
S1	AIE	44 (46%)	13 (13.5%)	2 (2%)	37 (38.5%)
S1	IES	8 (33.5%)	9 (37.5%)	1 (4%)	6 (25%)
S2	AIE	15 (15.5%)	12 (12.5%)	2 (2%)	67 (70%)
S2	IES	2 (8.5%)	6 (25%)	3 (12.5%)	13 (54%)

Table 2.4 Separated results of AIE (96 respondents) and IES (24 respondents) with regard to older patient (Case 2). Difference in treatment planning in absence (Scenario 1 – S1) or presence (Scenario 2 – S2) of symptoms illustrated.

2.4.3 Operator-related results

2.4.3.1 What vital pulp treatment materials were most commonly chosen?

HCSMs were the most commonly selected (**Figure 2.3**), with Biodentine and MTA combined accounting 81% of IES and 69% of AIE selected VPT materials. A smaller percentage of IES members choose Biodentine (17%) over MTA (64%), compared with AIE respondents (Biodentine 28% and MTA 31%). Both hard and non-setting $\text{Ca}(\text{OH})_2$ materials were also commonly used (40% of AIE, 19% of IES), with hard setting materials more common in both societies (**Figure 2.4**). No other materials were commonly used by either society

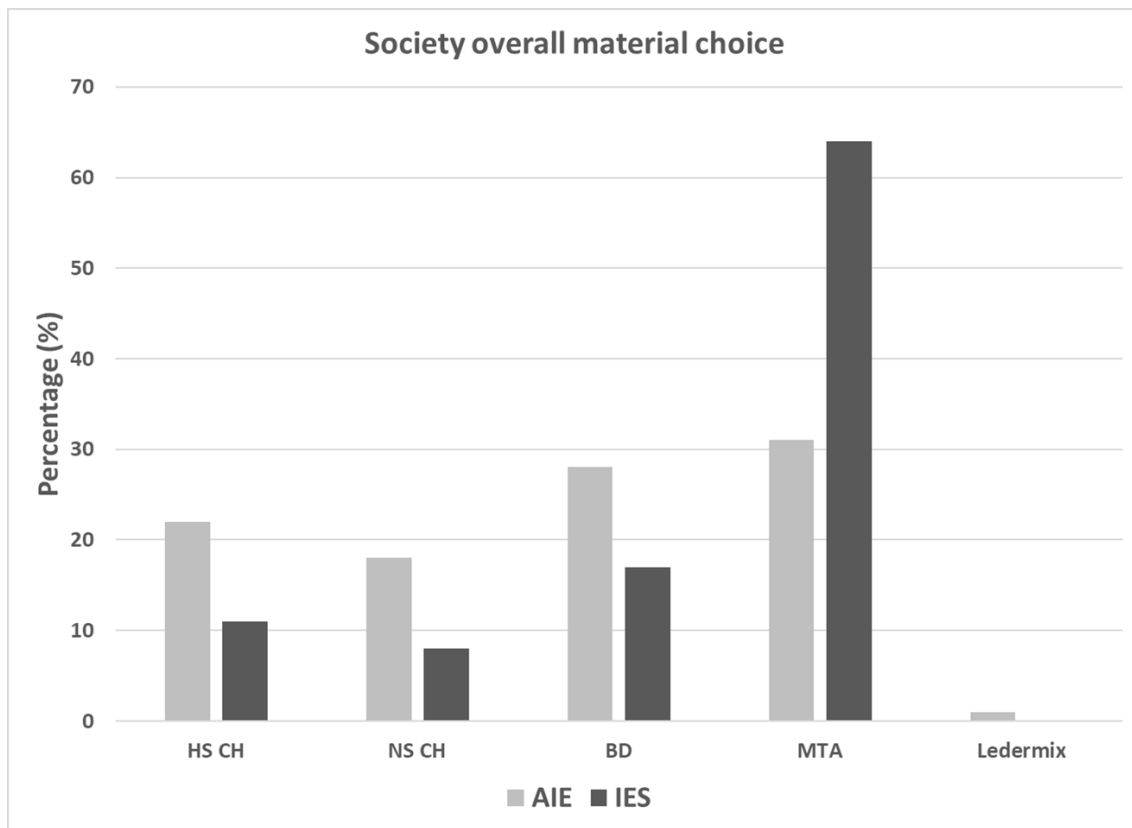


Figure 2.3 Overall VPT material choice in both societies. HS CH = Hard setting calcium hydroxide; NS CH = Non setting calcium hydroxide; BD = Biodentine; MTA = Mineral trioxide aggregate

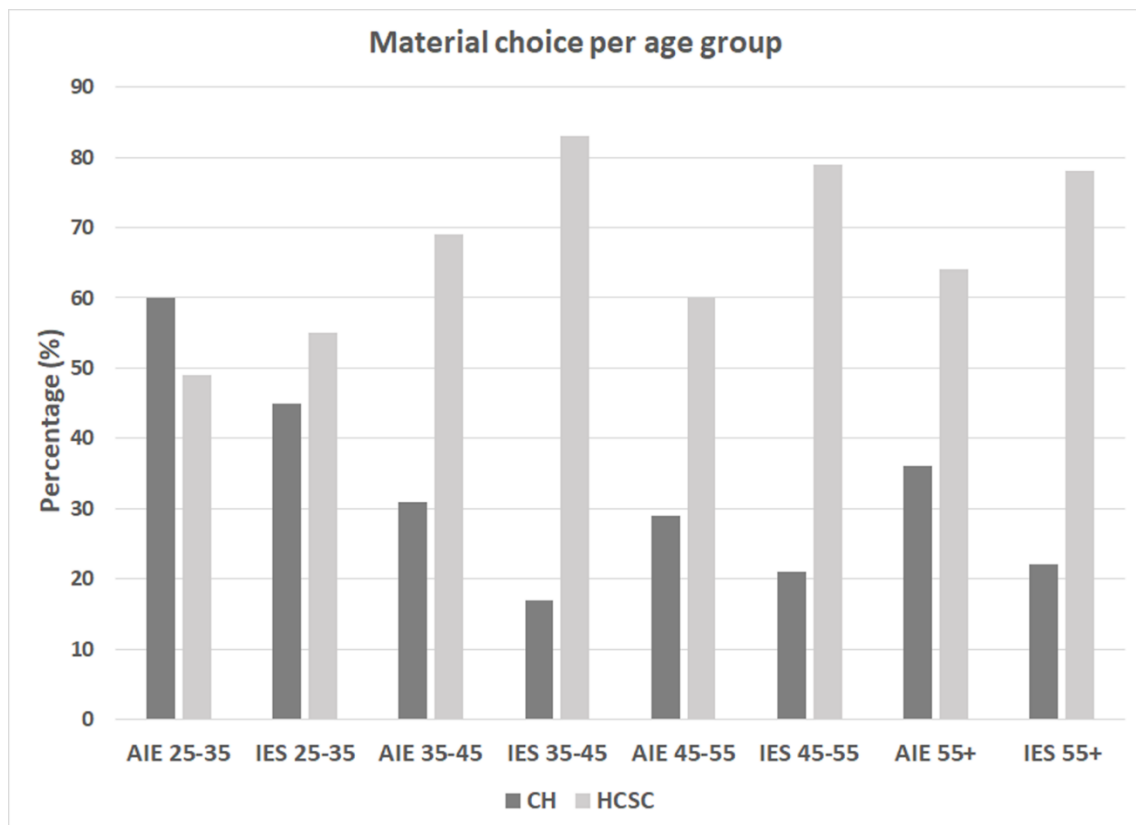


Figure 2.4 Choice of vital pulp treatment materials in the AIE and IES by age group

2.4.3.2. Did the age/experience of the operator make a difference to treatment plan?

To gauge the influence of experience on the treatment plan, respondents from both societies were categorized by age group. In general, younger dentists (25-35 years and 35-45 years) prescribe less RCTx than the older groups (45-55 years and 55+ years) (**Table 2.5**). When the youngest age group (25-35 years) was compared with older dentists (45-55 years and 55+ years), the older cohorts selected RCTx as the most appropriate choice in all Cases and Scenarios in both societies. Overall the most invasive cohort for both societies was the 45-55-year group, while for all age groups, symptomatology increased invasiveness in all groups. (**Table 2.5**).

2.4.3.3 Did the age/experience of the operator alter material choice?

In the AIE, CaOH₂ (hard and non-setting) was chosen as the most suitable material by 60% of the 25-35 year-olds, while HCSMs (Biodentine and MTA) were the first choice in all other age groups (35-45 years 63%; 45-55 years 62%; 55+ years 76%) (**Figure 2.4**). Biodentine and MTA were chosen similarly within all age groups (25-35 years BD 19%, MTA 21%; 35-45 years BD 37%, MTA 32%; 45-55 years BD 27%, MTA 33%; 55+ years BD 24%, MTA 36%).

For the IES, the age group 25-35 years selected Ca(OH)₂ as material of choice in 45% of cases and a HCSM in 55%, while in every other age group choose calcium silicates was the overwhelming choice (35-34 years 83%; 45-55 years 79%; 55+ years 78%) (**Figure 2.4**). For Irish respondents, MTA was selected more than Biodentine in all age groups with 83% of 35-45 year-olds, 63% of 45-55 year olds and 56% of 55 years+ selecting MTA over Biodentine.

2.4.3.4 What was the reason for choosing a material?

The dominant factor for the material choice was the predictability of outcome (AIE 59%, IES 86%), followed by the handling of the product (AIE 17%, IES 5%). Cost was only considered an issue by less than 5% of both society members.

2.4.3.5 Were antibiotics prescribed?

The use of antibiotics was considered unnecessary by almost all the respondents. Only two respondents indicated using antibiotics, both for case 2 (older patient, with or without symptoms). Both respondents were members of the AIE.

2.5 Discussion

The questionnaire was designed to be easy to read and straightforward to complete. The survey was carried out online, avoiding the need for member addresses, postage, printing and stamped envelopes for subsequent return of the questionnaire. It was hoped that this would increase engagement with the study. Indeed, the response rate of nearly 50% is good in comparison with similar recent postal studies carried out in this subject area (Chin *et al.* 2016, Ha *et al.* 2016, Schwendicke *et al.* 2017). Although effectively half of the membership of the two societies responded, the IES and AIE differ markedly in their member

numbers, so it is accepted that the study is limited by the fact that many more views were analysed from the Italian cohort. Notably, more respondents from the IES reported having a taught Master's degree in Endodontics, while the majority of AIE members have no structured postgraduate education. By contrast, five AIE members had a PhD, while no IES respondents possessed a doctorate. This is all likelihood reflects different traditions in the two jurisdictions as there is no history of structured endodontic training in Italy and equally no history of postgraduate research activity by endodontists in Ireland. A potential limitation of this study is the representativeness of the sample chosen, which is not a general dentist population. This was by design as although several studies have analysed views of general dental practitioners in VPT (Oen *et al.* 2007, Stangvaltaite *et al.* 2017, Schwendicke *et al.* 2017), there is an absence of studies analysing the view of endodontists. As neither Italy or Ireland officially recognise the speciality of Endodontics, members of the endodontic societies represented a group aligned to the subject in those countries. As endodontists have recently become the principle group driving minimally-invasive VPT for the exposed pulp (Bjørndal *et al.* 2019, Duncan *et al.* 2019) this group view on VPT was of particular interest.

Over the last 10-15 years MTA has become the international 'gold standard' material for use in VPT (Damaschke 2008, Nair *et al.* 2008, Ricucci *et al.* 2020). As a result, it was hypothesised during the planning stages of this study that MTA and other HCSMs (e.g. Biodentine) would be the most popular material choices, particularly amongst younger respondents. Particularly within Italy, calcium hydroxide materials remained as popular as MTA and Biodentine, and perhaps more surprisingly younger AIE and IES members preferred Ca(OH)_2 over HCSMs, compared with older respondents. The reasons for this are unclear, but is likely to relate at least in part to undergraduate teaching and training, where the influence is still stronger for those just qualified than for those who have been qualified for a longer period. Older practitioners may be more influenced by experts speaking at conferences and practical courses, as there has been much evolution in the area of VPT since they qualified. MTA and Biodentine are significantly more expensive than Ca(OH)_2 ; however, this was considered a minor reason not to use the material being highlighted by less than 4% of respondents in both territories. Of course, younger dentists in reality are more

likely to be working for someone else and are often not in the position to select the materials they would prefer. HCSMs are awkward to handle clinically and therefore we were interested in whether this was a factor determining material choice. Although, it was noted to be important by 17% of AIE respondents, it was only a minor concern for IES respondents with only 5% suggesting it influenced their choice of material. It may be a reason as to why younger people are using the more recently introduced Biodentine, which has shown to be easier to handle (Careddu & Duncan 2018, Rajasekharan *et al.* 2018).

Biodentine was used relatively more by AIE members than IES members in all except the youngest age group. Again, this may reflect differing teaching practices in the two jurisdictions, but it may also reflect the impact of the marketing on material choice and it is possible that in Italy the distribution and advertisement for Biodentine has been more consistent and aggressive than in Ireland. Septodont as a company have traditionally been involved in the sale of dental anaesthetic products rather than dental materials in Ireland, although a recent change in direction has started the promotion of biomaterials such as Biodentine and Bioroot RCS in dental schools.

In the AIE VPT techniques were more common carried out with an almost equal number of responses for the three groups (1-5, 5-10, 10+ VPT in a month). On the contrary, Irish respondents were less inclined to perform VPT with the majority of them performing not more than 5 treatments in a month and many respondents reporting that they did not to carry out these procedures at all. This could be related to a difference in teaching received at University as well as the fact that in Italy, Endodontists often perform some general practice while in Ireland there is a stronger referral-based system in place.

In general, the presence of pulpal symptoms increased the number of respondents who would perform a RCTx, rather than VPT both in AIE and IES. This is a clear indication that, even mild/moderate symptoms are considered a characteristic that may lead to failure of a conservative approach. This is in light of the fact that there is not a good correlation between diagnosis based on clinical symptoms and actual status of the pulp (Mejàre *et al.* 2012). This has been challenged recently when a good correspondence between clinical findings, and the reversible/irreversible pulpitis histologically was demonstrated (Ricucci *et al.* 2014). In this study, the cases highlighted the presence of mild symptoms, more

indicative of reversible than irreversible pulpitis; however, this was sufficient for respondents to select pulpectomy more often than a VPT procedure if symptoms were present.

Patient age appeared to have a considerable influence on the decision-making process in both societies. In both the presence or absence of pulpal symptoms, participants adopted a less conservative approach in older patients, which cannot be attributed to the level of root formation as in both cases root formation was complete. This supports other questionnaire-based studies examining operator choice in a range of scenarios, which have highlighted more invasive choices in older patients (Frisk *et al.* 2013). When considering why patient age is considered important, it should be noted that there is no firm clinical evidence to suggest that age is a significant factor for the success of VPT. Although, a recent prospective pulp capping study compared results in patients above and below 40 years and concluded that treatment was less successful in older patients (Marques *et al.* 2015); other studies with a similar age ranges have showed no difference (Matsuo *et al.* 1996, Mente *et al.* 2014). There is certainly a perception that young people and indeed pulp heals more predictably with prospective studies analysing the histological response to VPT materials invariably involving young patients (Hørsted-Bindslev *et al.* 2003, Accorinte *et al.* 2008, Nair *et al.* 2008) as do clinical pulp capping outcome studies in carious teeth (Farsi *et al.* 2006, Taha & Khazali 2017). Younger patients, under the age of 20, are selected due to the greater blood supply, the potential for open root apices and increased cellularity of their pulps; it has been suggested that these characteristics should result in more predictable healing (Massler 1972).

A further point of interest, was that when we analysed the age since qualification older groups were more inclined to perform RCTx than the younger, especially in absence of symptoms. This may be due to younger dentists being more aware of the latest developments in VPT (Bjørndal *et al.* 2010; Wolters *et al.* 2017), or it may reflect experience dictating that RCTx was more predictable in their 'hands' than VPT. On a positive note it appears that the need to reduce antibiotic prescribing and the lack of rationale for using antibiotics for 'toothache' has been effective at least for this cohort of participants (Agnihotry *et al.* 2019).

2.6 Conclusions

Within the limitations of this study it is clear that VPT is carried out frequently by both members of the IES and AIE. There is no consistency in approach about the most appropriate management for the exposed pulp or the VPT material of choice. Patient symptoms and age influence the decision-making process. Ca(OH)_2 was the most commonly used material only in the youngest AIE respondents, while MTA was the most commonly selected material from all ages of IES respondents. Members of both societies suggested that the predictability of the results, rather than cost or handling was the principal reason behind material choice.

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Chapter 3.

Histological Comparative Investigation of Two Hydraulic Calcium Silicate Cements in a Randomised Experimental Pulp Capping Study

3.1 Introduction

Preservation of dental pulp vitality and the prevention of apical disease, form the basis of operative dentistry. Recent advances in material science and a deepening understanding of pulp reparative responses have resulted in the promotion of biologically-based therapies and increasingly predictable outcomes for vital pulp treatment (VPT) (Smith *et al.* 2016, Kundzina *et al.* 2017). Historically, carious-exposures in permanent teeth were treated either by extraction of the tooth or root canal treatment (Swedish Council on Health Technology Assessment 2010, Bjørndal *et al.* 2019). However, a shift of focus to minimally-invasive strategies using hydraulic calcium silicate cements, including Mineral Trioxide Aggregate (MTA) and Biodentine, allied with careful case selection, vital pulp techniques such as direct pulp capping (Harms *et al.* 2019, Ballal *et al.* 2020 & 2021), partial pulpotomy (Barrieshi-Nusair & Qudeimat 2006, Taha & Khazali 2017) and full coronal pulpotomy have all shown to be successful (Taha *et al.* 2017). Although potentially altering decision-making for management the exposed pulp (Careddu *et al.* 2021), questions remain about the nature of the cellular response to vital pulp procedures (Ricucci *et al.* 2014), the relative efficacy of one material over another (Nair *et al.* 2008) and the quality of the repair within the dentine-pulp complex after several months (Dammachke *et al.* 2019).

Although the absence of clinical and radiographic signs and symptoms and continued response to pulp sensibility tests are indicators of the clinical success of VPT (ESE 2019), histological success principally relates to the absence of soft tissue inflammatory changes in the pulp and presence of a hard tissue bridge over the exposure (Schröder *et al.* 1972, Hargreaves & Berman 2016). In VPT, the hard tissue bridge formed over the pulp exposure is generally considered reparative rather than regenerative (Simon *et al.* 2014); however, there remains a lack of consensus on the exact composition of the hard tissue

and indeed the nature of the secretory cell responsible for its formation (Ricucci *et al.* 2014, Duncan *et al.* 2019). The traditionally advocated theory suggests a cyto-differentiation of progenitor cells under the influence of bioactive molecules, sequestered from both the dentine matrix and released from dental pulp cells, in which progenitor cells are recruited from central pulp, perivascular areas or migrate into the tooth and undergo subsequent differentiation into odontoblast-like cells, before secreting a dentine-like hard tissue bridge (Lesot *et al.* 1993, Frozoni *et al.* 2012, Smith *et al.* 2016, Duncan *et al.* 2019). Notably, for odontoblast-like cells to be considered morphologically similar to odontoblasts they need to demonstrate the presence of an odontoblast process, requiring the hard tissue repair to possess tubularity; a feature which is invariably absent in reparative hard tissue bridges examined histologically (Nair *et al.* 2008, Ricucci *et al.* 2014, Dammaschke *et al.* 2019). Other authors propose an alternative theory, that the hard tissue formation is part of a reparative scar process sustained by mineral secreting fibroblasts (Ricucci *et al.* 2014). Fibroblasts have been shown to have an ability to produce mineral *in vitro* (Yamamoto *et al.* 2015 & 2018) and can contribute to calcification elsewhere in the body (Ronchetti *et al.* 2013); however, in order to form a hard tissue bridge would also have to be induced to undergo a cyto-differentiation as they do not naturally secrete mineral. This has not yet been shown to occur in the dental pulp (Duncan *et al.* 2019).

Notably, experimental pulp capping studies employ unrestored teeth and short recall periods (Fransson *et al.* 2016); however, clinically both traumatic and carious pulpal injuries are affected by infectious, inflammatory and operator events, and as a result the interplay between pulp injury, infection, pulpitis and altered host defence responses over several months will be critical to the final outcome (Duncan *et al.* 2019). A focus of multiple experimental pulp capping studies has been on role and type of capping material (Aeinehchi *et al.* 2003; Nair *et al.* 2008; Fransson *et al.* 2016) rather than other variables such as disinfection (Ballal *et al.* 2020), bacterial leakage (Cox *et al.* 1985) or technical aspects of the procedure itself. Indeed, the bulk of studies investigating the comparative quality of pulp capping materials do not comment on the technical aspects of the procedure or verify the histological absence of microorganisms associated with failed or poor healing outcomes (Damaschke *et al.* 2019).

3.2 Study aims and objectives

The aim of this study was to investigate the pulpal response to experimental pulp capping using two hydraulic calcium silicate cements, Biodentine (Septodont, Saint-Maur-des-fossés, France) and a commercial MTA product (White Angelus-MTA [Angelus, Londrina, Brazil]), in 'sound' human teeth. The objectives were to;

- Objective 1: To study the nature and the quality of the mineralised bridge formed over the pulp exposure after 6 months using light microscopy.
- Objective 2: To compare the hard (mineralised bridge) and soft tissue response (pulpal inflammation) after application of Biodentine and MTA.
- Objective 3: To study the presence of bacteria adjacent to the capping material and in the pulp tissue as well as analysing the morphological nature of the cells in contact with the mineralised bridge.

3.3 Materials and Methods

This was an experimental pulp capping study on 'sound' human teeth with subsequent qualitative histological assessment. The clinical component was carried out in dental practice in Dublin, linked to the Division of Restorative Dentistry, Dublin Dental University Hospital (DDUH) and the histological component carried out in the private dental practice in Cetraro, Italy.

3.3.1 Experimental clinical trial

3.3.1.1 Ethical and pseudo-anonymous data storage

The study was granted Research Ethics Committee approval from St James's Hospital (JREC), Dublin on 25th April 2017 (REC ref: 2017/04/01). This involved a patient information leaflet for minors and adults and a consent form for minors and adults. On the treatment day the procedure was discussed again and the patient's willingness to participate confirmed. The operator went through the consent form and encouraged discussion about the most important aspects, answering possible questions and clarifying doubts. Once the patient was satisfied with the answers received they were asked to sign a consent form. Three copies were signed, one for the patient, one for the patient's dental chart

and one for the researcher's own study record. The data was collected and stored on an encrypted computer under an anonymous code. The key to the code was kept on a separate encrypted computer held by the study gatekeeper, hence keeping any data in a pseudo-anonymised state. During the procedure pictures of the inside of teeth (pulp chamber) were taken with a camera attached to the microscope to compare the size of the pulp exposure. The pictures were not associated to names (only codes), did not include the face of the patient and did not have any identifiable features only showing the pulpal chamber.

3.3.1.2 Patient sample and preoperative assessment

The sample group consisted of patients with healthy premolar or third molar teeth (mandibular/ maxillary), which were scheduled for extraction. Patients were recruited by one operator directly after the treatment plan had been decided. The inclusion criteria were male or female patients aged between 14-50 years (patients under 18 years required the consent of parent/guardian) with an experimental tooth responding positively to pulp sensibility testing (cold thermal and electric), which was scheduled for extraction. The tooth was to be extracted for either orthodontic or prevention reasons and needed to be completely caries-free. Teeth excluded from participation in the trial were: non-vital or carious teeth, teeth that could not be isolated with rubber dam or teeth that required sectioning of the roots in order to be extracted (**Table 3.1**).

After the decision to extract one or more of the teeth was made, the patient's teeth were examined for suitability for inclusion in the study. If the patient conformed to the inclusion/ exclusion criteria they were informed verbally of the nature of the study. The aim of the study was reinforced, as was the fact that their participation was voluntary, would not affect their treatment in the practice and that they were free to withdraw at any time. If at this stage the patient expressed an interest in the study, the procedure itself was discussed along with the likely drawbacks of any participation. Afterwards the patient was asked to confirm that they understood about what had been said and asked if they had any questions. Every interested patient was given a written patient information sheet for him or her to study at home and discuss further with his or her family or partner. They were informed that they could digest the material for a period and that they would be contacted by phone approximately one week later to ask if

they had any more questions and if they were willing to participate in the study. If at this stage they were willing to participate, an appointment was arranged and confirmed in writing. It should be noted that patients were not financially remunerated for participation in the study.

INCLUSION CRITERIA	EXCLUSION CRITERIA
- Patient aged 14 to 50 - Vital teeth (positive response to pulp sensibility testing) - Caries free teeth - Teeth scheduled for extraction for orthodontic/prevention/third molar reasons	- Patient aged <14 or >50 - Non-vital teeth - Presence of decay - Pregnant women - Not possible to isolate with rubber dam - Teeth that needs to be sectioned in order to extract

Table 3.1 Inclusion and exclusion criteria

3.3.1.3 Randomisation and power calculation

A randomised allocation was employed within this study to ensure that the two materials were allocated free of any operator bias; however, a power calculation was not carried out. This was a qualitative sample using histological comparison of two pulp capping materials and not an outcome study with a quantifiable end-point in terms of success and failure. For practicality reasons, including the complexity of carrying out histological evaluation of serial sections on large numbers of samples, the numbers of patients in the study was based on previous research projects of a similar nature investigating the biological qualitative response to pulp capping materials (Aeinehchi *et al.* 2003, Nair *et al.* 2008). Due to the difficulties in sourcing teeth, it was deemed permissible for two teeth to be used in one patient, with one tooth allocated to each material. A minimum of 6 teeth were designated to be required in both the MTA and Biodentine groups at six-month review, which allowing for drop out 8 teeth was considered adequate in each group. Random sequence generation was performed using a computer-generated number (www.random.org) and to provide allocation concealment a dental nurse not involved in the recruitment of patients carried out the online randomisation. The virtual coin was 'flipped' before each appointment to decide which material should be used. This was a double-blinded study as although it was possible to blind the patient and histological evaluator to the material, it was not possible to blind the operator as the two materials handle differently.

3.3.1.4 Operative procedure

The patient was clinically examined again and the radiograph studied. Tooth sensibility was reassessed preoperatively using thermal and electric stimuli. All teeth were isolated with cotton wool and dried prior to a -50°C thermal test (Endofrost, Roeko, Langenau, Germany) and electric pulp test (EPT) (Vitality Scanner Model 2006, Analytic Technology, Redmond, Washington, USA) being used. The EPT reading was repeated after two minutes and the readings recorded in the notes and research data. If possible, the EPT reading was compared with that of the corresponding tooth on the same arch or if this is not possible with the tooth immediately anterior to this. Local anaesthetic (2% Lignocaine hydrochloride with adrenalin 1:80,000) was administered around the experimental tooth being treated. The tooth was isolated with rubber dam and

liquid dam (Ena-dam [Micerium, Avegno, Italy] and the area (tooth, dam and clamp) swabbed with 3% hydrogen peroxide followed by 0.2% chlorhexidine (Hörsted-Bindslev *et al.* 2003) prior to an occlusal cavity being prepared. The cavity size was standardised approximately to 4 × 3 mm (width by height). The depth of the occlusal cavity varied from tooth to tooth depending on the size of the pulp chamber but was generally around 4-5 mm. Drilling was performed intermittently using a diamond bur in a high-speed handpiece (280,000 rpm) under waterspray until the cavity was close to the pulp.

Deepest penetration was carried out with a high-speed handpiece and a round diamond bur. An exposure was made with a sharp explorer and every attempt was made to standardize this to a size of 2 x 3 mm. The cavity was irrigated with 2% NaOCL (5 mL, 1 min), use of side-vented needle and a cotton wool NaOCl soaked pledget pressed against the pulpal tissue to ensure haemostasis. The pulp capping medicament used was Biodentine (Septodont, Saint-Maur-des-Fosses, France) or White Angelus-MTA (Angelus, Londrina, Brazil). The Biodentine and MTA-Angelus were mixed according to the manufacturer's instructions and placed over the exposure site with an appropriate plugger. As per the manufacturer's instructions Biodentine was used to fill the entire class one cavity in all specimens. Once material placement was complete, the manufacturers advertised setting time of 12 minutes was allowed to elapse, prior to testing the material with a small amalgam plugger under light pressure. This test was repeated every minute until setting was achieved. The material was considered set by pressing lightly with a small amalgam plugger, if the material was hard enough to not allow penetration of the instrument deeper than 1 mm it was considered set. MTA-Angelus was placed and in keeping with the manufacturer's instructions a wet cotton wool pellet was placed over it to facilitate setting prior to temporary restoration with Cavit (3M, Maplewood, USA). At a second visit, the wet cotton pellet was removed from the cavity and a glass-ionomer cement (Chemfil Dentsply, York, USA) restoration placed.

Preoperatively, post-restoration and at the extraction appointment, the colour of the crown was recorded on the buccal and palatal/lingual aspect of the tooth. The conditions were standardized for light and visibility with the shade of the tooth taken under 10x magnification and maximum light intensity using a microscope with a LED white light (Labomed Prima) and the Vita Classic shading

guide (VITA). At the extraction appointment, discolouration was further monitored and recorded.

3.3.1.5 Extraction procedure

Extractions were carried out after 6 months. All experimental teeth were carefully followed up and clinically assessed before extraction. This assessment included a history of symptoms (if any) and an examination recording tenderness to percussion and response to sensibility test. These thermal and EPT tests were carried out in exactly the same manner as preoperatively and the readings recorded. The teeth were extracted as atraumatically as possible, without sectioning of the teeth and avoiding damage to the pulp capping interface. Where appropriate (e.g. third molars) a muco-periosteal flap was reflected and buccal /distal bone was removed to facilitate their extraction. Immediately after extraction the teeth were briefly rinsed in cold water to remove any blood which might interfere with fixative penetration. The roots were cut off 3 mm apical to the root canal orifices and the crowns were carefully ground under magnification with high-speed diamond burs using water spray in a mesio-distal or bucco-lingual plane until one or two pulp horns are encountered (**Figure 3.1**). The tooth crowns were then immersed in a 10% neutral buffered formalin solution (Sigma-Aldrich, Arklow, Ireland) for at least 48 hrs prior to storage and transport for histological analysis.

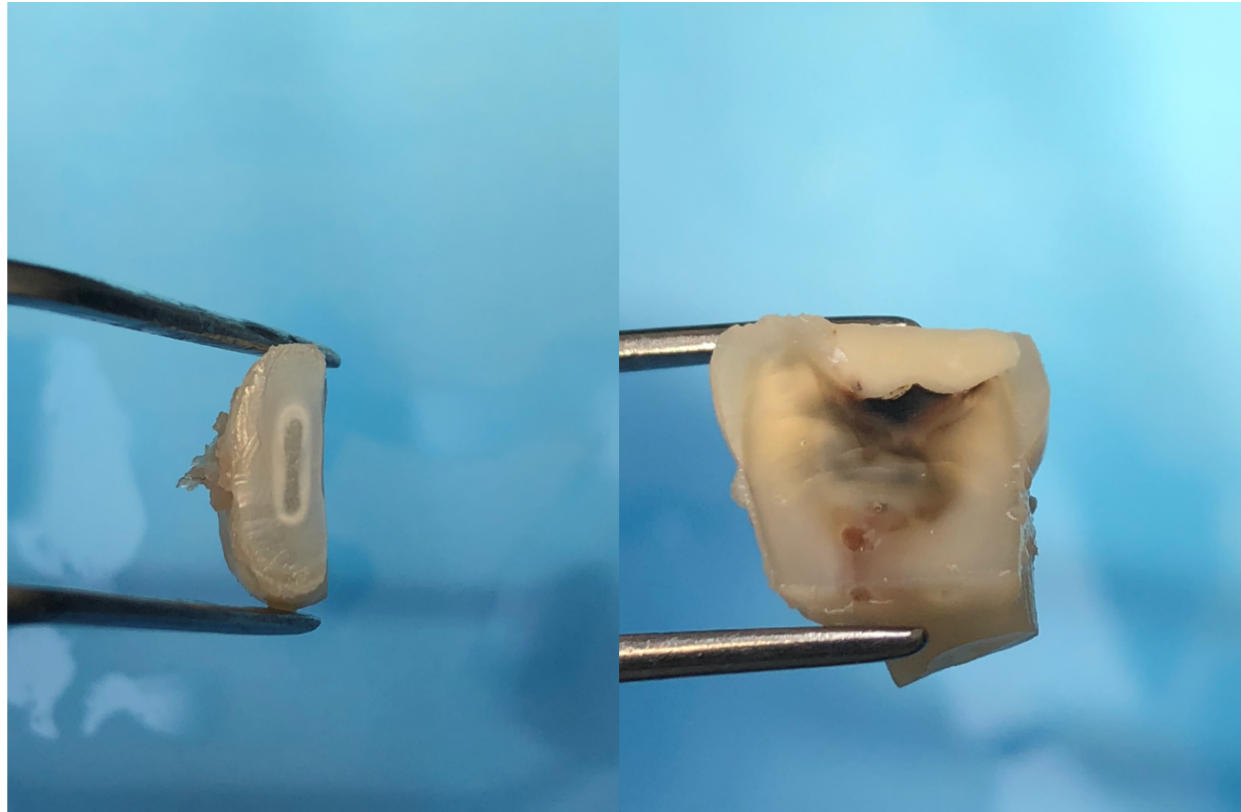


Figure 3.1 Section of extracted third molar teeth trimmed with a high-speed bur under water coolant to expose the pulpal tissue (red arrow), facilitating formalin penetration

3.3.2 Histological assessment

The teeth remained stored into 10% neutral buffered formalin solution at 4°C for a period up to 3 weeks prior to transportation for histo-morphological processing. After rinsing the teeth for 20 minutes in a phosphate-buffered-saline (PBS) solution, demineralisation was carried out in an aqueous solution consisting of a mixture of 22.5% (v/v) formic acid and 10% (w/v) sodium citrate for 3-4 weeks, with the end point of demineralisation determined radiographically. All specimens were then washed under running water for 48 hrs, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated and embedded in paraffin prior to sectioning. Longitudinal serial sections of 4-5 µm were prepared until sectioning of the pulp interface was completely exhausted. This resulted in the generation of 500–600 serial sections for each molar tooth. Initially, every fifth slide was stained with haematoxylin and eosin (H&E) for screening purposes to locate comparative areas of interest. Selected slides were stained with the Taylor's modification of the Brown and Brenn technique, which detects for bacteria (Taylor 1966).

3.4 Results

3.4.1 Patient sample demographics and clinical evaluation at 6 months

Twenty-one 'sound' unrestored teeth from 17 patients were enrolled into the study with 10 experimentally pulp-capped with MTA and 11 teeth treated with Biodentine. Five were excluded from the comparative histological evaluation; as three patients failed to re-attend, despite numerous attempts to contact, within the designated time period (one Biodentine and two MTA), and two other teeth were excluded one because the pulp was not exposed during the procedure (MTA) and the other due to symptoms of pulpitis occurring two weeks after the procedure (Biodentine) necessitated extraction (**Table 3.2**). That left a final sample of 16 experimental pulp capped teeth in 12 patients with the capping material being 9 Biodentine and 7 MTA. The patients age range of the included teeth was from 18 to 43 years of age with 14 of the teeth being third molar teeth (13 maxillaries, 1 mandibular) as well as two maxillary premolars (**Table 3.3**). Clinical evaluation at the time of extraction revealed no other patients in pain, but a variable response to pulp sensibility testing (**Table 3.3**).

All Biodentine samples (9/9) maintained the same colour throughout the procedure, which was in contrast to all MTA samples (6/7), which demonstrated dark stains generally involving one side of the tooth. The MTA discoloration was generally noticeable at the second appointment during which the resin-based composite was placed (**Figure 3.2**).

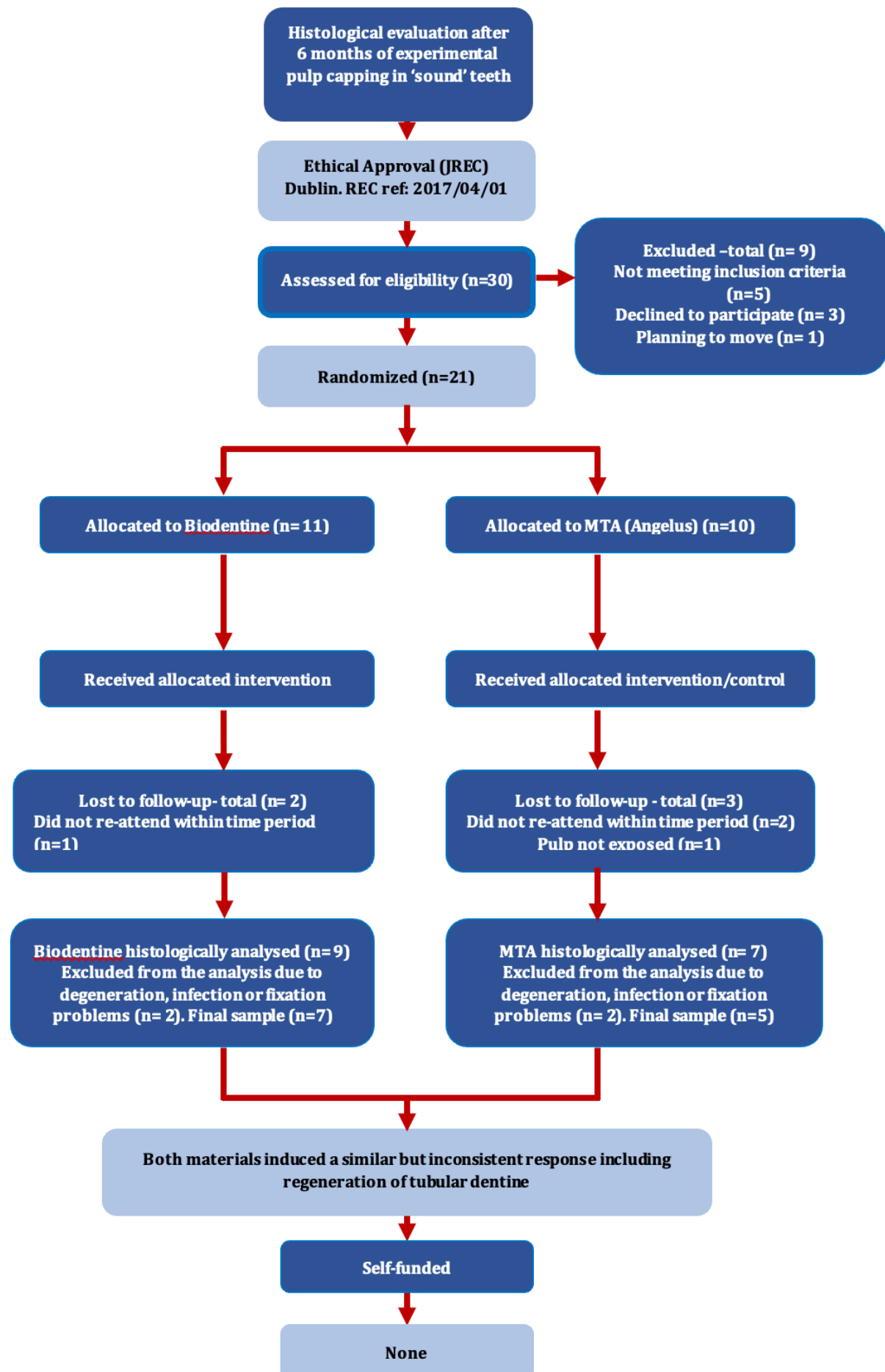


Table 3.2 Flowchart of patient recruitment and journey

Included Patients	Age (years)	Tooth (FDI)	Capping Material	Pulp sensibility test (P/N/U)*
1	18	15	MTA	P
		25	BD	P
2	42	28	BD	N
3	39	28	BD	U
4	23	28	MTA	N
		18	BD	U
5	42	38	MTA	P
6	22	28	BD	U
7	22	18	BD	P
		28	MTA	P
8	24	18	BD	N
9	21	28	BD	U
10	28	18	MTA	P
		28	BD	U
11	27	28	MTA	P
12	43	18	MTA	P
Excluded Patients	Age (years)	Tooth (FDI)	Capping Material	Reason for exclusion
13	22	18	BD	Did not attend recall within suitable time
14	41	28	MTA	Did not attend recall within suitable time
15	36	48	MTA	Did not attend recall within suitable time
16	18	28	MTA	Not exposed
17	19	18	BD	Symptoms of pulpitis

Table 3.3 Demographics of 21 teeth included within study. All teeth were observed for 6 months and extracted at 6 months (+/- 1 week). Unclear (U)* = Test was repeated on three occasions with unclear response to EPT and cold test. Positive (P) response to EPT and cold test. Negative (N) response to EPT and cold test.

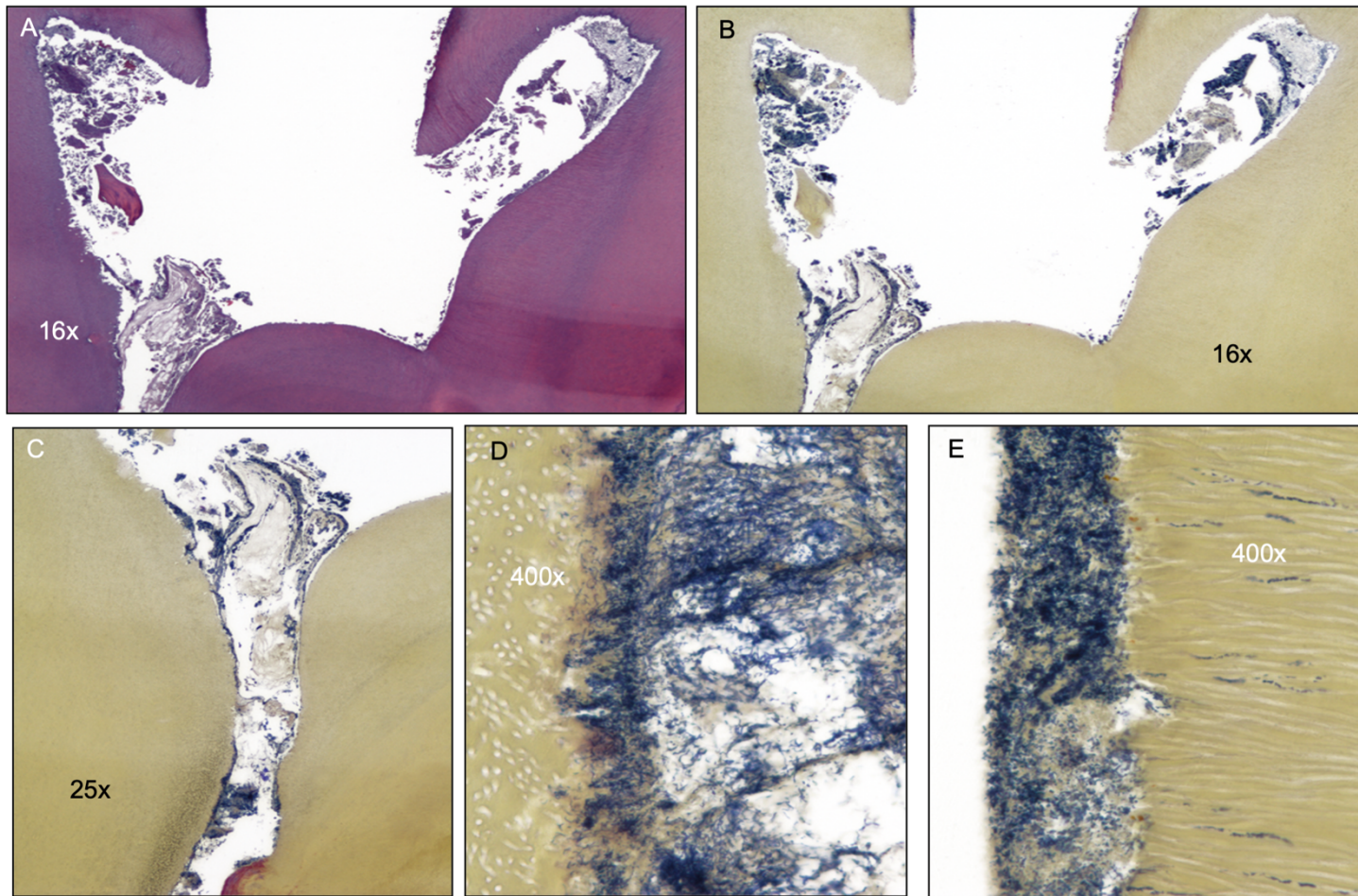


Figure 3.2 Biodentine pulp cap of the upper right third molar (18) **A)** H & E staining shows destructive preparation with necrotic tissue evident at periphery of pulp chamber (original magnification 16x), **B)** Same section stained with Brown Brenn highlights dark staining bacteria presence in the necrotic areas (original magnification 16x), **C)** Brown Brenn stained section of root canal system highlighting the progress of bacteria into the root canal 25x, **D)** Higher magnification identifies large microbial deposits aggregated on dentine surface within the root canal (original magnification 400x), **E)** Tubular infiltration by bacteria evident (original magnification 400x)

3.4.2 Histological evaluation

Of the 16 teeth that were processed for histology, two Biodentine and two MTA, had technical problems either during extraction or fixation and showed clear signs of pulp degeneration, infection on initial histological examination. The remaining 12 teeth responded positively to pulp sensibility testing and were extracted uneventfully at 6 months. No artefacts were observed in the mineralised tissues and there was no shrinkage in the tissue at the pulp-dentine interface. In general; however, there was a lack of consistency between samples in terms of exposure size, depth and aggression of the preparation (**Figures 3.2 – 3.8**).

Histological response of the remaining 12 teeth could be broadly separated into three types of response: (1) three teeth demonstrated infection and pulpal breakdown associated with the presence of bacterial infiltration and an over-destructive technical preparation (**Figure 3.2**); (2) five teeth (three Biodentine and two MTA) displayed a complete hard tissue bridge covering the exposure that was lined with cells and variable evidence of tubular dentine (**Figures 3.2 – 3.6**); (3) four teeth with a degree of mineralised tissue bridge that was disorganised and resembled osteodentine, with porosity, cellular inclusions in lacunae and the absence of any tubular structure indicative of dentine (**Figures 3.7 & 3.8**). The quality and thickness of the hard tissue bridge was not uniform between samples or materials; however, the quality of the hard tissue was always poorer in the earlier formed mineralised tissue in contact with the capping material (both Biodentine and MTA), which by contrast the mineralised tissue on pulpal side which was deposited later tended to be of higher quality with an absence of soft tissue in bridge and cellular inclusions.

All included teeth were vital with no evidence of caries. Furthermore, as they were aseptically treated under rubber dam, the presence of severe inflammatory change and destruction in three teeth and evidence of significant bacterial and necrotic pulp tissue (Brown Brenn, **Figure 3.2 B-E**) indicates a technically poor capping procedure and lack of 'postoperative' sealing restoration (**Figure 3.2**). This occurred in three Biodentine and one MTA pulp cap on upper third molar teeth (**Table 3.3**), highlighting the likely influence of careful pulp tissue handling, technical skill and meticulous restoration during pulp capping, which is not always easy using third molar teeth.

In the five teeth exhibiting evidence of regeneration of tubular dentine this occurred with both Biodentine and MTA, with no obvious morphological differences between the Biodentine (**Figure 3.3**) and MTA samples (**Figures 3.4 & 3.5**). Within this group of three MTA and two Biodentine, the morphology of the cells at the dentine-pulp interface could be examined and presented a distinct cellular phenotype, with the cells varying from flat in structure, but with evidence of tubules (**Figure 3.3G [inset], Figure 3.4G**) to more columnar cells, resembling odontoblast-like cells (**Figure 3.5**) that often-displayed processes extending into tubular structures (**Figure 3.6C & D**). In these cases, the reparative tertiary dentine resembled a true tubular dentine (**Figures 3.3 and 3.6**), which although not as well structured as primary dentine still possessed tubules and odontoblast processes (**Figure 3.6**). There was also less destructive pulp chamber access and tissue damage with accompanying evidence of a lack of inflammatory cells in the cases demonstrating hard tissue regeneration.

In the four cases with amorphous mineralisation the mineralisation was inconsistent with cellular soft tissues inclusions, porous bridges and evidence of inflammation in the pulp tissue (**Figure 3.7C-E**). The cellular layer lining the pulp/barrier interface was either absent or composed of cells with flattened bodies and the absence of an odontoblast processes, indicating a cellular morphology similar to a fibroblast rather than an odontoblast cell (**Figure 3.8**). Although, minor differences in morphology were evident in the cells lining the bridge in these samples, the layer was not always continuous with no evidence of a columnar cell structure, odontoblast process or tubular formation in the hard tissue repair. The periphery of the exposure area in these cases demonstrated dentine with tubular structure typical of dentine unaffected by the operative procedure.

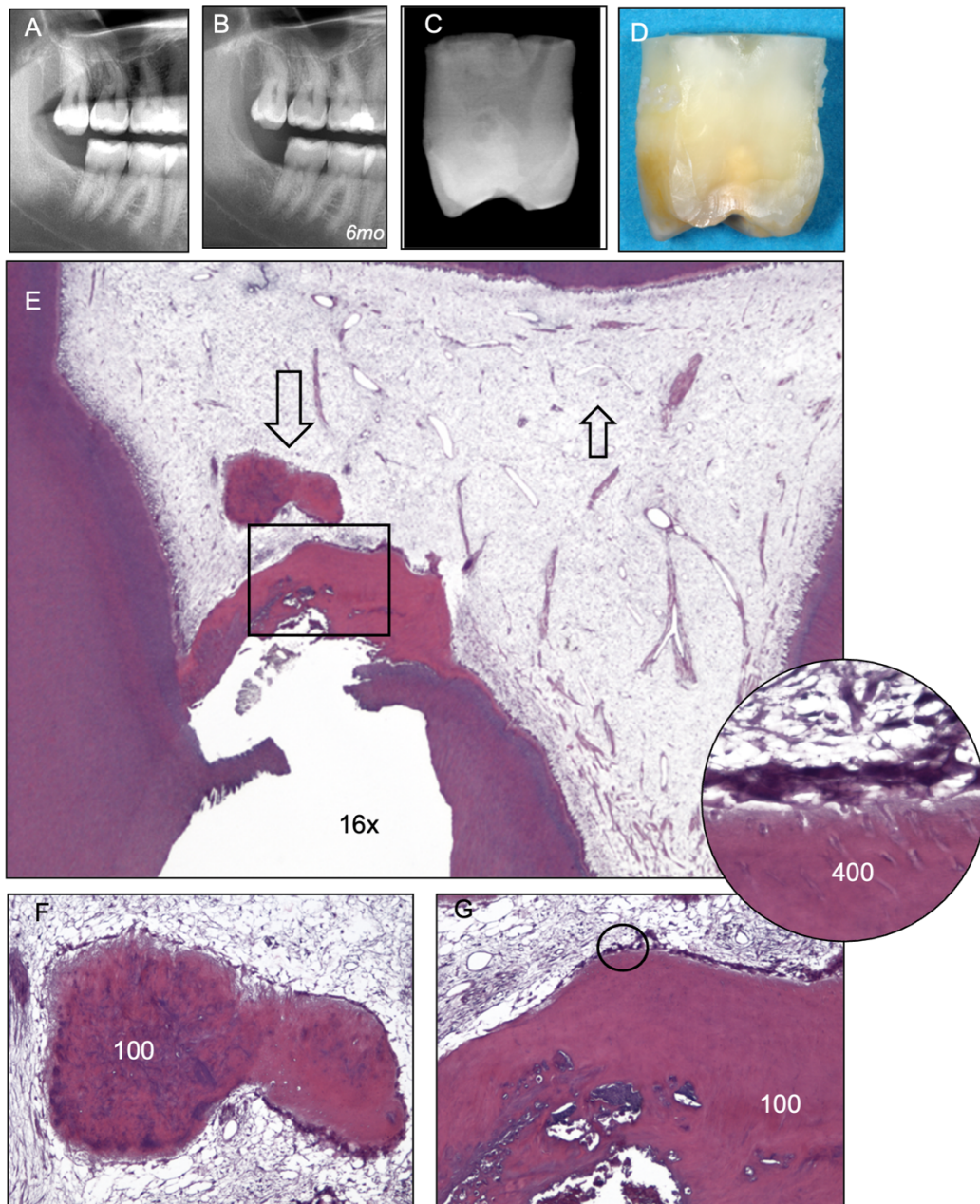


Figure 3.3 Biodentine pulp cap of upper right third molar (18) which had an unclear pulp sensibility test at extraction. H & E stain. **A)** Hemi-panoramic radiograph right side showing over-erupted third molar tooth, **B)** Hemi-panoramic radiograph 6-month after pulp capping procedure, **C)** Radiograph of the crown of the extracted tooth, **D)** Macro-photograph of fixed stain-free crown, **E)** Complete hard tissue bridge formation evident over pulp wound of varying thickness with calcific deposit in pulp chamber (White arrow - large) (original magnification 16x), **F)** Amorphous, porous calcific deposit in pulp surrounded completely with flattened cells (original magnification 100x), **G)** Poorer quality mineralised tissue evident at the material interface with cellular inclusions (original magnification 100x). Circular inset taken at higher-magnification (inset original magnification 400x) from the pulp tissue at the interface showing tubule formation and the presence of a flattened cells against bridge.

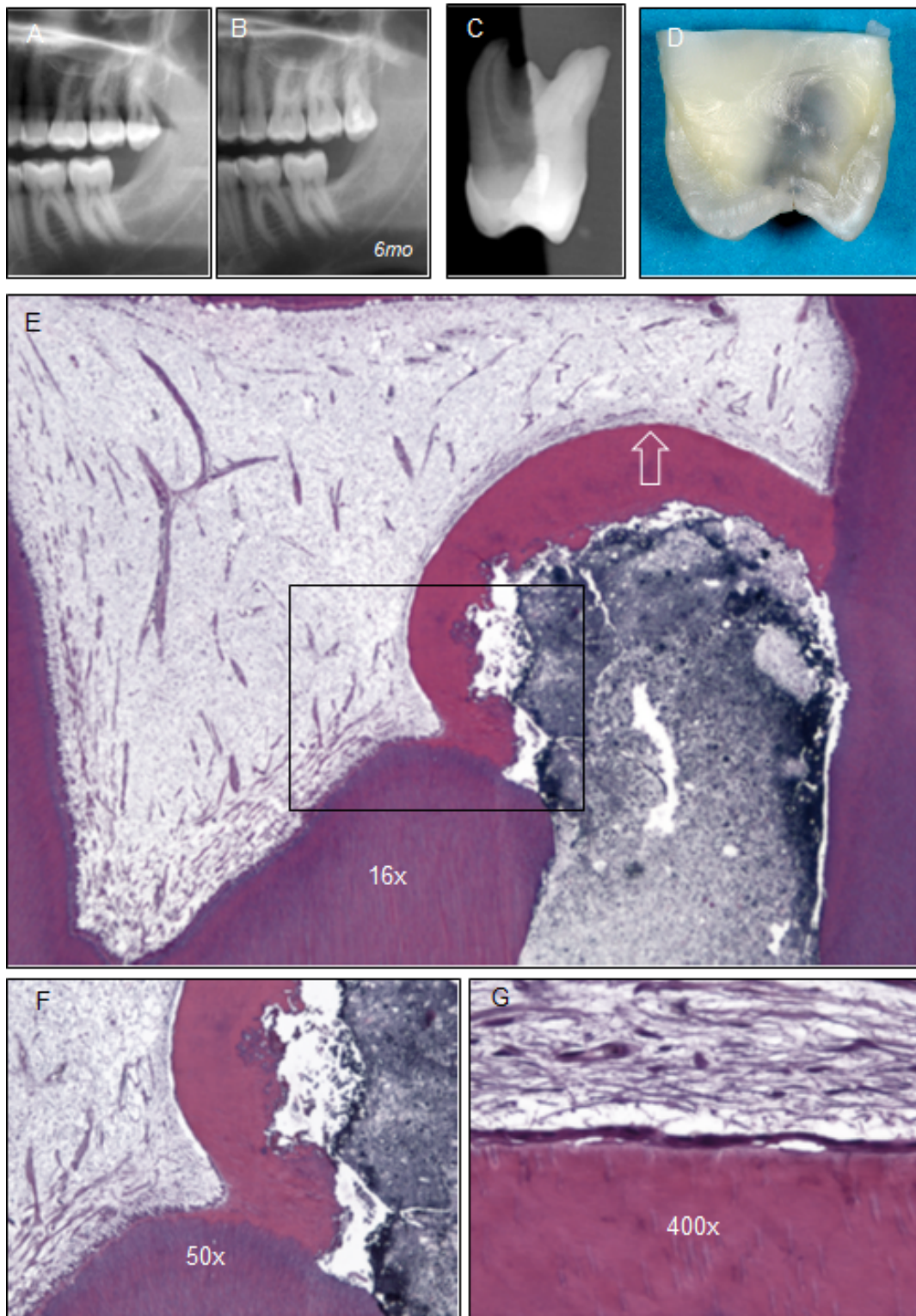


Figure 3.4 MTA pulp cap of upper left third molar (28) with a positive pulp sensibility test at extraction. **A)** Hemi-panoramic radiograph left side showing over-erupted third molar tooth, **B)** Hemi-panoramic radiograph 6-months after experimental pulp cap placement, **C)** Radiograph from mesial aspect of whole extracted tooth demonstrating orientation of pulp cap procedure, **D)** Macro-photograph of crown demonstrating black stain from MTA, **E)** Thick complete hard tissue bridge formation evident over pulp wound (original magnification 16x), **F)** From square inset (F), lower quality mineralisation evident in contact with pulp capping material (original magnification 50x), **G)** Higher magnification image showing flattened continuous cell layer lines hard tissue bridge with some indication of tubular formation (original magnification 400x).

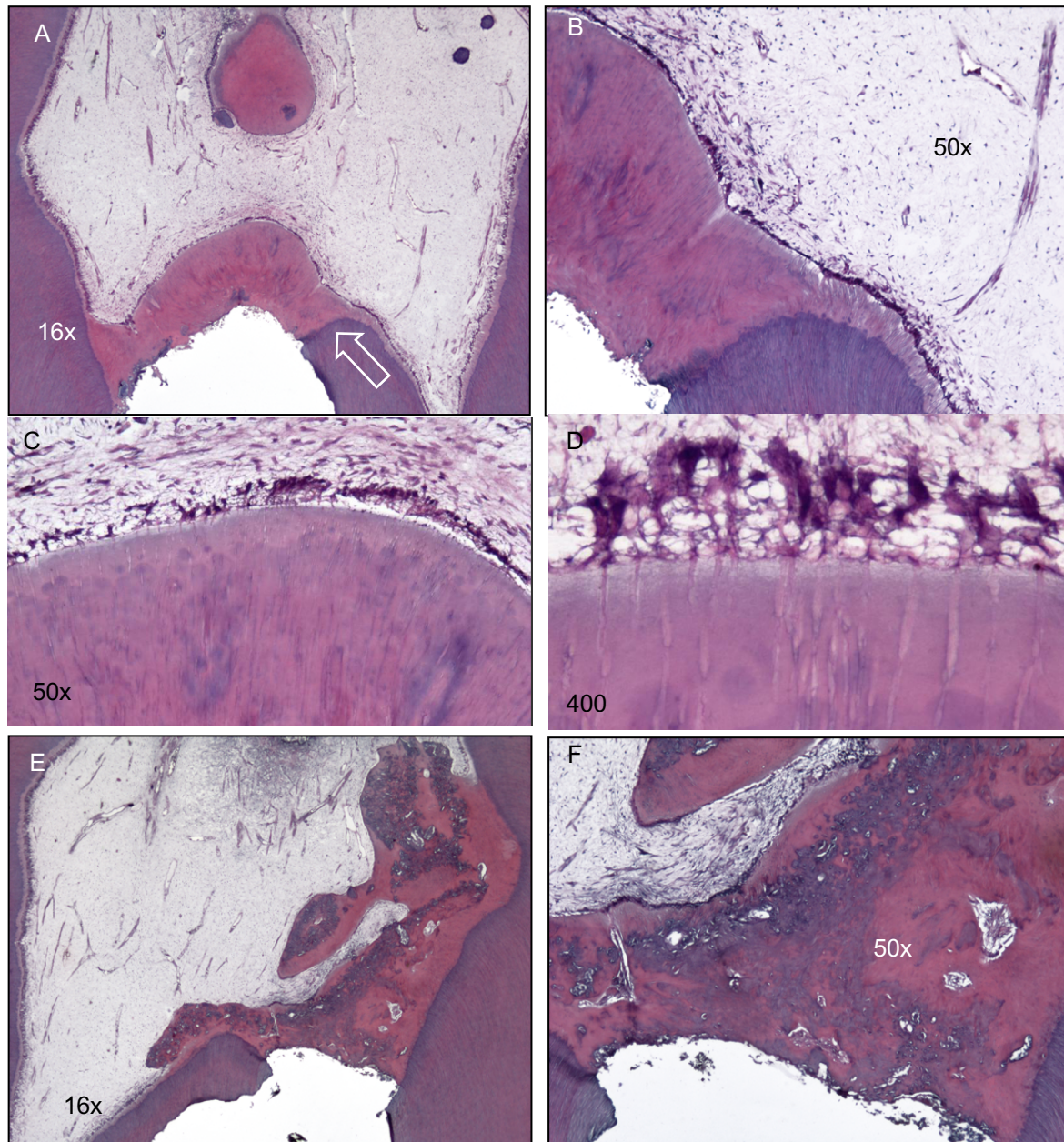


Figure 3.5 MTA and Biodentine pulp caps on upper right and left second premolars (15, 25) from same patient. **A)** MTA pulp cap on 15 highlights a complete thick hard tissue bridge with interface to primary dentine noted with a white arrow (original magnification 16x). **B)** Higher magnification view of hard tissue bridge with continuous cell layer evident on pulpal aspect (Original magnification 50x) **C)** View of central aspect of hard tissue bridge with tubules evident and processes extending into the newly formed tubules (original magnification 50x) **D)** High power magnification view of the same part of bridge as C, highlights clear tubular structure, presence of cuboidal cells and processes extending into tubules (original magnification 400x) **E)** Biodentine pulp cap on 25 demonstrates a significant volume of low quality amorphous mineral material deposited over pulpal wound site and extending into pulp tissue (original magnification 16x). **F)** Higher magnification view of bridge shows amorphous calcific materials with soft tissue inclusions (original magnification 50x)

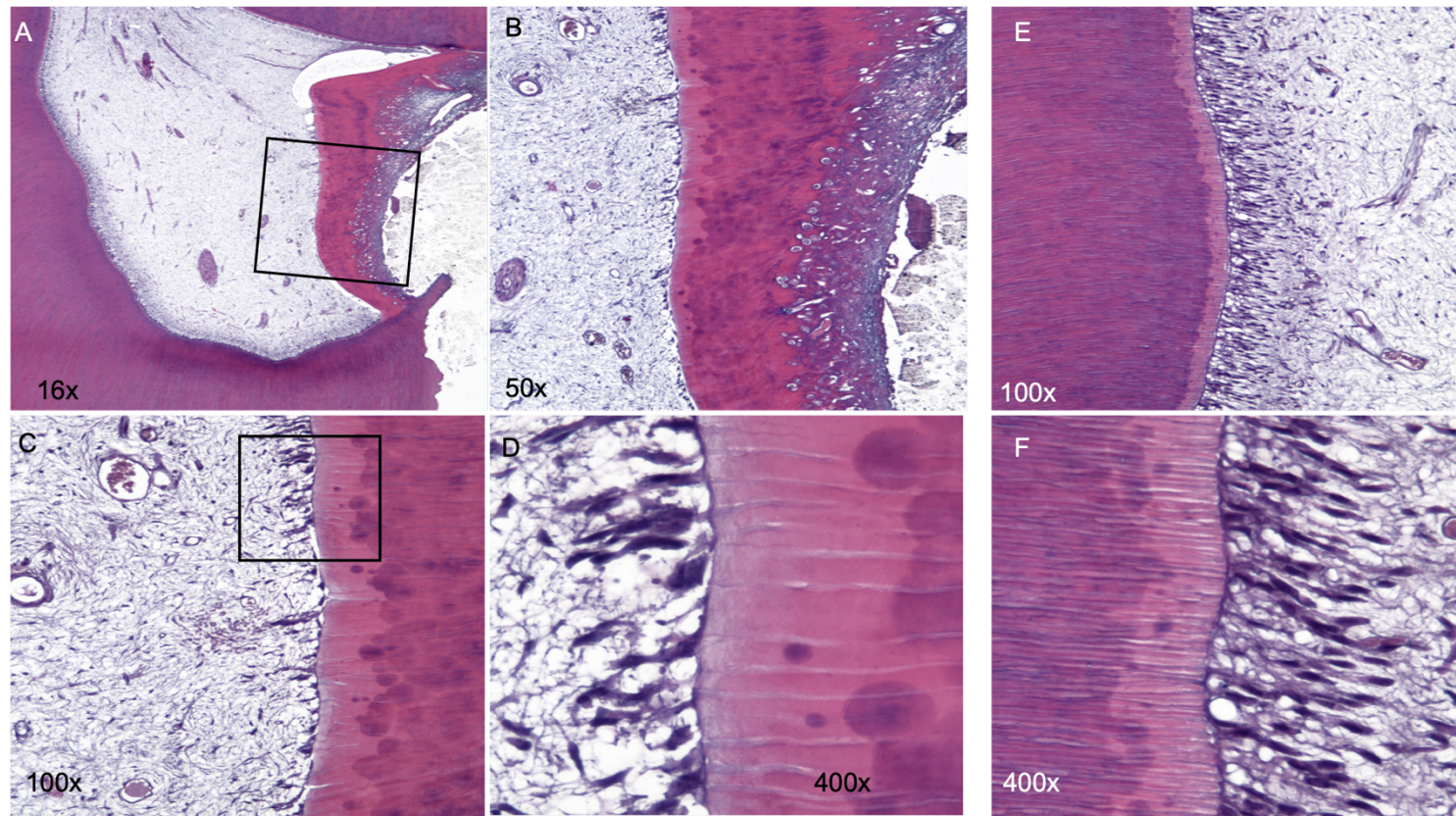


Figure 3.6 Biodentine pulp cap of the upper left third molar (28). **A)** Thick hard tissue bridge formed over large pulp wound with primary dentine evident on left of slide (original magnification 16x). **B)** Inset cuboidal area of higher magnification demonstrates higher quality mineralisation on the pulp aspect and amorphous deposits on the material aspect. Continuous cell layer lines hard tissue bridge (original magnification 50x) **C)** Higher 100x magnification of hard tissue bridge over the pulp wound highlights tubular structure and pulp tissue free of inflammatory infiltrate (original magnification 100x). **D)** Inset rectangular area (original magnification 400x) shows clear dentinal tubule formation, associated with columnar cells and processes extending into the tubules. **D)** Rectangular inset of different aspect of the slide in A highlighting primary dentine (original magnification 16x). Inset shows structured tubules, predentine layer and dense odontoblast layer organised against the dentine (original magnification 50x). **F, G)** Higher 100x and 400x magnification of primary dentine highlighting cuboidal cells and odontoblast processes in comparison to C).

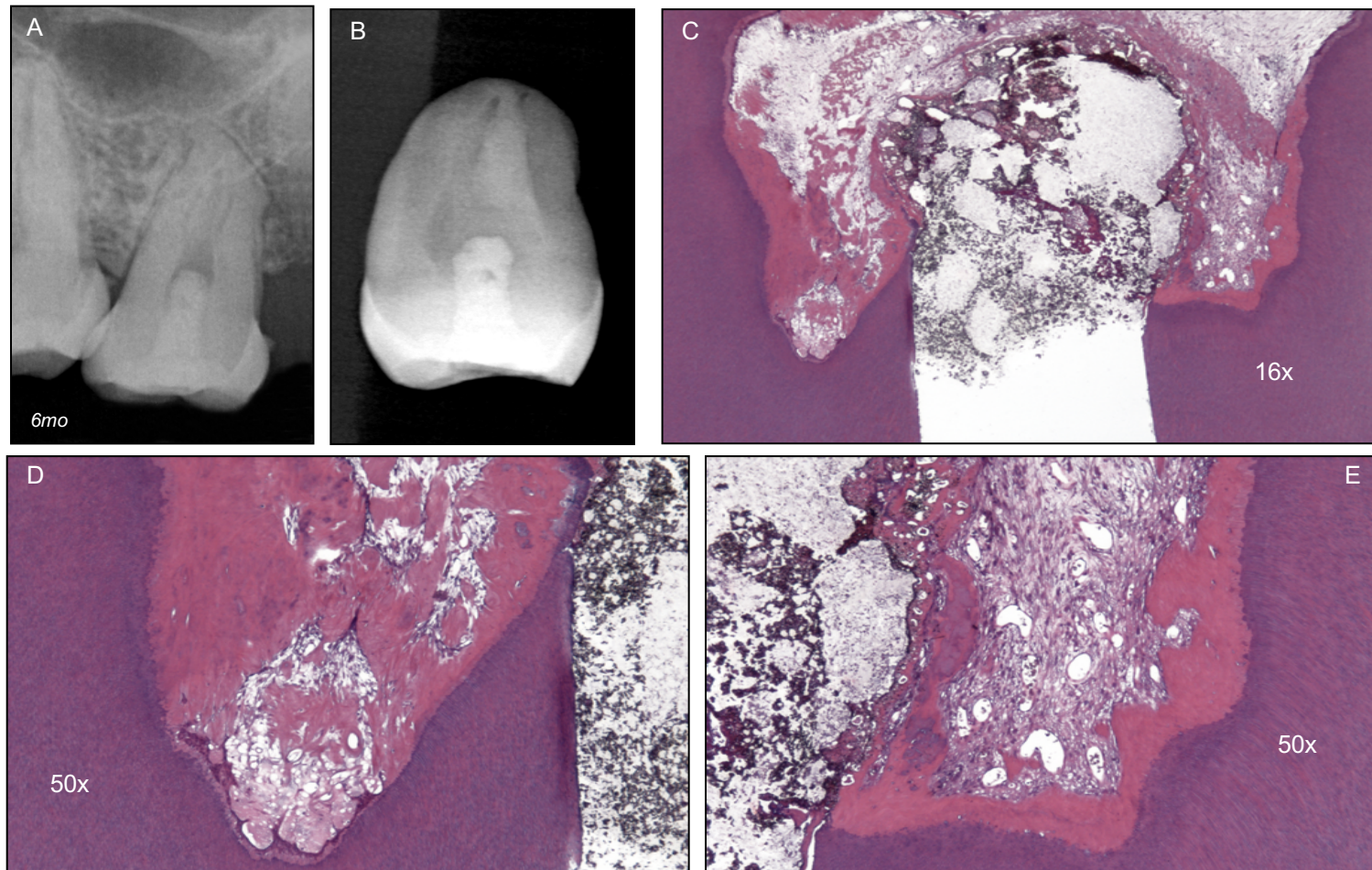


Figure 3.7 MTA pulp capping of upper left third molar (28). **A)** Periapical radiograph showing over-erupted third molar tooth and pulp cap extending into pulp chamber **B)** Radiograph from mesial aspect of whole extracted tooth demonstrating orientation of pulp cap procedure and pulpal extension, **C)** Amorphous poor quality mineralised bridge, incomplete in places and associated with pulp inflammatory exudate (original magnification 16x), **D)** Extensive mineral deposition at cusp tip highlighting areas of pulp tissue and soft tissue inclusion within the dystrophic mineral (original magnification 50x), **E)** Similar effect evident in another cusp tip with large areas of soft tissue and lower quality mineral resembling scar tissue repair material (original magnification 50x)

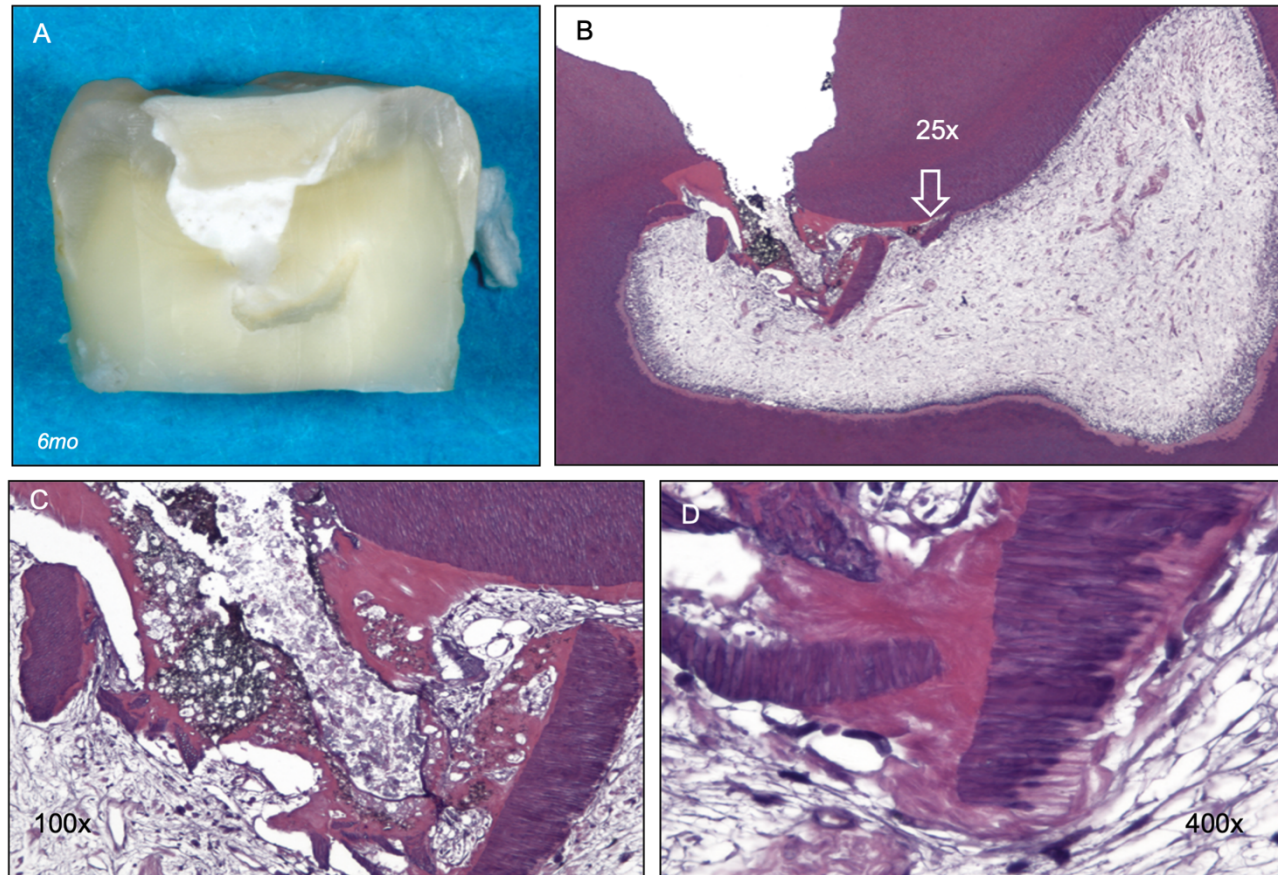


Figure 3.8 MTA pulp capping of lower left third molar (38). **A)** Macro-photograph of crown highlighting capping material and interface with resin-based composite. **B)** H & E staining demonstrating dentine 'chips' displaced into pulp tissue surrounded by amorphous mineral deposits (original magnification 25x). **C)** Higher magnification of area identified by white arrow in B). Amorphous mineral links displaced tubular primary dentine from with soft tissue inclusions and areas of necrosis evident. Inflammatory cells and vascular engorgement are also evident around the mineralised tissue (original magnification 100x). **D)** Higher magnification view (original magnification x400) shows atubular mineralised material linking displaced primary dentine fragments.

3.5 Discussion

The absence of soft tissue inflammatory changes in the pulp and presence of a hard tissue bridge over the exposure are considered the salient histological features associated with success of pulp capping techniques (Hargreaves & Berman, 2016). There is, however, a lack of consensus on the structure of the hard tissue and indeed the nature of the secretory cell responsible for its formation (Duncan *et al.* 2019; Dammaschke *et al.* 2019). The traditional theory involves tertiary dentine being formed after a cyto-differentiation process under the influence of bioactive molecules, sequestered from both the dentine matrix and released from dental pulp cells, in which progenitor cells are recruited from central pulp or perivascular areas and undergo subsequent differentiation into odontoblast-like cells, before secreting a dentine-like hard tissue bridge (Lesot *et al.* 1993, Duncan *et al.* 2019). Notably, for odontoblast-like cells to be considered morphologically similar to odontoblasts they need to demonstrate the presence of an odontoblast process, requiring the hard tissue to possess tubularity; a feature which is considered absent in reparative hard tissue bridges (Damaschke *et al.* 2019). As a result, other authors propose an alternative theory, that the hard tissue formation is part of a reparative process sustained by mineral secreting fibroblasts (Ricucci *et al.* 2014). Fibroblasts have been shown to have an ability to produce mineral *in vitro* (Yamamoto *et al.* 2015 & 2018) and contribute to calcification elsewhere in the body (Ronchetti *et al.* 2013); however, in order to form a hard tissue bridge they would have to be induced to undergo a cyto-differentiation as they do not naturally secrete mineral in their basal state. This has not yet been shown to occur in the dental pulp and the findings are based on morphological observations (Duncan *et al.* 2019). Indeed, the formative cells observed lining the pulp interface with the hard tissue bridge represent those cells functioning at that location at the time of the morphological assessment (in this case 6 months) and may of course be cells that have replaced other cells that were present earlier, in other words regeneration and repair *in vivo* is temporal. Research in this area is also hampered by a lack of unique molecular markers for physiological odontoblasts, and coupled with alterations in the nature of odontoblast throughout their life (Couve 1986), makes definitive identification or replacement odontoblast-like cells difficult. Presence of dentin-sialophosphoprotein (DSPP), nestin or dentin matrix acidic

phosphoprotein 1 (DMP-1), while representing a component of the odontoblast's molecular profile, are also not definitive markers of cell phenotype (Duncan *et al.* 2019).

A principal aim of this study was to morphologically assess the quality of the hard tissue bridge at longer term intervals (6 months) and the nature of the cells lining the hard tissue. When the pulp remained infection-free (9 cases) a range of histological responses were evident, from poorly formed amorphous mineral with soft tissue inclusions to thick consistent bridges with evidence of tubularity. The response notably appeared to be independent of capping material, but was linked to the technical quality of the preparation. A consistent finding was the increased quality of the hard tissue bridge from the pulp to the capping material, suggesting that earlier formed dentine was of poorer quality and improved over time was an increased number of secreting cells differentiated. Indeed, at the interface with both MTA and BD an atubular component was invariably predominant with a progressive increase in cyto-differentiation and the formation of clearly tubular tissue at the interface with the pulp in selected samples (**Figures 3.2-5**). MTA has previously been demonstrated to be histologically superior to calcium hydroxide in unrestored human teeth, generally producing complete homogenous hard tissue bridges in time intervals up to 3 months (Nair *et al.* 2008); however, in that study ProRoot MTA (Dentsply, Ballaigues, Switzerland) was used and there was no evidence of dentine tubularity albeit over a shorter time period. Furthermore, in a 7-month study examining experimental pulp capping with RetroMTA the authors concluded that there were no cells exhibiting evidence of regular dentine or evidence of odontoblast-like differentiation (Damaschke *et al.* 2019). This is clearly not the case in the current study as the formation of tubular dentine and presence of odontoblast-like cells is evident in selected, but not all samples. Other previous studies have reported Biodentine and MTA to have superior thickness of bridges than calcium hydroxide at 6 weeks (Nowicka *et al.* 2013) and that Biodentine and MTA were capable of inducing odontoblast-like cells and tubular dentine also at 6 weeks (Nowicka *et al.* 2015). Unfortunately, within the latter study on examination of the small number of histological slides presented (Nowicka *et al.* 2013) it is impossible to comment as to whether odontoblast-like cells are present or tubular

dentine is reformed. The quality of the histological analysis in the current study provides more robust support of dentine regeneration.

That said, although the presence of tubularity in the hard tissue repair was evident in five samples, in many others the bridge was amorphous, atubular with multiple 78nm mineralized areas and cellular inclusions. The lack of tubularity, supports the concept of an osteodentine or dystrophic calcific scar being formed over the pulp exposure in these cases (Dammaschke *et al.* 2019). Furthermore, although the cells in several sections are flat, without tubular extensions and phenotypically resemble fibroblasts without further advanced molecular evaluation it is impossible to confirm the exact origin of the cell-type, which forms the hard tissue (Duncan *et al.*, 2019). It does seem though that depending on a range of factors that may affect the pulpal tissue niche, that an array of cellular responses from inflammation to repair and regeneration are possible. A positive aspect of the current study is the length of time between the procedure and extraction. The majority of clinical studies, perhaps for practical reasons employ shorter times (reviewed in Fransson *et al.*, 2016) of 6 weeks (Nowicka *et al.* 2013) or 3 months (Nair *et al.* 2008) with fewer studies evaluating the response in at least some (Aeinechi *et al.* 2003) or all samples (Dammaschke *et al.* 2019) at 6 months or later. There was a lack of inflammatory cells in the pulp tissue of the cases that displayed a regeneration of tubular dentine, however, there was acute inflammation and associated bacterial invasion evident in other samples (**Figure 3.1**). It must be remembered that these procedures were carried out in sound unrestored teeth in which the pulp tissue is expected to be uninflamed with no pathological change. This highlights an important issue that VPT procedures, in this case experimental pulp capping are technically sensitive and only when carried out with minimal trauma to the dentine-pulp complex and meticulously restored tissue response is improved.

An issue during this study was patient recruitment. Ethically sourcing patients with 'sound' teeth that require extraction and are willing to volunteer to participate in an invasive research study that delays the extraction for 6 months is challenging. The most suitable teeth for practical reasons including isolation, access, and management are maxillary and mandibular premolars that need to be removed for orthodontic treatment. Within the confines of this study very few patients were willing to delay their orthodontic treatment for six months prior to

starting their therapy. Ethically it was not possible to propose an additional treatment, which has a detrimental effect on the patient's care, in this case delaying orthodontic therapy. As a result, the majority of teeth recruited in the study were over-erupted maxillary third molar teeth scheduled for routine extraction. These 'sound' teeth commonly require extraction; however, for an experimental pulp capping their use creates other challenges related to effective rubber dam isolation, reduced access and visibility, which inevitably increases the likelihood of technical errors during the clinical operative procedure (e.g. depth and width of pulp exposure due to poor visibility, inadequate cooling). These clinical challenges offer a potential explanation for the identification of several samples displaying pulpal necrosis after treatment, perhaps relating to a lack of water coolant and resulting pulpal damage. When working in a narrow cavity in a restricted area of the mouth, it may be difficult for the water jet to adequately cool the high-speed bur, leading to overheating and resulting damage of the pulp tissue. Additionally, locating the coronal pulp tissue, while maintaining a standardised cavity access in third molars is difficult as they may possess altered anatomy or indeed be malpositioned. The use of malpositioned third molars inadvertently presented an unexpected finding, that the regenerative response in sound teeth may not only be related to the condition of the pulp and pulp cell niche, but also as much to do with the technical procedure of pulp capping as the material used.

Accepting the limitations of these qualitative results, comparison of the coronal staining of the two calcium silicate cements presented obvious differences. While MTA-Angelus presented a visual range of discolouration in all the samples treated, Biodentine did not induce any visible colour alteration. It has been highlighted elsewhere in the literature that MTA products induce staining, due to the radiopacifier additive bismuth oxide, which has been replaced by zirconium oxide in Biodentine (Mozyńska *et al.* 2017, Yoldas *et al.* 2016). A limitation in the methodology of assessing discolouration was evident in this study. The assessment was carried out by comparing the tooth with a shading guide, which for practicality purposes subjectively relied on the clinical judgement of a single operator. A more accurate way to assess this would be using a spectroradiometer or a digital shade mensuration device (Shokouhinejad *et al.* 2016; Yoldas *et al.* 2016); however, for reasons of practicality was not available within this study.

Finally, from a translational perspective, this study is somewhat limited by the use of healthy 'sound' teeth. Although, scientifically it is essential when comparing materials to limit confounding factors e.g. bacterial challenge, pulpitis (Ricucci *et al.* 2014 & 2018) and aids comparison it reduces the ability to extrapolate the results to the clinical reality (Fransson *et al.* 2016) in which the pulp is generally exposed due to deep restoration or caries.

3.6 Conclusions

This is one of the first human histological studies to demonstrate that the formation of odontoblast-like cells and regeneration of tubular dentine is possible at least in healthy teeth after experimental pulp capping. However, the regenerative response is not predictable with the healing response independent of the hydraulic calcium silicate material used, but linked to the technical aspects of the procedure.

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Chapter 4.

Prospective Clinical Study Investigating the Predictability of Partial Pulpotomy after Relating Preoperative Symptoms to a New Classification of Pulpitis

4.1 Introduction

The dental pulp can be irritated by a range of chemical, thermal and traumatic stimuli; however, microbial challenge represents the principle threat to pulp vitality (Möller *et al.* 1981). In response to this threat, the dentine-pulp complex mounts a complex immune response triggered initially via bio-sensing by odontoblast cells and thereafter by an orchestrated inflammatory process by dental pulp cells and circulating immune cells recruited locally to the injury site (Farges *et al.* 2015, Duncan *et al.* 2019, Duncan & Cooper 2020). The early stages of pulpal inflammation are generally limited to the coronal pulp tissue with other areas of the pulp relatively healthy (Ricucci *et al.* 2014); however, current ways to categorise pulpitis are absolute considering the pulp to be either salvageable or not (American Association of Endodontists [AAE] 2013).

Inflammation of the dental pulp is classically categorised as being either reversible and irreversible pulpitis based on the relationship of signs and symptoms to management (AAE 2013). Irreversible pulpitis indicates that the pulp tissue is so damaged that it is impossible to maintain vitality, thus root canal treatment (RCTx) is necessary. On the contrary, reversible pulpitis is a condition in which the pulp, even if damaged, is capable of recovery if the irritant stimulus is removed and the tooth adequately restored (Tronstad & Mjör 1972). Unfortunately, this classification simplifies the complex and often-unpredictable nature of pulpitis and fails to reflect the recently reported success of partial and full pulpotomy in cases with signs and symptoms indicative of irreversible pulpitis (Özyürek & Demiryürek 2016, Taha & Khazali 2017, Uesrichai *et al.* 2019). As a result, there has recently been a call for new classifications of pulpal status to be proposed in an attempt to link the signs and symptoms of pulpitis to vital pulp management strategies (Wolters *et al.* 2017) and to reflect the localized nature of pulp damage (Ricucci *et al.* 2014, Rechenberg & Zehnder 2020). Recently, Wolter's proposed a system based on symptoms with four different categories of pulpitis, initial, mild,

moderate and severe; with pulpal exposure and pulpotomy only indicated in selected categories. Notably, the word 'irreversible' has been removed from all diagnoses in this proposed classification (Wolters *et al.* 2017). Although this represents a potentially more relevant diagnostic system, at present, there are no studies investigating its usefulness in determining the predictability of the vital pulp treatment (VPT) procedures.

Hydraulic calcium silicate cements (HCSCs) are recommended as the 'gold standard' material for conservative treatment of the exposed pulp (ESE, 2019). HCSCs are characterised by high biocompatibility and hydrophilic nature, which assists their use in VPT procedures; however, mineral trioxide materials (MTA) have been shown to be associated with extended setting and tooth discolouration (Careddu & Duncan 2018). HCSCs, such as ProRoot MTA (Dentsply Sirona, Ballaigues, Switzerland) modulate the pulp response reducing pulpal inflammation and increasing the quality of hard tissue bridge formation over the exposed pulp, compared with hard-setting calcium hydroxide materials (Nair *et al.* 2008). A recently introduced HCSCs, Biodentine (Septodont, Saint Maur des Fosses, France) is specifically marketed for VPT with manufacturer claims that it sets in 12 minutes, does not cause tooth discolouration with clinical research indicating good success in pulp capping over 2-3 years (Harms *et al.* 2019) and also pulpotomy (Taha & Abdelkhader 2018a).

4.2 Study aims and objectives

The purpose of this study is to prospectively investigate the predictability of partial pulpotomy using a calcium silicate cement in adult teeth presenting with symptomatic deep carious lesions and classified according to the AAE and Wolters diagnostic systems (AAE 2013, Wolters *et al.* 2017).

- Objective 1: To study the response of symptomatic cariously-exposed teeth treated using a partial pulpotomy technique and capped with Biodentine
- Objective 2: To assess the practicality and reliability of Wolters (Wolters *et al.* 2017) and the AAE (AAE 2013) classification for diagnosis of pulpitis and subsequent management.

- Objective 3: To record practical aspects of Biodentine use, including discolouration, setting time and postoperative symptoms.

4.3 Materials and methods

The proposed study was a longitudinal cases series investigating the reliability of Biodentine in the treatment of adult teeth presenting with deep caries and symptoms of pulpitis. For the purposes of this study, 'deep caries' was defined as reaching the inner quarter of dentine, but with a zone of hard or firm dentine between the caries and the pulp, which was radiographically detectable when located on an interproximal or occlusal surface (Duncan *et al.* 2019a). The clinical component was carried out in dental practice in Dublin (Roberto Careddu [RCD]) linked to the Division of Restorative Dentistry, Dublin Dental University Hospital (Hal Duncan [HD]). All clinical procedures were performed by RCD.

4.3.1. Ethical approval

As the nature of this study is a clinical intervention on human subjects, an ethical approval was necessary. An application was submitted to the Research Ethics Committee at St James's Hospital (JREC), Dublin and Ethical Approval (REC ref: 2017/04/01) was obtained on the 25th April 2017.

4.3.2 Size of patient cohort

This study was not designed, as a randomised clinical trial comparing two materials, but rather as a practicality-based clinical study investigating the usefulness of a new classification system in general dental practice. As a result, a power calculation was not carried out. The sample size was decided largely by practical issues, as during the planning stages it was unclear how many patients would present with this diagnosis to RCDs practice, how many would consent to have the procedure carried out and what the level of follow up would be. Furthermore, as there was a necessity to complete the study within the confines of overall doctorate project, a patient recruitment period of 30 months was considered a sufficient amount of time in order to collect as many cases as possible and not less than 50 teeth. The recruitment started in March 2017 and continued until August 2019. The included teeth were reviewed at 3, 6 and 12

months as part of this study. A second phase of the same project plans to analyse the outcome at 24 months.

4.3.3 Patient sample and inclusion criteria

The sample group consisted of patients with a deep carious lesion (Duncan *et al.* 2019a). Symptomatology was recorded and used to categorize the clinical features into mild, moderate or severe pulpitis (Wolters *et al.* 2017). In order to compare the results with a more established classification the teeth were also categorised as either reversible or symptomatic irreversible pulpitis (AAE 2013). Although 'Wolters' classification (Wolters *et al.* 2017) was used to categorised pulpal condition, the proposed management strategies also advocated in the proposal were not rigidly followed and all cases were treated by non-selective caries removal and partial pulpotomy. This was carried out in order to standardise treatment and to assess the validity of Wolters' diagnosis as well as evaluating the efficacy of partial pulpotomy and non-selective caries removal in deep carious lesions. The material chosen as pulp capping agent was Biodentine a hydraulic cement marketed for VPT procedures.

The sample group consisted of patients aged between 14 and 60 years old, presence of deep carious lesion (evident from radiograph), pulpal symptoms ranging from none to moderate pain, positive response to cold pulp sensibility test, accidental exposure during non-selective caries removal. Pregnant women, teeth that were giving excruciating pain and sleeping problems for several days, teeth not responsive to vitality test and teeth that were not exposed after caries non-selective removal were excluded (**Table 4.1**).

Patients were recruited by the operator (RCD). After taking a detailed pain history, and clinical/ radiographic examination, if a case was considered suitable for the study, he/she was informed about the possibility of being involved in this research project and a full explanation about the procedure, its risks and benefits were discussed. A clinical information leaflet was also provided for the patient to digest later.

INCLUSION CRITERIA	EXCLUSION CRITERIA
- Patient aged 14–60 years - One tooth per patient - Premolar or molar tooth - Presence of a deep and extremely deep carious lesions on radiograph - Symptoms: range from mild, non-spontaneous pain to spontaneous severe pain - Positive response to pulp sensibility test - Pulp exposure after non-selective caries removal	- Patient aged <14 or >60 years - Incisors or canines - Absence of deep carious lesions radiographically - Preference: patient has moderate-severe pain and preferred root canal treatment - Pregnant women - Negative response to vitality test - Presence of “sound” dentine over pulp - Pulp not exposed intraoperatively - Pulpal haemorrhage could not be arrested - Pulp necrosis evident on exposure

Table 4.1 Inclusion and exclusion criteria

4.3.4 Consent and pseudo-anonymous data storage

After a patient was considered potentially suitable for the study and expressed an interest in participating, an informed consent was obtained. During the consent procedure it was stressed that the current 'gold standard' for the treatment for the cariously-exposed pulp in a mature adult tooth was root canal treatment (RCTx). Due to the emergency nature of 'toothache', it was not possible in the bulk of cases to allow a 'cooling off' period as the patient was in pain and required immediate treatment. For this reason, the operator (RCD) carefully discussed consent with the patient on the day of presentation, clarifying doubts and explaining aspects of the procedure that the patient was unclear about. Afterwards, if the patient remained willing to participate he/she was asked to sign the form. Within the consent procedure it was further explained that the patient would only be enrolled into the study if the pulp was exposed after non-selective caries removal. If a pulp was not exposed, an indirect pulp capping would be carried out, equally if on entry to the pulp there were clear signs of necrosis a RCTx would be performed. The number of these cases were recorded in the experiment flow chart (**Figure 4.1**). Three copies of the consent were signed; one for the patient, one for the patient's dental chart and one for the researcher's own study record. Any data collected by RCD was stored on an encrypted computer. The key to the code was kept on a separate encrypted computer held by the principal investigator (HD), hence keeping any data in a pseudo-anonymised state. All the images (excluding radiographs) taken before, during and after the procedure were not associated to names (only codes) and did not include the face of the patient and did not have any identifiable features. This material was stored under the same code as described above. All data will be deleted after 5 years.

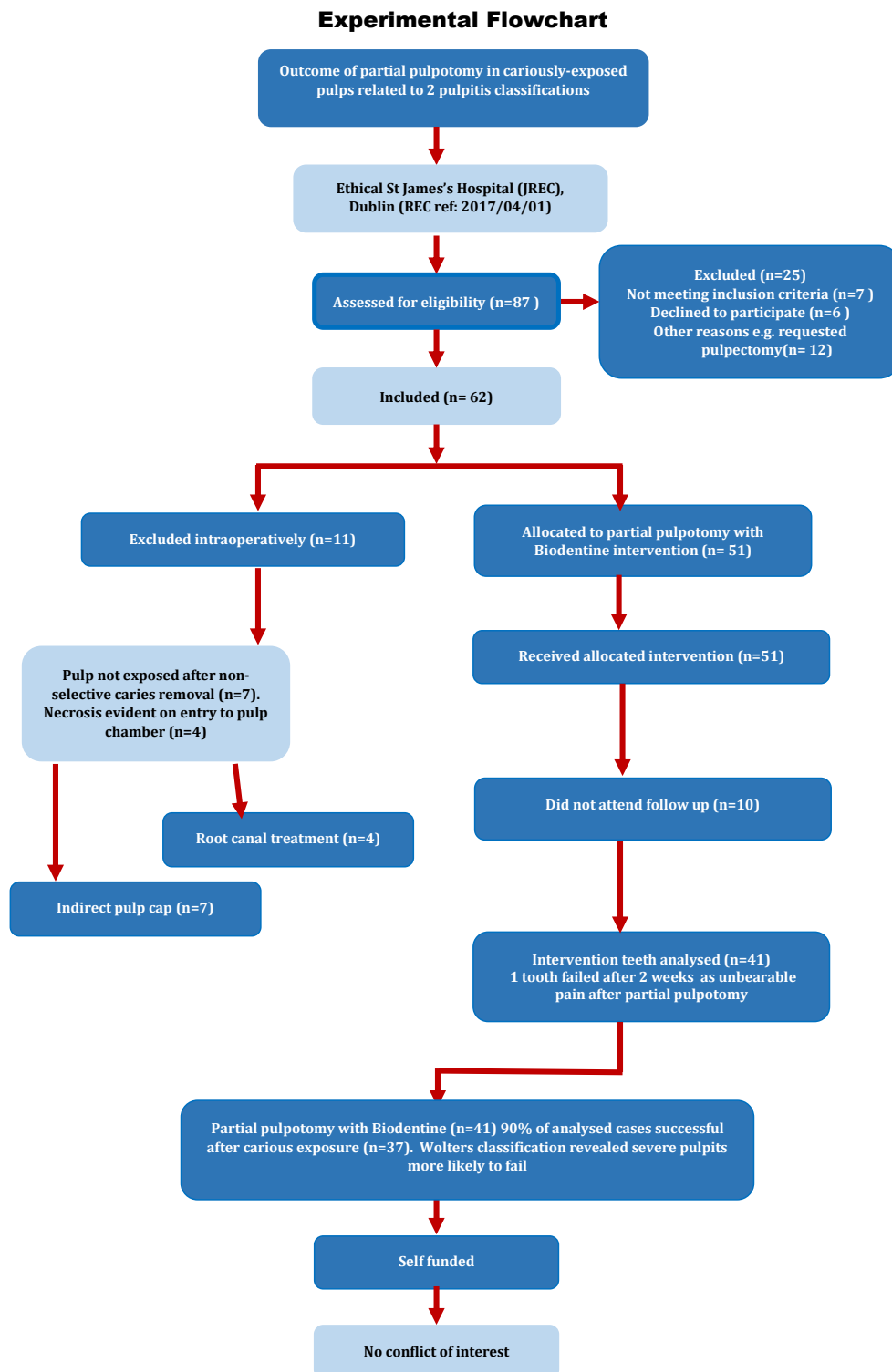


Figure 4.1 Experimental Flow Chart

4.3.5 Patient assessment and operative procedure

Before beginning the procedure the patient was assessed and the tooth categorised according to Wolters *et al.* classification (2017) as: 'Mild Pulpitis' if the tooth was asymptomatic until triggered by cold stimuli or percussion; 'Moderate Pulpitis' if the tooth demonstrated clear symptoms triggered by cold stimuli or percussion that lasted for minutes, but the pain could be stopped using medications; or 'Severe Pulpitis' if the tooth displayed severe spontaneous pain and clear strong reaction to thermal stimuli as well as pain to percussion. The tooth was also categorised as having either reversible or symptomatic irreversible pulpitis (AAE 2013) (**Table 4.2**).

In order to assess preoperative vitality, the teeth were isolated with cotton wool and dried prior to a -50°C thermal test (Endofrost, Roeko, Langenau, Germany) being applied. After administration of local anaesthetic (2% Lignocaine hydrochloride with adrenaline 1:80,000), the tooth was isolated with rubber dam and liquid dam (Ena-dam, Micerium, Avegno, Italy) (**Figure 4.2A**). Thereafter, the isolated area was cleaned using a cotton pellet soaked in 3% hydrogen peroxide followed by 0.2% chlorhexidine (Hörsted-Bindslev *et al.* 2003). Using an operative microscope (Labomed Prima, Labomed, Los Angeles, USA) (**Figure 4.2B**), the gross caries was non-selectively removed, first using a diamond bur in a high speed handpiece under water coolant, prior to using a sterile round steel bur in a slow handpiece (**Figure 4.2C**). The absence of carious dentine was confirmed using FIND (Fluorescence Inspect and Determine system) present in Ledex WL90+ (DentMate, New Taipei City, Taiwan) (**Figure 4.2D**). The area in closest proximity to the pulp was cleaned manually with a sharp hand excavator. If after non-selective caries removal, the pulp was exposed (**Figure 4.3A**), a further 2-3 mm of pulp was removed with a fresh sterile carbide bur in a hand speed handpiece under abundant water coolant (**Figure 4.3B**). After rinsing the pulp with NaOCl 2.5% solution, a cotton pellet soaked in a 2.5 % NaOCl was pressed against the exposed pulp and haemostasis was checked every minute up to 6 minutes (**Figures 4.2E & 4.3C**). The time required for the pulpal tissue to stop bleeding was recorded.

Biodentine was mixed according to the manufacturer instructions and applied with gentle pressure directly onto the pulpal wound surface (**Figures 4.2F & 4.3D**). Biodentine was used to fill the entire cavity and setting time of the

material was recorded. Setting time was defined, as the time taken for the material to harden to the extent that light pressure with an amalgam plugger did not penetrate more than 1 mm. The patient was scheduled to return after one week, when 2-3 mm of the Biodentine restoration were removed and a permanent resin-based composite restoration was placed (**Figures 4.3E & 4.F**). Pulp sensibility (Endofrost) was checked at one week, three months, six months, one year and was considered positive if the patient consistently reported even mild sensitivity to cold. After one year a periapical radiograph was taken.

Preoperatively and post-restoration of the tooth the colour of the crown was recorded on both the buccal and lingual aspect of the tooth. In order to standardise the conditions of light and visibility, the shade of the tooth was taken under magnification 10x and maximum light intensity using a microscope with LED white light (Labomed Prima) and the Vita Classic shading guide (VITA, Bad Säckingen, Germany). During the subsequent follow up appointments discolouration was further monitored and recorded.

WOLTERS CLASSIFICATION Wolters <i>et al.</i> 2017	STANDARD CLASSIFICATION AAE Endodontic diagnosis 2013
Mild pulpitis: Heightened and lengthened reaction to cold, warm and sweet stimuli that can last up to 20 s, but then subsides. Tooth may be percussion sensitive. Moderate pulpitis: Clear symptoms, strong, heightened and prolonged reaction to cold, which can last for minutes, possibly percussion sensitive and spontaneous dull pain that can be suppressed with pain medication. Severe pulpitis: Severe spontaneous pain and clear pain reaction to warm and cold stimuli, often, sharp to dull throbbing pain, patients has trouble sleeping because of the pain (worsens when lying down). Tooth is sensitive to touch and percussion.	Reversible pulpitis: Discomfort triggered by cold/sweet stimuli, which relieves within seconds after the removal of the stimulus. The pain is non spontaneous Symptomatic irreversible pulpitis: Sharp and lingering pain triggered by thermal stimulus (often 30 s or longer after stimulus removal), spontaneous pain and often referred pain. The pain can be accentuated by postural changes such as lying down or bending over and over-the-counter analgesics are typically ineffective.

Table 4.2 Pulpal disease classification systems used



Figure 4.2 Clinical armamentarium used. **A)** Isolation: rubber dam and liquid dam were used to isolate the tooth. **B)** Operative microscope. **C)** For carious tissue removal and pulpotomy, softened tissue was removed first with sterile diamond burs mounted on high speed handpiece and then with steel sterile burs on a slow handpiece. Pulpotomy was performed with a new sterile carbide bur on high speed handpiece. All the burs were used under abundant water coolant. **D)** The cavity was inspected with a fluorescence based dental caries detector Ledex WL90+. **E)** NaOCl 2,5%: used to rinse the cavity after pulpotomy and to soak cotton pellet used to achieve haemostasis. **F)** Biodentine (Septodont) used as pulp capping agent and temporary filling material.

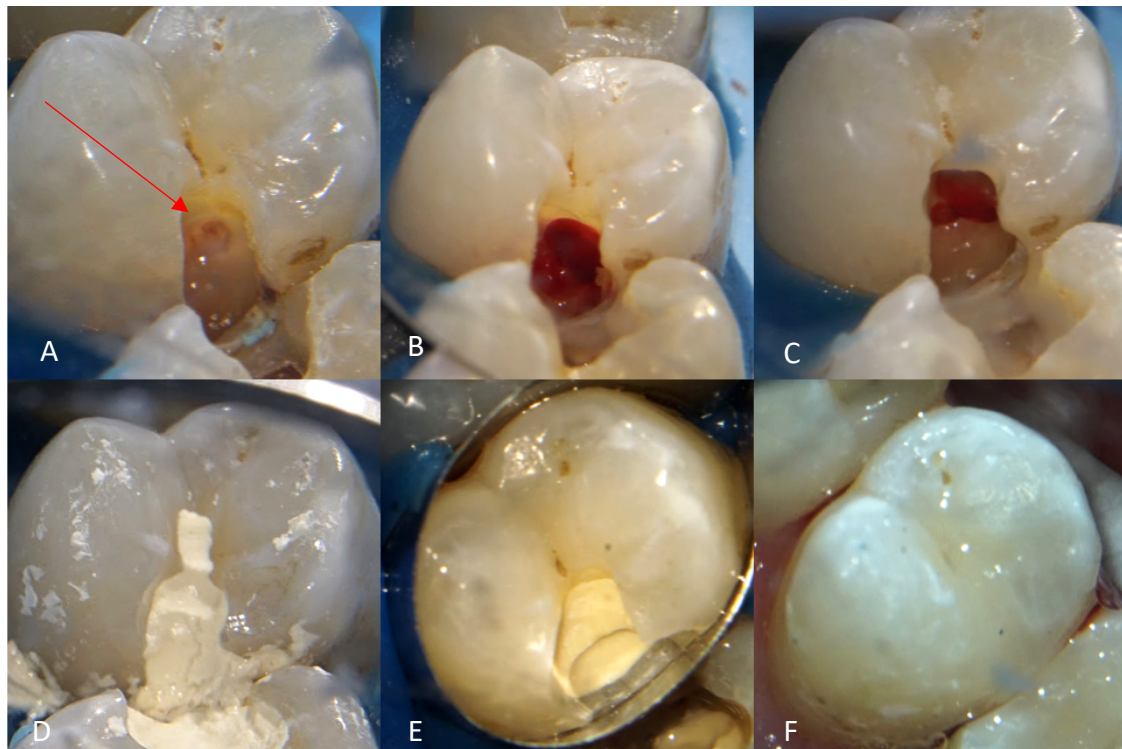


Figure 4.3 Partial pulpotomy clinical protocol. **A)** Pulp exposure after non-selective caries removal (red arrow). **B)** Superficial pulp tissue removed (to depth of 2-3 mm). Pulpal haemorrhage evident. **C)** Haemostasis obtained after several minutes by placing cotton pellet soaked in 2.5% NaOCl, pressed against the exposed pulp tissue. **D)** Biodentine used to restore the entire cavity. **E)** Superficial removal of Biodentine at the second visit. At least 3 mm of material is left above the exposure site before restoration. **F)** Tooth permanently restored with resin-based composite material.

4.4 Outcome evaluation

At every follow up appointment (3, 6 and 12 months) the teeth were carefully assessed for the presence of signs and symptoms of pulpitis, while vitality was verified and recorded. This involved carrying out a percussion test and a cold test at -50°C (Endofrost). The integrity of the coronal seal was verified visually under the microscope. After 12 months a periapical radiograph supplemented the history and clinical examination. At all review points the teeth were categorised as either 'successful', 'unresponsive' or 'failed'. Treatment was considered 'successful' when the tooth responded positively (including a consistently reduced response) to thermal test, the response did not linger, was symptom-free, not tender to percussion and there was no evidence of an apical radiolucency. Unresponsive teeth, did not demonstrate any apical radiolucency and were symptom-free but did not respond to vitality tests. A treatment failure was noted if either the patient reported pain, the tooth was tender to percussion and/or radiolucency was present.

4.5 Statistical Analysis

Arrest of bleeding and Biodentine setting time were submitted to a Shapiro–Wilk test of normality ($p < .05$ samples are not normally distributed). A non-parametric Kruskal–Wallis test was used to analyse differences in bleeding time between Wolter's diagnostic groups and Mann–Whitney U -tests were used to analyse bleeding time between reversible and irreversible pulpitis. The Wilcoxon rank-sum test was used for differences in setting time. Differences in the outcome of partial pulpotomy with each diagnosis of pulpitis were tested and cross-tabulated with contingency tables, chi-square analysis and Fisher's exact test. Data analysis used SPSS (v25; IBM) ($p < .05$). For radiographic caries, depth assessment interobserver agreement was calculated using Cohen's κ test (K) according to the scale for strength of agreement $K < 0.20$: poor, $K = 0.21–0.40$: fair, $K = 0.41–0.60$: moderate, $K = 0.61–0.80$: good, $K = 0.81–1.00$: very good (Altman 1990).

4.6 Results

4.6.1 Sample Characteristics

Eighty-seven patients were assessed for eligibility having presented with a symptomatic carious lesion in a premolar or molar tooth. Of those patients, 25 were excluded due to preferring RCTx/extraction (12 patients), refusing to participate (6 patients) and not meeting inclusion criteria for deep/extremely deep caries (7 patients) (**Figure 4.1**). Sixty-two teeth were enrolled for this study and 11 were subsequently excluded intraoperatively. Of those 11 cases, seven (one with severe, two with moderate and four with mild pulpitis) did not have carious pulp exposure after non-selective removal of caries and they were treated either with RBC restorations or with an indirect pulp capping and a calcium hydroxide lining (**Figures 4.4 A & B**). Subsequently, one of the teeth without pulp exposure but with a diagnosis of moderate pulpitis required RCTx after 5 months as it displayed signs of pulpal necrosis and apical radiolucency. Of the remaining 4 cases, one case provisionally diagnosed with mild pulpitis and three cases with severe pulpitis were treated immediately with RCTx as after access to the pulp chamber partial pulpal necrosis was diagnosed (**Figures 4.4C & D**).

Fifty-one teeth were finally included and treated within this study, with 41 teeth reviewed after 1 year (**Figure 4.1**). The diagnostic breakdown of the initial sample by Wolters criteria (Wolters *et al.* 2017) includes five cases of severe pulpitis, 22 of moderate pulpitis and 24 with mild pulpitis. By the traditional AAE pulpal diagnostic system, there were 23 reversible pulpitis and 28 irreversible pulpitis (AAE 2013). All the cases were treated with partial pulpotomy (removal of 3–4 mm pulp tissue), rinsed with 2.5% NaOCl and capped with Biodentine. All treated patients reported mild postoperative discomfort on the day of the treatment, which was managed with “over the counter” analgesics as required. Postoperative pain generally disappeared within 24 h; however, in the 7 days following the procedure more than 50% of the sample reported an increased sensitivity to cold stimuli that gradually subsided during the following 3 months. One patient with irreversible/ severe pulpitis developed unbearable pain within a week and had a pulpectomy carried out which was categorized as failure; this was the only painful failure within the 1-year period. Ten patients did not re-attend for 1-year review and were excluded from the study (5 reversible, 5 irreversible pulpitis; 5 moderate, 5 mild pulpitis) (**Figure 4.1**). Specifically, four of the ten

patients did not attend the second appointment for permanent filling placement; however, they subsequently attended the clinic as an emergency presenting with pain and sensitivity due to dislodgement of the Biodentine restoration. All four teeth had subsequent RCTx. The remaining 6 patients returned to get the permanent restoration placed, but not the subsequent 1-year review.

Generally, the response to pulp sensibility testing was less intense after treatment. As a result, in four cases because of lack of response at the second appointment, the permanent filling was performed without administering local anaesthetic and the cold test was repeated after removing part of the Biodentine; the response was positive in all four cases. Three patients were initially classified as unresponsive, but at the subsequent review appointment at 6 months demonstrated a mild response to a cold test. The visualization of a hard tissue bridge radiographically was a variable finding being present on only 12 postoperative review radiographs at 1 year (**Figure 4.5**). Two consecutive patients, one 15-year-old and one 16-year-old patient presented with deep carious lesions in two mandibular second molar teeth (**Figure 4.6**), which after completion of partial pulpotomy remained responsive to pulp sensibility testing at review. The mandibular left second molar in the 15 years old, although remaining positive to sensibility testing developed internal resorption in the middle third of both the mesial and distal roots and was classified as a treatment failure (**Figure 4.6F**).



Figure 4.4 Deep carious lesions that were excluded from the study. **A)** Upper left second premolar with a deep carious lesion, which after non-selective removal did not have a pulp exposure. **B)** Another tooth 25 where the pulp was not exposed after non selective caries removal and was treated with indirect pulp capping. **C)** Tooth 46 responsive to vitality test presenting a deep carious lesion. **D)** Same tooth in figure C treated with RCTx and temporary restored with GI as presenting clear signs of necrosis in the exposed pulp on visual inspection



Figure 4.5 Tooth 35 presenting moderate pulpitis treated with partial pulpotomy and pulp capping with Biodentine. **A)** Preoperative radiograph of a tooth presenting moderate pulpitis. **B.** Six-month radiographic evaluation showing hard tissue bridge formation (red arrow). The tooth responded positively to pulp sensibility testing and was symptom free.



Figure 4.6 two separate patients presented with deep carious lesions on teeth 47 and 37 respectively. Both teeth treated with partial pulpotomy using Biodentine. **A)** Deep carious lesion on tooth 47 presenting with incomplete root formation. **B)** 6 months after partial pulpotomy hard tissue bridge evident (red arrow). Pulp sensibility testing maintained positive response. **C)** 12 months after partial pulpotomy the root formation is complete (red arrow). Presence of radiopaque material indicative of ectopic hard tissue deposition in pulp chamber (blue arrow). **D)** Deep carious lesion removal and partial pulpotomy procedure on tooth 37. **E)** At 3 months, sensibility was maintained at a positive reading and there are no adverse signs or symptoms. **F)** 12 months after partial pulpotomy the tooth is responding to pulp sensibility tests, root formation is completed (yellow arrow), but areas of internal resorption are evident in the 37 (red arrow).

4.6.2 Outcome

For the 41 patients attending at 1-year review, of the 19 mild pulpitis cases treated, 14 were deemed successful and five unresponsive but successful, whilst of the 17 cases of moderate pulpitis 12 were considered successful, three unresponsive but successful with two failures. For teeth presenting with severe pulpitis, two cases were considered as success, one was unresponsive but successful and two failed (one within a week) (**Table 4.3**). Grouping success and unresponsive but successful, the outcome for partial pulpotomy at 1 year was 90%. Relating outcome to preoperative diagnosis, revealed that teeth with reversible pulpitis and pulp exposure treated by partial pulpotomy were 100% successful and irreversible pulpitis 78% successful at 1 year. Using Wolters classification, a preoperative diagnosis of mild pulpitis was 100% successful, 88% moderate pulpitis successful and severe pulpitis 60% successful at 1 year. If the results are reconsidered and unresponsive is a failure, reversible pulpitis were 72% successful and irreversible pulpitis cases 61% successful at 1 year, whilst using Wolters classification mild pulpitis was 74%, moderate pulpitis 65% and severe pulpitis 40% successful (**Table 4.3**).

The 1-year outcome with unresponsive considered a *success* was compared between groups classified as being reversibly and irreversibly inflamed, with no significant difference being reported ($p = .32$). Outcomes at 1 year were also compared using the three Wolters diagnostic groups with no significant difference between “mild” and “moderate” pulpitis ($p = .49$) and “moderate” and “severe” pulpitis groups ($p = .19$). When “mild” and “severe” pulpitis success rates were compared at 1 year, the “mild” group had a significantly better outcome than the “severe” pulpitis group ($p = .04$). When unresponsive was a *reconsidered*, a *failure* overall success dropped to 66% at 1 year; however, there were no significant differences between reversible and irreversible cases ($p = .52$) and any of the “mild,” “moderate” or “severe” groups ($p > .28$) (**Table 4.3**). Notably, when the two classification systems were cross-tabulated according to symptoms (**Table 4.4**), it can be seen that whilst there was good correlation between the classification systems ($p < .001$), moderate and severe pulpitis essentially represented a subdivision of irreversible pulpitis; with the subdivision related to treatment outcome with 12% failure in the

moderate pulpitis group and 40% in the severe pulpitis group at 1 year (**Table 4.3**).

The time taken for pulpal haemostasis ranged from 1 to 4 min. In all cases, pulpal haemorrhage could be arrested. Notably, there was only a small difference between the average duration of bleeding for mild and moderate pulpitis (mild pulpitis—average 1 min 9 s; moderate pulpitis—average 1 min 8 s); however, bleeding time was increased in the cases with severe pulpitis to an average of 2 min 6 s (**Table 4.3**). This difference between the groups was not significant ($p = .26$). There was also no significant association between outcome and time to achieve haemostasis ($p = .6$). Although all severe pulpitis cases were tender to percussion, compared with 82% of samples with moderate pulpitis and 37% of cases with mild pulpitis (**Table 4.3**), there was no significant correlation between tenderness and increased failure rate ($p = .61$).

The results were subdivided by preoperative caries depth (judged radiographically) into “deep” and “extremely” deep caries where it can be seen the majority of cases included in the study were “extremely deep” ($n = 50$), rather than “deep” ($n = 12$) caries (**Table 4.5**). Of the 12 “deep” cases, 7 were later excluded as the pulp was not exposed after non-selective caries removal, whilst all the “extremely” deep cases were exposed; 4 were excluded as the pulp was necrotic on entry to the pulp chamber. The 4 deep “caries” cases were all successful, albeit one case with irreversible/moderate pulpitis was unresponsive to sensibility testing, whilst all 5 failures presented within the “extremely” deep caries group (**Table 4.5**). The two “blinded” evaluators demonstrated a moderate level of agreement in assessing caries depth from an intraoral periapical radiograph ($\kappa = 0.57$).

This study was designed as a single-visit partial pulpotomy procedure with a second visit scheduled 1–2 weeks later for definitive restoration placement. Restoration of the entire cavity with Biodentine is recommended by the manufacturers; however, this is reliant on the material setting. In only two cases did the material set within the manufacturer's stated time of 12 min, with one case taking 45 min to set. The average setting time for Biodentine was 22 min. A Wilcoxon rank-sum test revealed between the average setting time to be significantly different from the manufacturer's claim ($p < .05$). In all 41 cases reviewed at 1 year, no visual discolouration of tooth substance was detected.

Classification (number of cases)	Subdivision (number)	Bleeding time (average)	Tenderness to percussion (%)	Success at 1 year (%)	Success at 1 year (%) If unresponsive classed as failure
AAE (2013) (41)	Reversible pulpitis (18)	1 min 32 s	7/18 (39%)	13 Success 5 Unresponsive success (100%)	13 Success 5 Unresponsive failure (72%)
	Irreversible pulpitis (23)	1 min 37 s	19/23 (83%)	14 Success 5 Unresponsive success 4 Failures (83%)	14 Success 5 Unresponsive failure 4 Failures (61%)
	Mild (19)	1 min 9 s	7/19 (37%)	14 Success	14 Success

Classification (number of cases)	Subdivision (number)	Bleeding time (average)	Tenderness to percussion (%)	Success at 1 year (%)	Success at 1 year (%) If unresponsive classed as failure
Wolters et al. (2017) (41)				5 Unresponsive success (100%)	5 Unresponsive failure (74%)
	Moderate (17)	1 min 8 s	14/17 (82%)	11 Success 4 Unresponsive success 2 Failures (88%)	11 Success 4 Unresponsive failure 2 Failures (65%)
	Severe (5)	2 min 6 s	5/5 (100%)	2 Success 1 Unresponsive success	2 Success 1 Unresponsive failure 2 Failures

Classification (number of cases)	Subdivision (number)	Bleeding time (average)	Tenderness to percussion (%)	Success at 1 year (%)	Success at 1 year (%) If unresponsive classed as failure
				2 Failures (60%)	(40%)

Table 4.3. Record of clinical features and 1-year success rate

Pulpitis	Mild	Moderate	Severe	Total
Reversible	18	1	0	19
Irreversible	1	17	5	23
Total	19	18	5	

Table 4.4 Cross tabulation of pulpal classifications according to preoperative symptoms and tests. This table highlights that by symptoms moderate and severe form effectively a sub-classification of irreversible pulpitis. $P < 0.001$ significant correlation between the two classification systems.

Cases meeting inclusion criteria	Subdivision due to caries depth (number)	Exclusions (reason)	Number enrolled	Number reviewed (reason for drop out)	Preoperative diagnosis	Outcome (success %)
62	Deep (12)	7 (non-selective caries removal not exposed)	5	4 (1 did not attend follow-up)	<i>Reversible: 3</i> <i>Irreversible: 1</i>	<i>Reversible: 3</i> Success (100%) <i>Irreversible: 1</i> Unresponsive-success (100%)
					<i>Mild: 3</i> <i>Moderate: 1</i> <i>Severe: 0</i>	<i>Mild: 3</i> Success (100%) <i>Moderate: 1</i> Unresponsive-success (100%) <i>Severe: 0</i>
	Extremely deep (50)	4 (necrotic on entry to pulp)	46	37 (9 did not attend follow-up)	<i>Reversible: 15</i> <i>Irreversible: 22</i>	<i>Reversible: 10</i> Success; 5 Unresponsive-success (100%) <i>Irreversible: 14</i> Success; 4 Unresponsive-success; 4 Failures (82%)
					<i>Mild: 16</i> <i>Moderate: 16</i> <i>Severe: 5</i>	<i>Mild: 11</i> Success; 5 Unresponsive-success (100%) <i>Moderate: 11</i> Success; 3 Unresponsive-success, 2 Failures (87%) <i>Severe: 2</i> Success, 1 Unresponsive success, 2 Failures (60%)

Table 4.5 Cases subdivided on caries depth (deep or extremely deep). Unresponsive cases were classed as a success.

4.7 Discussion

Partial pulpotomy is defined as the removal of a small portion of coronal pulp tissue after exposure, followed by application of a biomaterial directly onto the remaining pulp tissue prior to placement of a permanent restoration (ESE 2019). Partial pulpotomy has been advocated as the preferred treatment for cariously exposed pulp tissue as it removes both the biofilm on the surface of the pulp tissue and the superficially inflamed pulp and it has been recognized as suitable treatment for permanent teeth with signs of pulpitis and even irreversible pulpitis (Massler 1959, Taha & Khazali 2017). It has been advocated as a possible management strategy for young cariously exposed teeth with no clinical symptoms, with success in excess of 90% reported at 1 year for cases treated with partial pulpotomy and capped with calcium hydroxide (Mejäre & Cvek 1993, Zilberman *et al.* 1989). Indeed, the results of this study using HCSC in a range of ages are in accordance with the outcome of these studies (Mejäre & Cvek 1993, Zilberman *et al.* 1989) highlighting an overall success of 90% for partial pulpotomy after carious exposure, but with associated symptoms. Indeed, the high rate of success in the current study may be partly attributed to the use of an enhanced technique using an operating microscope, disinfection of the pulpal wound, strict asepsis using rubber dam and placement of a HCSC in what has been described as a class II VPT procedure (Bjørndal *et al.* 2019).

A principal problem of treating particularly deep, and to a lesser extent, extremely deep caries is the decision as to whether carious dentine should be left in a one visit or stepwise excavation technique with the avoidance of pulp exposure (Bjørndal *et al.* 2010 & 2019); or whether the carious dentine should be completely removed and the pulp exposure examined and managed (Ricucci *et al.* 2020). In this study, all teeth presented with symptoms associated with a deep or extremely carious lesion, so a decision was made to non-selectively remove the carious tissue in all cases. Although this could be considered overtreatment by some (Schwendicke *et al.* 2016), others have reported excellent outcome by non-selective carious removal and careful restoration of the exposed pulp (Marques *et al.* 2015). Notably, in the current study all teeth were symptomatic so partial pulpotomy was selected and both deep and extremely deep caries groups were included. Indeed, only cases that were not exposed after non-

selective removal were treated by indirect pulp capping and excluded from the study even though they were symptomatic. This was carried out for methodological reasons as within the design it was critical that “sound” dentine was not breached in order to expose the pulp. Interestingly, 2 moderate and 1 severe pulpitis (or 3 irreversible pulpitic teeth) were contained within this group and although one case of moderate pulpitis failed, the other two cases did not fail within the designated review period. It may be stressed, however, that these cases were not included within the review protocol and the outcome of indirect pulp capping in symptomatic pulpitis was not an aim of this study. Nonetheless, it is an interesting observation that warrants future study. Further subanalysis of the results highlights that the majority of the included cases were extremely deep (90%) rather than deep caries. This likely represents an increased chance of extremely deep caries being symptomatic, which was an inclusion criterion for this study. Notably, all extremely deep cases were exposed, whilst only 42% of the deep cases were exposed after non-selective removal and all failure cases were in the extremely deep caries group highlighting the importance of caries depth in the outcome of VPT (Bjørndal *et al.* 2019). Two experienced dentists, independently and blinded to outcome evaluated the radiograph dividing them into deep and extremely deep caries with a moderate level of inter-operative agreement ($\kappa = 0.57$). This agreement may have been expected to be higher, but perhaps reflects the difficulty (and arbitrary nature) of objectively analysing a two-dimensional radiograph and separating into distinct categories, particularly for borderline cases as well as periapical radiograph distortion. In this study, discussion afterwards resulted in consensus regarding the depth of caries. Finally, all the failure cases occurred in the extremely deep caries group, which highlights the potential prognostic benefit of establishing and recording radiographic caries depth preoperatively.

As a result of an improved understanding of the pulp's response to injury (Smith *et al.* 2016) and the advent of HCSCs such as MTA and Biodentine, there has been renewed interest and improved outcomes in the area of conservative treatment of pulp exposure (Aeinehchi *et al.* 2003, Nair *et al.* 2008, Parirokh *et al.* 2017). However, a continual challenge for strategies aimed at preserving the pulp is the establishment of an accurate assessment of the clinical features that reflect the “true” histological condition of the pulp, as it has been shown that

infection and inflammation reduce the outcome of the therapy (Al-Hiyasat *et al.* 2006). It has long been considered that the correlation between signs and symptoms of pulpitis and histological cell infiltrate was poor (Dummer *et al.* 1980); however, a recent study highlighted a good association between symptoms and histological status of pulpitis (Ricucci *et al.* 2014). The finding that inflammation is generally localized to the area sub-adjacent to the carious lesion stimulated new research into the preservation of pulp tissue and conservative management in cases with reversible and irreversible pulpitis (Simon *et al.* 2013). Indeed, even more recent data on the histological and bacterial profile of the carious teeth in advance stages of disease could also support the data that inflammation is localized to the pulp chamber and relates to the carious lesion penetration, whilst underlining the importance of caries depth (Demant *et al.* 2021).

In the current pragmatic study carried out in one general dental practice, partial pulpotomy was generally effective with an overall success rate of 90% with only one painful failure occurring within the year-long review. These results differ from a recent study investigating pulp capping of asymptomatic carious exposures with HCSC, which reported a high level of early failure (Ballal *et al.* 2020). This can perhaps be explained by the fact that all pulpal wounds within this study were washed with NaOCl, which has been shown to reduce painful failure in the study (Ballal *et al.* 2020) and also that partial pulpotomy was used rather than simply pulp capping, a technique that removes the superficially inflamed and infected dentine and pulp tissue and is recommended for procedures with increased pulpal damage (ESE 2019).

As a result of the success of VPT procedures being advocated in cases of irreversible pulpitis, a new classification system has been proposed and linked to treatment indications (Wolters *et al.* 2017). The diagnostic system proposed by Wolters and co-workers was used in this study as a way of investigating how the nature and severity of symptoms affected outcome. The influence of exposure on the outcome of VPT remains controversial with multi-centre trial evidence reporting a negative prognostic effect (Bjørndal *et al.* 2010), whilst several principally single-arm studies carried out in Endodontic practice reporting high VPT success after carious exposure (Bogen *et al.* 2008, Marques *et al.* 2015, Taha & Khazali 2017). In this study of symptomatic teeth, it was elected to non-selectively remove all carious dentine in every case and if the pulp was exposed

carry out a partial pulpotomy. This approach was previously adopted when treating both symptomatic and asymptomatic teeth with partial pulpotomy (Mejäre & Cvek 1993).

Any classification of pulpitis should ideally guide a subsequent decision as to whether the pulp can be maintained; however, the task of accurately ascertaining whether the pulp is actually irreversibly inflamed remains a significant challenge (Bjørndal *et al.* 2019, Mejäre *et al.* 2012). Multiple recent reports highlighting high success rates for partial and full pulpotomy in teeth with signs and symptoms of irreversible pulpitis (Simon *et al.* 2013, Taha & Khazali 2017, Uesrichai *et al.* 2019) underline the need for a more sensitive classification of pulpal disease. This need has stimulated research groups to suggest new classifications or subdivisions of pulpitis that may be more reflective of the likelihood of the pulp to respond to conservative treatment (Hashem *et al.* 2015, Wolters *et al.* 2017). One such classification (Wolters *et al.* 2017) divided pulpitis into four practical categories, three of which were associated with symptoms. On closer examination (**Table 4.2**), two of Wolters proposed groups moderate and severe pulpitis simulate a division of the AAE irreversible pulpitis group (AAE 2013, Wolters *et al.* 2017). The current study demonstrates that such a division may be helpful in predicting which cases are likely to fail after treatment as the severe group had significantly more failure (60% successful) than the mild group; however, the moderate group was not significantly different from the mild group. Notably, these differences were lost when the broader descriptor irreversible pulpitis was employed. This offers the potential for symptoms and the use of Wolters subdivisions to offer a prognostic guide for clinicians when deciding whether to embark on VPT. Indeed, within the limitations of this work subdivision of patient preoperative symptoms was a more useful indicator of success than preoperative features such as TTP or the intraoperative assessment of pulpal haemostasis. Furthermore, the increased complexity by adding more divisions to the new classification system was considered simple to use and in practical terms only involved small extensions to existing pain history relating to the nature and duration of the pain (**Table 4.2**).

Bleeding has long been considered important to both the success and practicality of VPT (Accorinte *et al.* 2008, Matsuo *et al.* 1996). Recently, time to arrest haemorrhage was highlighted as a potential prognostic indicator in primary

teeth (Mutluay *et al.* 2018). In the current study, variation in bleeding was evident with copious haemorrhage evident in teeth displaying very mild symptoms, and alternatively symptomatic teeth displaying only a small amount of bleeding. Although bleeding time was increased in severe and irreversible pulpitis categories (**Table 4.3**), there was no significant correlation between bleeding time and outcome. Clearly uncontrolled haemorrhage can indicate a severely damaged pulp as well as influencing the placement of certain restorative materials, for example resin-based materials (Accorinte *et al.* 2008); however, this may not be so critical for hydrophilic materials such as HCSCs. Notably, in this study there were no cases in which the bleeding could not be arrested prior to material placement.

When considering the clinical features to assess pulpitis, it was decided to use the most reliable findings in order to label the teeth. Indeed, pain and response to sensibility test were the principal characteristics assessed. It is difficult to predictably quantify the level of TTP, so this variable was dichotomized into TTP positive or negative. Surprisingly, TTP was frequently evident in mild and moderate pulpitis, whilst it was present in all severe pulpitis cases; however, its preoperative presence was not significantly linked to failure in this study. An unusual finding in this study was the fact that 10 patients (1 deep and 9 extremely deep caries (**Table 4.5**) were unresponsive to pulp sensibility testing after 1 year, which is at odds with previous partial pulpotomy studies using cold testing (Taha & Khazali 2017), which did not highlight this as a feature in their results. Within this study, these cases were grouped as a success as there was no sign of an emerging apical radiolucency or other sign of failure, which in line with a recent ESE position statement (ESE 2019). Interestingly, pulp sensibility responses were temporarily lost in several cases, which is relatively common after dental trauma (Bastos *et al.* 2014). Longer term follow-up demonstrated recovery in the majority of cases, highlighting the necessity for continuing review (ESE 2019). However, it is conceded that tooth sensibility tests may have been restored in more cases if the follow-up had been extended to 2 years. Success in this study was a composite measure of patient-reported and clinician-reported outcomes (Duncan *et al.* 2021), that included signs and symptoms of failure, response to pulp sensibility testing (responsive/ unresponsive) and radiographic analysis. It is possible that even in the absence of signs, symptoms and radiographic apical

breakdown that the pulp may be necrotic and for this reason the results were recalculated as a “worst-case” scenario with all “non-responsive” cases having failed (**Table 4.3**). If unresponsive cases are considered as a failure, the overall success of partial pulpotomy after 1 year drops to 66%; however, the same trends related to pulp classification are evident with mild pulpitis 84%, moderate pulpitis 71% and severe pulpitis 40% successful, respectively. The problem with reliable sensibility testing after VPT although, not previously highlighted in partial pulpotomy studies of symptomatic cases (Taha & Khazali 2017), has been highlighted as a concern after complete pulpotomy in the recent deep caries position statement, which suggested that a positive response to sensibility testing was unlikely in these cases (ESE 2019).

Ten of the 51 included teeth were excluded because they failed to attend recall appointments or have the definitive restoration placed (19.5% drop out). Of the 10 exclusions, four attended the clinic in pain within 6 months and were managed with RCTx. In all four cases, the patient did not return for the second visit as planned, and after several months, the pulp was necrotic and the Biodentine was dislodged. These anecdotal data question the manufacturer's claims that the material is suitable as a long-term temporary restorative material. In one molar, internal resorption was evident in both roots, which although the tooth was otherwise a success this is still classed as an unusual type of failure (ESE 2006).

Biodentine is marketed as a single-visit VPT material with a setting time that should be 12 min. Mixing and dispensing the material according to the manufacturer instructions did not prevent differences in the consistency of the Biodentine once the mixing process was complete. Some samples demonstrated a “creamy” aspect whilst some others had a dry and brittle consistency. Once placed in the cavity, Biodentine assumed a soft and flowable consistency. The material generally took significantly longer to set than would be practical in one session with an average setting time of 22 min, which is almost double the manufacturers indicated time. As VPT is often carried out as an emergency procedure, the potential attractiveness of a single-visit treatment is considerable; however, a longer setting time may lead to the dentist to opt for a two-stage procedure, reducing the advantage of Biodentine over the classic formulation of MTA (which sets in several hours). Notably, Biodentine was not associated with

any postoperative discolouration or staining which have been a feature of other MTA materials (Camilleri 2015). With the limitations of this study, the results confirm the studies carried out on Biodentine discolouration (Camilleri 2015, Kohli *et al.* 2015, Yoldaş *et al.* 2016).

Although this work is the first to analyse the effectiveness of a new classification of pulp condition, it has certain limitations. The study had only one operator, which although reducing intraoperative variability and increasing practicality, as it was set in primary care, does reduce generalizability of the data as only one individual carried out the operator elements of the work. In order to reduce potential bias, the radiographs were blinded and assessed by a second operator; however, for practical reasons the review appointments were carried out by the operator. It is accepted that larger studies with multiple operators will be required in the future to verify the results of this study. Furthermore, there was no control group in the study as the study was designed as an outcome study to assess the relative effectiveness of a new pulp classification to which the patients could not be randomized. Finally, Wolters and co-workers designed a new classification system as part of an overall strategy to guide subsequent endodontic management (Wolters *et al.* 2017). In the current study, the management recommendations were not followed and every pulp that was exposed after non-selective caries removal was treated by partial pulpotomy. Although this limits the applicability of the management arms of that study, it was carried out to limit bias and allow comparison of the management of the exposed pulp using two different pulpal diagnosis classifications.

4.8 Conclusions

Partial pulpotomy using Biodentine was successful in 90% of analysed cases for the treatment of symptomatic carious exposures of the pulp after 1 year, but included cases where pulp vitality could not be confirmed. Within the limitations of this study, it could be concluded that cases with signs and symptoms indicative of irreversible pulpitis were as successful as cases with reversible pulpitis; however, using Wolters classification cases with severe pulpitis all presented with extremely deep caries and were significantly less successful than cases with mild pulpitis, which highlights a potential prognostic benefit in diagnostically subdividing cases with more severe symptoms. Bleeding time and tenderness to

percussion were not significant indicators of the outcome of partial pulpotomy procedure and although Biodentine did not induce discolouration after 1 year, the setting time was significantly longer than the manufacturers claim.

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Chapter 5.

Next-Generation Pulpal Diagnostics: A CRP Chairside Test to Assess the Level of Pulpal Inflammation

5.1 Introduction

Preservation of pulp vitality is a principal goal of endodontics with VPT recently becoming a realistic treatment strategy, even in teeth exhibiting moderate to severe pulpitis (Duncan *et al.* 2019). However, obtaining predictable results in pulpitis cases can be challenging as symptoms do not correlate closely with the histological state of the pulp (Dummer *et al.* 1980, Gopikrishna *et al.* 2009). Although, diagnostic tests have been developed with the hope of accurately establishing an inflammatory 'threshold' between reversible and irreversible pulpitis, recently introduced tests including pulse oximetry (PO) and laser doppler flowmetry (LDF) have their own limitations and have not superseded 'classic' thermal and electric sensibility tests (Alghaithy & Qualtrough 2017). Recently molecular techniques aimed at analysing pulpal biomarkers have been proposed as a potential solution (Rechenberg *et al.* 2016); however, there is a lack of robust data to support their use clinically (Karrar *et al.* 2023). At present there is a paucity of evidence investigating their applicability *in vivo*.

The majority of biomarkers assessed to date in the literature are locally produced by dental pulp cells (Karrar *et al.*, 2023); however, accessing the dental pulp for diagnostic purposes involves intervention and access to either dentinal fluid or pulpal blood. Other sensitive markers of inflammation are synthesised systematically in the liver, which presents an opportunity to assess pulpal inflammatory changes from systemic blood. One systemically produced inflammatory marker C-reactive protein (CRP) is an acute phase inflammatory protein whose blood plasma levels rise dramatically and dynamically in the presence of tissue damage, inflammation or infection with CRP values commonly measured in medical practice to gain information on disease activity (Thompson *et al.* 1993). A normal level of CRP in healthy young adults is calculated to be approximately 0.8 ml/l, but CRP does rise in expression in the presence of certain diseases including autoimmune disorders, cardiovascular diseases and infection (Pepys & Hirschfield

2003, Sproston & Ashworth 2018). CRP gene polymorphism may alter the basic plasma level in different subjects, as well as environmental cues such as obesity and smoking or other factors including gender and cellular distress; however, even these confounders do not appear to compromise the efficacy of CRP as an acute phase inflammatory marker (Kushner *et al.* 2006, Hage & Szalai 2007). Indeed, in cases of an acute inflammation or tissue damage, blood levels of CRP can rise quickly with an increase of even 1000-fold compared with baseline (Hage & Szalai 2007).

As indicated previously CRP is principally produced by hepatocytes in response to a stimulation by inflammatory cytokines including interleukin 6 (IL-6) and interleukin 1 (IL-1), however other mediators can also have roles in its synthesis (Du clos & Mold 2004). Even though the liver is the main producer of CRP, the protein has been shown to be synthesized in other tissues as well, including periodontal tissues Baidoshvili *et al.* 2002, Garrido *et al.* 2015); however, not to date in pulpal tissues. After synthesis the protein is distributed systemically and in sites where acute inflammation is present (Du clos & Mold 2004). It has been postulated that dental infection could potentially have systemic manifestations and a correlation between oral disease and general health have been reported elsewhere (Li *et al.* 2000, Rautemaa *et al.* 2007, Harjunmaa *et al.* 2018). Severe infection of dental origin may produce an increase of CRP expression and generalised symptomatology; however, it is not clear if an acute or subacute inflammation can be detected looking at CRP levels or if it is useful as an indicator of pulpitis and pulp infection (Boucher *et al.* 1967, Georgiou *et al.* 2019). Previous studies have reported an increase of CRP in cases of dental inflammation, as well as an altered expression of CRP in serum and dental pulp, however these studies were preliminary and limited by a small cohort size (Proctor *et al.* 1991, Ren & Malmstrom 2007)

5.2 Study aims and objectives

The aim of this preliminary *in vivo* study is to investigate the levels of CRP, present in healthy teeth with no symptoms, symptomatic teeth with pulpitis as well as teeth associated with pulpal necrosis and apical abscess. The following objectives were addressed;

- Objective 1: To establish a negative control level of CRP in 'sound' teeth with a healthy pulp by which to compare inflamed and necrotic pulps.
- Objective 2: To establish a positive control of CRP in teeth with severe dental infections and evidence of acute apical abscess.
- Objective 3: To investigate the expression of CRP in patients with symptomatic pulpitis.
- Objective 4: To compare CRP levels before and after treatment for pulpitis and apical abscesses.

5.3 Materials and methods

5.3.1 Power calculation

This preliminary study aimed to assess whether CRP was useful for assessing endodontic-related inflammation and swelling. Therefore, the power calculation was based on the primary outcome of finding a significant difference between patients with acute infection and swelling and irreversible pulpitis group. Assuming the effect size of 0.4, 5% level of significance and 80% power, the required sample size for each group was established as 7. It was planned that the results of this study would also be used to ascertain an appropriate power calculation to discriminate between the other groups.

5.3.2 Patient recruitment, ethical approval and consent

After ethical approval was obtained patients were recruited by the principal investigator (RCD) in private practice or by the principle investigator (HD) in DDUH. Whenever a suitable patient was identified, he/she was informed about the study and they were asked if they wished to consider participation in the study. An informed consent was obtained. There were common inclusion criteria and specific per each group (**Table 6.1**).

For the Control Group (CG) and all groups the patient were adult, male or female, without systemic diseases including heart diseases, auto-immune diseases, cancer, viral or bacterial infection. The patient also reported an absence of acute disease in the past two weeks.

Group One (G1) included patients that presented with severe symptoms associated with intra and extraoral swelling, pain and need for immediate emergency dental care. In this group, patients were included who had started an antibiotic course less than one day before consultation without undertaking any dental emergency treatments yet.

Group Two (G2) included patients with symptoms of irreversible (severe) pulpitis including excruciating pain worsening at night, no relief from over the counter painkillers and tenderness to percussion.

Group Three (G3) included patients with reversible (Initial/Mild/Moderate) pulpitis: these patients do not report strong pain but more discomfort, pain is triggered by hot/cold stimuli but then goes away.

Group Four (G4) included cases where the pulp was necrotic, an abscess/sinus tract was present but the patient experienced just mild symptoms or was asymptomatic.

GROUP	INCLUSION CRITERIA	EXCLUSION CRITERIA
All groups	>18 years of age. Absence of any chronic disease including cardiac, autoimmune disorders, cancer or bacterial/viral infections. Absence of any acute disease or low-grade infection in the previous two weeks No history of recent trauma.	<18 years. Presence of chronic diseases/conditions or acute infection, inflammation in the previous two weeks; Recent intake of antibiotics/steroids. Pregnancy. Obesity
Group 0 (G0)	Absence of dental caries. Absence of periodontal disease and good oral hygiene.	Presence of dental cavities, apical pathology and periodontal disease.
Group 1	Presence of severe symptoms and intra/extraoral swelling. Need for immediate care.	Use of antibiotics for more than one day or patient who had already had emergency operative dental treatment
Group 2	Symptoms of irreversible (severe) pulpitis, sleepless nights and severe pain not controlled by analgesia	Patients who had recent treatment of that condition (e.g. pulpectomy), patients on antibiotics
Group 3	Symptoms of reversible pulpitis, pain triggered by hot/cold/mastication but bearable and controlled by analgesia	Cases where the pain could be related to periodontal issues such as gingivitis, cases where the tooth causing pain was necrotic
Group 4	Presence of apical pathology, necrotic teeth, abscess/sinus tract. Asymptomatic or mild symptoms	Pain or extraoral swelling associated with apical pathology

Table 5.1. Inclusion and exclusion criteria

5.3.3 CRP chairside test

The test chosen for this study is an immunoturbidimetric assay for the measurement of CRP levels. In order to perform this test chairside, a commercially available rapid measuring system was used. The CRP QuickRead go (Aidan Oy, Espoo, Finland) system is a point-of-care device designed for the rapid assessment of CRP levels in blood from a finger-tip sample. The system is based on the principle of antigen-antibody reactions, to measure CRP concentrations. The CRP QuickRead go system features a 20 µl sample collector (**Figure 5.1**), a disposable pre-filled cuvette with a reagent cap (**Figure 5.2**) and a chairside reader machine (**Figure 5.3**). The pre-filled cuvette contains a buffer solution, while the cap contains lyophilised active reagents. The cuvette and cap have a bar code, which contains calibration data that the machine reads before starting analysing the sample (**Figure 5.2**). The testing kit is kept refrigerated at 2-8°C and the cuvette needed brought to room temperature (18-25°C) shortly before the test is carried out. Once the blood comes in contact with the reagents, the blood cells are haemolysed: the CRP present in the sample binds with the micro-particles coated with human CRP antibodies F(ab)₂ fragments present in the cartridge. This reaction forms antigen/ antibodies complexes that initiate a change in the solution's turbidity. The chairside reader then measures the level of turbidity using a bespoke optical reader and compares it with the pre- calibrated standards contained in the barcode present on each cuvette. Through this comparison, a quantitative measurement of CRP levels expressed in milligrams per litre (mg/L) is provided within approximately 2-minutes. The results are automatically recorded in the internal memory of the QuickRead go reader machine, and linked to the operator and patient's details. For the purposes of this study the data was pseudo-anonymised by use of a code before being copied to an encrypted device (**see section 5.3.4**).



Figure 5.1 Sample collector. 20 μ l of the patient's blood (taken from the second finger) fills the transparent portion of the collector through capillary action



Figure 5.2 Cuvette and reagent cap. Both present with bar code labelling.

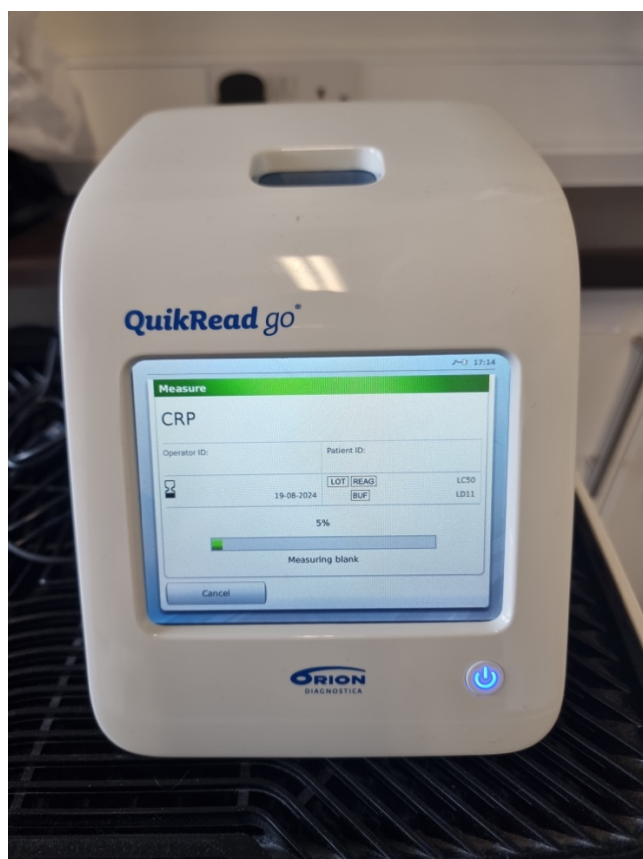


Figure 5.3 QuikRead go (Aidan Oy) chairside reader

5.3.4 Patient consent and data storage

As part of the experimental plan and ethical approval, the data was collected and stored on an encrypted computer under an anonymous code (RCD). The key to the code was kept on a separate encrypted computer held by the study gatekeeper (an administrator not involved in the project), hence keeping any data in a pseudo-anonymised state. All collected data were recorded dividing them by category and associating a number to each case in order to anonymize the patients.

5.3.5 Clinical sample collection and analysis

After discussing the procedure with the patient and gaining consent, the fingertip of the patient's second finger was wiped using a 70% isopropyl alcohol swab (Alcohol Preps, Hibernia Medical, Dublin, Ireland). Bleeding was then induced using a single use contact activated lancet (BD microtainer, Franklin Lakes, USA) (**Figure 5.4**), pressed gently until the skin was punctured (**Figure 5.5**). The first drop of blood was removed and gentle pressure was applied in order to promote the formation of a

'pea-sized' blood drop. The 20 µl sample collector was then used to simply touch the blood drop that then filled the collector by capillary action (**Figure 5.6**). The collector was then inserted inside the cuvette through a 'guide', allowing the release of the blood in the solution while avoiding the sample collector touching the base of the cuvette (**Figure 5.7**).

The patient was given an antiseptic cotton wool swab to press against the wound in order to stop the bleeding and a small 'plaster' was applied. Thereafter the cuvette was closed with the reagent cap and inserted in the commercial reader, which indicated the results on the display after approximately 2 minutes. After the analysis was completed, the reader automatically discharged the used cuvette, which was then discarded according to the manufacturer's instructions. The results were recorded for each case and the treatment commenced. If CRP level was superior to 1 mg/L the patient was invited to attend the practice for a follow up appointment after the procedure in order to evaluate if a change in CRP level occurred after treatment.



Figure 5.4 BD microtainer contact activated lancet



Figure 5.5 Bleeding is induced gently pressing the BD microtainer against the skin. A small blade/needle punctures the skin inducing bleeding



Figure 5.6 Blood fills the collector by means of capillary action



Figure 5.7 The collector is inserted in the cuvette to release the sample. A system of guides and stoppers ensures that the sample collector does not touch and potentially damage the base of the cuvette.

5.3.5 Statistical analysis

The preliminary results were statistically analysed in order to provide proof of principle data and inform a power calculation for a future study. All four groups were tested with a Shapiro- Wilk, which showed a non-normal distribution of results with ($p<0.05$).

As a result, the groups were assessed through a non-parametric ANOVA test and post-hoc Tukey's. The control group (G0) was thereafter compared with the other three groups by using a one-sample t-test and Mann-Whitney U (non-parametric) test. The parametric paired-sample t-test and non-parametric Wilcoxon signed rank test were used to calculate the difference between the CRP levels obtained before and after intervention in the swelling group ($p<0.05$). A power calculation was only carried out for the primary outcome swelling compared with irreversible pulpitis not considered for these samples. All data significance = $p<0.05$.

5.4 Results

This study included one healthy control group (negative control), one swelling group (positive control) and three other study groups.

The control group (G0) consisted of five healthy individuals that did not present any current dental disease and had a routine dental check up with radiographic examination within 3 months and that did not show any sign of dental pathology. The common finding was a level of CRP at or below 1mg/L (**Table 2**).

In Group 1 (G1) there were eight patients that attended the practice for emergency appointments presenting intra or extra oral swelling associated with excruciating pain. Of these eight cases, six of them presented with abscesses of endodontic origin, one case was related to an impacted 'wisdom' tooth and another one was a severe 'flare-up' reaction after a root canal treatment procedure. All of these cases presented an increase of CRP levels ranging from 7.7 to 70 mg/L (**Table 2**). All the patients were taking over the counter NSAIDs and two of them had started an antibiotic course within 24 hours of the appointment. Due to the emergency nature of the swelling, immediate care was provided and the patient was seen after one week for a review. All cases showed a considerable reduction in the CRP levels and

the complete disappearance of symptoms (**Table 2**). The reduction in CRP after treatment was significant ($p < 0.05$).

The second group (G2) contained seven cases of irreversible (severe) pulpitis with strong symptoms (*i.e.*, excruciating nocturnal pain disturbing sleep, spontaneous pain, extreme reaction to thermal or mechanical stimuli). Of these seven cases one showed a normal value of CRP (1 mg/L), while all of the others had an increased CRP ranging from 2.3 to 15 mg/L (**Table 2**). All these patients received root canal treatment but unfortunately all but two failed to attend the one-week follow-up appointment making it impossible to analyse the results after therapy. In one case the patient received a root canal treatment and a partial pulpotomy on two adjacent teeth in the same visit. The patient reported immediate relief and after one-week CRP decreased from 2.3 to 1 mg/L or less. Notably one other patient had a root canal treatment that alleviated the symptoms; however, on follow-up the CRP level increased from 15 to 17 mg/L. In this case the patient fractured a bone in his foot between the first and second appointment.

The third group (G3) included five cases of reversible (mild/moderate) pulpitis, three of them had a normal value of 1mg/L, while one case had 2.6mg/L and another 1.6 mg/L (**Table 2**). The two cases with increased CRP in this group were reporting a more aggressive symptomatology in the days before the appointment. The symptoms then subsided and when they attended the surgery for consultation the clinical situation was compatible with moderate pulpitis.

The final group (G4) had six patients who had no symptoms but presented with chronic apical abscesses and a periapical radiolucency. Of these cases three of them had normal values of 1mg/L or below and after root canal treatment (**Table 2**).

Statistically there were significant differences between G1 (swelling) and the reversible pulpitis (G3), chronic apical abscess (G4) groups ($p < 0.05$). The difference between G1 (swelling) and G2 (irreversible pulpitis) was marginally non-significant ($0.05 < p < 0.10$), while there was no significant difference between G2 and G3/G4. The test groups were statistically compared with the healthy control (G0) showing that the groups with severe symptoms, G1 (swelling) and G2

(irreversible pulpitis) had significant differences with G0 (healthy) $p < 0.05$, while the other two groups (G3 and G4) were not significant.

Diagnosis	CRP pre-treatment mg/L	Treatment	CRP post-treatment mg/L
Group 0			
Healthy	<1	-	-
Healthy	1	-	-
Healthy	<1	-	-
Healthy	1	-	-
Healthy	<1	-	-
Group 1			
Endodontic abscess	70	Pulpectomy, drainage	23
Endodontic abscess on a root treated tooth	52	Lanced abscess, antibiotics	2.3
Vertical root fracture	20	Extraction	4.7
Endo-perio abscess	24	Extraction	4.2
Endodontic abscess	20	Pulpectomy, antibiotics	4.7
Endodontic abscess	11	Pulpectomy, drainage	2.2
Endodontic flare up	27	Antibiotics, steroids	4.2
Abscess due to impacted 'wisdom' tooth	7.7	Antibiotics	5
Group 2			
Irreversible/severe pulpitis	1	Root Canal Treatment	-
Irreversible/severe pulpitis	2.3	Root canal treatment + partial pulpotomy	1
Irreversible/severe pulpitis	5.1	Root Canal Treatment	-
Irreversible/severe pulpitis	2.4	Root Canal Treatment	-

Irreversible/severe pulpitis	4.2	Root Canal Treatment	-
Irreversible/severe pulpitis	15	Root Canal Treatment	17*
Irreversible/severe pulpitis	10	Root Canal Treatment	-
Group 3			
Moderate Pulpitis	1.6	Indirect restoration	-
Moderate Pulpitis	2.6	Direct Restoration	-
Moderate Pulpitis	1	Direct Restoration	-
Mild Pulpitis	<1	Direct Restoration	-
Mild Pulpitis	1	Direct Restoration	-
Group 4			
PARL on 2 teeth	1	Root Canal Treatment	-
PARL and sinus tract	1.6	Root Canal Treatment	1.2
PARL and sinus tract	2	Root Canal Treatment	3.8
PARL and sinus tract	<1	Root Canal Treatment	-
PARL, small swelling	1	Root Canal Treatment	-
PARL	3.7	Root Canal Treatment	4.8

Table 5.2. Dental issue and CRP levels before and after treatment for patients all groups

5.5 Discussion

5.5.1 General considerations

Pulpal diagnosis is currently based on clinical observations and radiographic findings, with supplementary molecular testing of blood or dentinal fluids not commercially available (Miller & Westgate 2014). Attempts to identify biological markers that can help in the determination of the pulp status has been made with limited translation to the clinic (Donnermeyer *et al.* 2022). Studies conducted on pulp fluids analysing Biomarkers such as MMP9, the IL family and PGE₂ highlighted the potential to assess the level of pulp damage and potentially the survival rate of the pulp (Zanini *et al.* 2017, Ballal *et al.* 2022, Donnermeyer *et al.* 2022). Although these markers can give accurate information on the actual inflammatory status of a dental pulp, which results in a possible better orientation of a treatment plan, they present several issues (Rechenberg *et al.* 2016). The first is the need for supplementary laboratory analysis, requiring time and facilities; the second is that the pulp must be exposed in order to collect a proper sample, making the test intraoperative (Ballal *et al.* 2022). In this aspect, testing CRP or another systemic marker testing presents advantages as the protein's levels can be tested quickly and chairside and it is not necessary to expose the pulp to get an appropriate sample. This would be similar to tests using saliva or gingival crevicular fluid. Theoretically, if it is possible to test specific biomarkers chairside, in a lateral flow type testing platform, the clinician could be immediately directed to a more appropriate treatment (Duncan 2022). Development of chairside tests is a priority in dentistry (Garrido *et al.* 2015, Mitsakakis *et al.* 2016, Baumgartner *et al.* 2021, Ko *et al.* 2021, Paqu  *et al.* 2021, Claesson *et al.* 2022). The ability to screen patient's blood and subsequently analyse their general health in dental practice may also represent a robust and potentially popular way of assessing the patient or checking for undetected medical conditions (Miller & Westgate 2014); however, it may well be beyond the remit of the dentist (Greenberg *et al.* 2010, Greenberg *et al.* 2012, Laurence 2012). Only in specific situations it is important to obtain laboratory results before embarking on dental treatments. For example, patients using traditional anticoagulants like Warfarin, the International Normalised Ratio (INR) needs to be assessed prior to surgical procedures (Perry *et al.* 2007, Shastry *et al.* 2014). Among the medical commercial chairside tests available the QuickRead go system can accurately test the haematic levels of CRP. CRP is a protein produced in the liver that rises quickly

and dynamically during inflammation (Papaevangelou *et al.* 2006, Marcus *et al.* 2008, Schousboe & Wiese 2023).

The main goal of this preliminary study was to understand if CRP systemic chairside assessment could detect inflammation or infection of endodontic origin and furthermore whether it can assess the severity of pulpitis through a correlation between symptoms and CRP levels. Previous studies have demonstrated that the CRP increases in presence of dental and endodontic pathology (Boucher *et al.* 1967, Ren & Malmstrom 2007, Georgiu *et al.* 2019). Accurately and objectively recording the degree of inflammation caused by a dental condition will assist in the decision-making process for dental treatment plan. Studies in other medical fields have shown that CRP testing lead to a decrease use of antibiotics for the inflammation of the respiratory tract (Cals *et al.* 2010, Little *et al.* 2013), which could also help in dentistry (Stein *et al.* 2018, Buonavoglia *et al.* 2021).

As a result of rapid and dynamic alterations in CRP-levels it can be confounded by other inflammation or infective processes in the body and as a result will likely not be effective as a single test but in combination with others (Pepys & Hirschfield 2003). This represented a limitation of the current work, because it was necessary to exclude from the cohort of patients, individuals who presented chronic disease or any episode of recent acute inflammation.

5.5.2 Study-related discussion

The patients were divided in groups according to the severity of the dental condition ranging from extraoral swelling to mild or no symptoms to healthy teeth. Firstly, it was necessary to assess if dental disease was able to be detected by the chairside CRP reader at any level. There is paucity of studies investigating CRP within dentistry; however, it was important to discern if there was a difference between different levels of inflammation such as an acute episode such as irreversible pulpitis or extraoral swelling and a chronic apical periodontitis or a reversible pulpitis.

While all the control group had no increase in the CRP levels, the groups with patients experiencing severe symptomatology (G1 and 2) showed a generalised increase in the protein levels. This increase generally reflected the severity of the oral signs and symptoms (**Table 2**), indeed, cases where swelling had significantly higher levels than the cases with no swelling even if a draining sinus tract was present.

In 2007 Ren & Malmstrom demonstrated an increase in CRP-levels on dental emergency patients using a similar, but older generation, chairside testing-machine, followed by a decrease after the treatment of the dental condition (Ren & Malmstrom 2007). This particular pattern was noted in G1 (swelling) where there was a significant rise of CRP and a subsequent decrease after treatment. The cases reported show that a range of dental interventions lead to a relatively rapid decrease of the levels of CRP in this group: dental extraction or pulpotomy and drainage were used in some cases while in other a pharmacologic approach was added to the treatment.

The novelty of this work lies in the fact it does not just focus on dental infection, but investigated if levels of pulpitis can be detected with a chairside test. Although a limited number of samples was considered a limitation, the finding that all cases of irreversible pulpitis, but one exhibited an increase of CRP is a novel discovery that may suggest that the severity of pulp inflammation is related to CRP expression. Another interesting finding is the fact that the reversible pulpitis group (G3) showed increased CRP only in the two cases which showed moderate pulpitis at the time of assessment, but where the patient reported important pain in the days before their appointment. This may indicate that they could have had severe pulpitis in the days before and that would explain the rise in CRP. Unfortunately, there are not objective data that could be used to verify this hypothesis. This highlights the need for objective markers of pulpitis as subjective markers such as symptoms lack reproducibility.

Unfortunately, only a limited number of cases in the irreversible (G2) group returned for the follow up appointment. One showed a normalisation of the test results after one week, while another one had an increased value. The CRP rise could be explained by the patient fracturing his foot, which highlights the difficulties in discerning between a persistent odontogenic inflammation and another cause. Some have reported that in severe odontogenic infections requiring hospitalisation it was noted how the CRP rise is not an adequate prognostic factor, and it is better to base the prognosis on clinical evidence (Mirochnik *et al.* 2017). Even in this case, the resolution of the signs of chronic abscess like the sinus tract was regarded as a much better prognostic factor than the simple increase or decrease of a specific marker, so CRP's role in response to therapy in dentistry may be of limited value in swelling but helpful after a pulpotomy where assessment of success is more difficult.

5.6 Future work within this study

The present research represents a preliminary study that can inform a larger adequately powered study. The results support the need for a larger study. A limitation of the present study is the small sample size, however, using the results of this study and assuming a 0.4 effect size, a 5% level of significance and 80% power the groups for a subsequent would need to be as follows:

- To detect a difference between G1 (swelling) and G2 (irreversible pulpitis): will require 10 samples per group.
- To detect a statistical difference between G3 (reversible) and G4 (chronic apical abscess): 130 samples per group will be required.
- To detect a difference between the healthy control (G0) and G3 (reversible), 35 cases are required,
- Finally, to detect a difference between G0 (healthy) and G4 (chronic apical periodontitis) a sample of 45 is required.

The plan in the future is to carry out an adequately powered VPT outcome study using the calculations to assess if a point-of care CRP assay can accurately discriminate between reversible and irreversible pulpitis. In addition, the effect of VPT on the level of CRP will also be analysed.

5.7 Conclusions

CRP is a systemic inflammatory biomarker that is actively used in medicine to detect the presence and severity of a generalised inflammation or infection. Chairside/ point of care diagnostic tools such as QuickRead go were introduced in the market in order to allow a quick and reliable readings of the CRP values in order to better direct decision-making and subsequent therapy. The use of this type of diagnostic assay uncommon in dentistry and currently unavailable in endodontics. Within the limitations of this preliminary study, the results highlight not only that it is possible to dynamically detect and quantify the level of CRP caused by a dental infection with associated swelling, but also that severe pulpitis can increase the protein levels, compared with lower levels of inflammation. This study not only 'paves the way' for

larger adequately powered diagnostic accuracy studies, but offers hope that objective chairside testing for pulpal disease may be available in the future.

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Chapter 6.

General Discussion and Future Research

6.1 Discussion

Recently, due advances in technology, new biomaterials and a better understanding of pulpal repair processes, endodontics has shifted towards minimally invasive approaches. This has resulted not only in a move away from more aggressive root canal instrumentation techniques towards more anatomical preparations that preserve dentine but, also the promotion of VPT in cases of carious exposure or symptomatic pulpitis (Bürklein & Schäfer 2015, Wolters *et al.* 2017). This change has been reflected by a recent position statement by the European Society of Endodontology (Duncan *et al.* 2019).

Pulp preservation strategies and VPT are not new, with pulp capping using different techniques and materials reported since the 18th century (Damaschke 2008). Pulp capping has had ‘surges and dips’ in popularity due to uncertain success, the lack of well-defined clinical protocols, a poor understanding of pulp repair processes as well as on-specific materials that do not promote pulp healing (Glass & Zander 1949, Nyborg, 1955, Nyborg 1958, Damaschke 2008). As a result, VPT was reserved for specific cases such as traumatic exposure of the pulp or as an interim procedure before definitive root canal treatment (ESE 2006).

Although gaining in popularity there is the urgent need to development the research base and recommendations regarding management strategies in VPT in order to improve the quality of these treatments (Duncan 2022). As a result, the purpose of the current work was to analyse a new HCSC, Biodentine (Septodont, Sant-Maur-des-Fosses, France), in the context of several relevant current issues in relation to; decision-making in VPT, histological response and pulpal healing, clinical outcome of partial pulpotomy in teeth with a range of symptoms as well as the potential of molecular testing to link to pulpal symptoms and guide treatment (Figure 6.1).

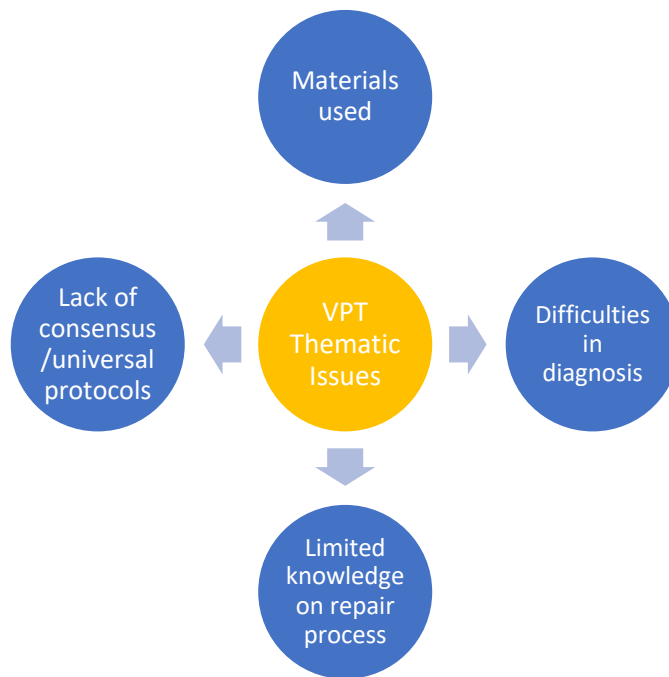


Figure 6.1. Thematic-issues affecting the use and development of VPT (Duncan 2022).

6.1.1 Materials

Historically, calcium hydroxide has been considered the gold standard VPT material, however, over the last 15 years Mineral Trioxide Aggregate (MTA) has shown improved histological and clinical outcomes (Dammaschke 2008, Nair *et al.* 2008, Hilton *et al.* 2013). MTA is not an ideal material, however, being limited by a long setting time, poor handling characteristics and the ability to discolour teeth (Yoldas *et al.* 2016, Careddu & Duncan 2018). As a result, other HCSCs, have been developed and marketed in order to try and overcome these drawbacks. Biodentine is one of the most popular ‘new’ HCSC materials and has been recommended specifically for VPT procedures (Taha & Abdulkhader 2018). As a result, a comparison between Biodentine and MTA was therefore necessary to consider whether it had similar biological responses and clinical outcomes to those attributed to MTA and whether could potentially have advantages over the existing material (Careddu & Duncan 2018)

The first Chapter of this thesis was a literature review analysing biological and clinical studies that compared MTA with Biodentine. Dental materials should be evaluated both clinically and in the laboratory: in this specific case, it was important to have information on both of these aspects. From a laboratory perspective, the

interactions between MTA, Biodentine and the pulp tissue were shown to be similar, inducing no or very little inflammation, being non-cytotoxic, as well as inducing migration, cytodifferentiation and mineralisation of dental pulp cell populations (Chang *et al.* 2014, Poggio *et al.* 2015, Collado-Gonzalez *et al.* 2017). Clinically, although there currently a limited pool of studies the comparative results are similar in terms of success rate, with many suggesting Biodentine as a valid alternative to MTA for VPT (Taha *et al.* 2022, Singh *et al.* 2023). It was observed that although both materials can be used for VPT, they have contrasting drawbacks (Bogen & Chandler 2008, Careddu & Duncan 2018). While, Biodentine presents a radiopacity below the ISO standard and is challenging to visualise, it does not induce discolouration, MTA is visible radiographically, but it discolours teeth (Tanalp *et al.* 2013, Kaup *et al.* 2015, Yoldas 2016, Parinyaprom *et al.* 2018).

It was concluded that, even though not yet ideal materials, both are suitable choices for VPT with increased success rates compared with traditional calcium hydroxide materials.

6.1.2 Decision-making in VPT

VPT includes a range of strategies used to maintain the vitality of all or part of the pulp, from techniques that avoid exposure of the pulp to full coronal pulpotomies after pulp exposure (Duncan *et al.* 2019). Although, new guidelines from the ESE and Cariology groups have highlighted the need for more minimally-invasive approaches, there remains a lack of consensus and a lack of defined protocols for practitioners to follow in VPT (Ericson 2007, Schwendicke 2016, Careddu & Duncan 2018, Duncan *et al.* 2019, Edwards *et al.* 2021).

The second Chapter of this thesis explores the nature of this observation in relation to those with further training in Endodontics. Understanding differences in clinical approaches towards caries management can assist future initiatives to target interventions and also to disseminate up to date evidence-based information. The selection of members of two endodontic societies was intentionally targeted to investigate the response of practitioners who deal daily with the exposed pulp and should be well placed to carry out VPT. This approach was also used in other survey-based investigations by the Chinese Society of Endodontology in Guangdong (Li *et al.* 2019). Within this study the age of the dentist determined by year of graduation appears to have a role on the type of approach and its

invasiveness. Other studies have also noted the tendency for a more conservative approach in younger dental groups (Weber *et al.* 2011, Croft *et al.* 2018, Careddu & Duncan 2018, Li *et al.* 2019).

The materials used for VPT were also assessed, with wide variability in the responses amongst dentists. Recent evidence has identified MTA as the gold standard material for management of the exposed pulp with additional cost-effectiveness studies highlighting its efficacy in the long term (Nair *et al.* 2008, Schwendicke *et al.* 2015). Surprisingly, calcium hydroxide in both setting and non-setting forms remained very popular and, in certain groups of dentists, the material of choice (Chisini *et al.* 2015, Li *et al.* 2019, Edwards *et al.* 2021).

The treatment chosen for the management of exposed pulp appears to be influenced by the age of the patient and by the presence or absence of symptoms. In general, the presence of symptoms and the older adult patient are viewed as barriers for VPT procedures. Indeed, although young pulps have a more diffuse blood supply, patient age and the presence of mild symptoms do not seem to significantly influence treatment outcome (Matsuo *et al.* 1996, Mente *et al.* 2014, Taha & Abdulkhader 2018, Careddu & Duncan 2021).

VPT has traditionally not be taught as systematically as root canal treatment in the undergraduate dental curriculum. Recently, the availability of clearer indications and protocols have led to protocols that detail less invasive approaches (Duncan *et al.* 2019, AAE 2021). While dissemination of this new information should be implemented, undergraduate teaching plays a critical formative role as there is a direct correlation between aggressiveness of treatment plans and University course attended (Croft *et al.* 2018). Currently, there is an insufficient VPT training provided and a call for improvement in the educational curriculum for VPT has been stressed (Croft *et al.* 2018, Duncan *et al.* 2021, Wisniewski *et al.* 2022, Duncan 2022).

6.1.3 Limited knowledge of pulpal repair processes

VPT assumes that if the irritation is removed and the tooth is adequately restored, the pulp can repair (Tronstad & Mjör 1972). The exact reparative mechanism remains to be elucidated, but has been attributed to a balance between inflammation and repair (Cooper *et al.* 2014, Galler *et al.* 2021). The secretion of a mineralised bridge over the exposed pulp tissue indicates a natural response of the damaged tissues and is generally viewed as a positive prognostic factor (Zarrabi *et al.* 2010,

Nowicka *et al.* 2015). The mechanism and cells responsible for mineralised bridge formation is therefore an issue of considerable interest within pulp biology (Bortoluzzi *et al.* 2015, Widbiller *et al.* 2016).

Chapter three of the thesis addresses histologically the response to Biodentine and MTA after experimental pulp repair capping. In keeping with other studies, MTA and Biodentine were able to produce a continuous, thick mineralised hard tissue bridge with little evidence of pulp inflammation (Nowicka *et al.* 2015). The study also sheds light on the type of cells forming hard tissue bridge. Some authors advocate that the mineralised bridge is a 'scar' formed by fibroblast cells (Ricucci *et al.* 2014b), which is at odds with the accepted theory, that proposes a cytodifferentiation of pulpal progenitor cells or DPSCs into 'odontoblast-like' cells under the influence of bioactive molecules (Sakai *et al.* 2010, Zarrabi *et al.* 2010, Duncan *et al.* 2019b). In the absence of molecular markers specific to odontoblasts, for the mineralised bridge to be considered dentine and for odontoblast-like cells to be considered responsible for its secretion, tubules must be identified histologically. Other studies report a well-organised, palisade structure of cells with accompanying tubular dentine in the bridge, however, the images in the manuscripts were not of sufficient quality to accurately judge this aspect (Zarrabi *et al.* 2010, Nowicka *et al.* 2013). Within this study selected samples showed tubularity, while others did not, highlighting that the regenerative response is not predictable and may be technically sensitive amongst other factors. The length of follow up time being 6-months may also be important as this was twice the length of time in other studies (Nair *et al.* 2008, Nowicka *et al.* 2013). As infection should not be an issue the available data though do not allow to discern the reason behind the different responses.

Histology only gives a snapshot view of the processes underlying pulp repair and it is therefore impossible to use the technique in isolation to assess cellular activity. In the future it should be used in combination with cell tracking studies and molecular assays to establish the true origin of cells contributing to bridge formation (Duncan *et al.* 2019b).

6.1.4 Diagnostic issues

One of the most challenging aspects in VPT, is the assessment of the pulp status to guide intervention.

The most widely recognized classification of pulpitis divides it in reversible and irreversible, which only recently was shown to correlate with histological findings (Ricucci *et al.* 2014). However, if a pulp is irreversibly inflamed it should not respond positively to a conservative treatment, and yet several recent cases have reported teeth with a so-called diagnosis of ‘irreversible pulpitis’ recovering after VPT (Asgary *et al.* 2015, Taha & Kahzali 2017, Careddu & Duncan 2021). The existing classification clearly requires change, as does the urgent need to develop tools which can objectively identify the threshold between a reversible and irreversible tissue damage (ESE 2019, Rechenberg & Zehnder 2020, AAE 2021).

In Chapter four, the outcome of partial pulpotomy using Biodentine was investigated by linking the preoperative signs and symptoms to a new classification system that aims to better guide the clinician when assessing pulpal inflammation (Wolters *et al.* 2017). The Wolters classification divides the inflammatory status in four (Initial, Mild, Moderate, Severe) rather than two in the traditional reversible/irreversible pulpitis nomenclature. In the current study it was demonstrated that teeth with severe pulpitis and extremely deep carious lesions were significantly more likely to have a fail after partial pulpotomies (Wolters *et al.* 2017, Demant *et al.* 2021). The study had a pragmatic design being carried out in private practice rather than in a University academic environment and used the recommended ‘enhanced’ protocol (magnification, bleeding control/disinfection with NaOCl and use of HCSCs) (Duncan *et al.* 2019).

Endodontic diagnosis relies on radiographic and clinical examination together with the assessment of the patient’s symptoms, which can of course be deceiving as they are subjective and may not be present even in cases of severe inflammation (Michaelson & Holland 2002, Zehnder & Belibasakis 2022). This makes accurate pulp assessment difficult.

A call for the development of specific objective lab tests for determining the pulp status has been made, but as yet no commercial kit is available for pulp diagnostic purposes (Duncan 2022, Zehnder & Belibasakis 2022).

Chapter five represents an attempt to ascertain the level of pulp inflammation and dental infection by assessing systemic CRP levels. CRP is a protein that increases in systemic blood when inflammation is present. It is not a specific marker for pulp inflammation so the goal was to establish a correlation between the severity of dental condition and CRP levels. Unquestionably, there are more specific markers for pulp inflammation such as members of the Interleukin (IL) family or MMP9, however these are intraoperative markers only attainable after pup exposure and require an external lab with associated additional costs (Zehnder *et al.* 2011, Zanini *et al.* 2017, Kearny *et al.* 2018, Ballal *et al.* 2022, Duncan 2022). The main advantage of the system used in this study, QuickRead go (Aidan Oy, Espoo, Finland), is the rapid results obtained chairside (2 minutes) that are compatible with clinical practice. The preliminary results are encouraging, with an increase in the CRP levels in cases of swelling as well as irreversible/severe pulpitis. Further study is required; however, this test could potentially orientate the decision-making process towards a root canal treatment or VPT procedure. Even if the preliminary results of this research are positive, at present the systemic testing methodology possesses many limitations with false positives and negatives likely, so in the future the aim should be to develop specific user-friendly tests with locally produced biomarkers (Duncan 2022).

6.2 Future research

This work has highlighted several areas for future study. Although, Wolters classification shows benefits over traditional used diagnostic nomenclatures, it relies on patient symptoms, which are by definition subjective. Furthermore, the study performed here was based on the work of only one operator, in the future larger cohorts of patients, and ideally multicentre studies are needed in order to definitively assess the best way to clinically assess the pulp to guide the clinical decision-making process.

The development of precise diagnostic chairside test to assess pulp inflammation continues. CRP testing has shown potential in detecting high levels of inflammation, however, Chapter five only represents a preliminary study. The power calculation highlights the size of study that is required to adequately assess the utility of CRP. Notably, the simplicity of CRP testing puts an accent on the need for

future research to develop complimentary tests for specific markers with the view to develop chairside tests that can be as easy to use as the CRP used in the chapter.

The histologic assessment of hard tissue bridge formation has highlighted some unresolved issues. Although there are multiple ethical challenges in using human teeth, sampling third molars and premolar teeth for future research is important to improve the quality of information. Notably, VPT is not performed on 'sound' teeth and development of a pulpitis model (animal, bioprinted or human) remains a priority for testing of dental material in this area.

Predictable mineralised bridge development and the response of the pulp tissue appear to be dependent on the length of follow up, with VPT clinical studies highlighting complications such as root resorption and pulp canal obliteration. Long-term clinical assessment on the outcome of pulpotomies should be a key focus area for future research as at present many of the comparative studies are short-term.

Biodentine and partial pulpotomy have shown a high success rate when combined with Wolters classification at least after 1-year. However, there remains many unexplored areas. For example, in Chapter 4, all the cases were treated with a partial pulpotomy, however it is unknown if a diagnosis of severe pulpitis would have responded better a full coronal pulpotomy. Equally, mild or moderate pulpitis may have succeeded even after a less aggressive treatment such as pulp capping. Adequately powered, robust, prospective comparative trials investigating a range of VPT treatment options and relating them to a range of diagnoses is important moving forward.

The international survey contained in Chapter 2 highlighted a lack of consensus on the most appropriate approach to deep caries management, a finding that is in line with the results of other studies (Chisini *et al.* 2015, Li *et al.* 2019). Improved dissemination of information is indeed needed, starting from undergraduate curriculum development. In the first instance the nature of undergraduate teaching carried out in regards to VPT could be ascertained across Europe or globally. The influence of lab training and dedicated preclinical sessions on student knowledge has already been highlighted (Chevalier *et al.* 2021).

6.3 Conclusions

This thesis addressed several different and topical issues in VPT as well as the ability of Biodentine to promote pulp healing. Within the limitations of this series of linked studies it can be concluded that Biodentine is a suitable material for carrying out VPT and is comparable with the current gold standard MTA. This is based on a thorough analysis of the literature, as well as primary clinical and histological studies that showed positive outcomes using Biodentine, and analysed the role of various prognostic factors in determining the outcome of partial pulpotomy. The use of CRP as marker for pulpal inflammation showed promising initial results, that could in selected cases be developed as an objective non-invasive diagnostic aid for VPT. The current work also highlights the importance of and current inconsistencies in education in the VPT area. The heterogeneity of responses among different clinicians indicates the urgent need for focussed dissemination of new evidence and clinical protocols in a coordinated manner.

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Appendices

Appendix I Ethical approval

THIS DOCUMENT MUST NOT BE USED FOR
ANY OTHER THAN THE GOVERNMENT PURPOSES

SIR/AM/NCB Research Ethics Committee Secretariat
Claire Hurin Ph: 4142199
email: clh@amncb.ie

Dr. Hal Duncan
Lecturer/Consultant in Endodontics
Division of Restorative Dentistry & Periodontology
Dublin Dental University Hospital
Trinity College Dublin
Lincoln Place
Dublin 2
Ireland

25 April 2017

**Re: Histological investigation of the dental restorative cements, biodentine and
proroot mta, as vital pulp treatment materials**

REC Reference: 2017/04/01
(Please quote reference on all correspondence)

Dear Dr Duncan,

The Committee decided to give ethical approval to this study subject to the following conditions:

- The committee are querying why the cut-off for entry into the study is 60 and those over 60 are excluded.
- Part D3 of the application states that *'the potential benefit (of involvement) is that the dental materials - Biodentine and ProRoot MTA – provide superior reparative results for vital pulp therapy this will lead to clinical improvements for patients. Specifically in the pulp capping procedure that will be: more predictable (so more root canals treatment could be avoided), quicker and cost effective.'* This is not entirely accurate though as regardless of the response within the individual themselves the tooth will be **extracted**. It would be more accurate to state that the only benefit will be improving the wider knowledge of the cellular response of teeth to these treatments.
- The PIL does not describe the procedure involved in filling the teeth. The application states that PO analgesia may be required afterwards but this is not mentioned either. It could also be construed as misleading in that potential participants may be led to think that the aim of the study is to assess effectiveness of the treatment rather than the cellular response under light microscopy. The committee feel that the PIL should outline this more clearly.
- The PIL for 18 years is almost identical to the general PIL. The committee feel that this should be redesigned in a more child friendly manner.



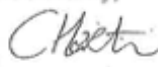
**THE ADELAIDE & MEATH
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- The committee note teeth will be extracted 6 months after filling. In those who are having extraction of sound teeth for orthodontic procedures, e.g. braces, does this fit in with the usual plan in these patients? Is 6 months longer than one would wait usually prior to having the braces inserted? Can this be explained?
- The PIL should clearly outline how long the procedure will take.
- The consent form should include consent to take x-rays

Yours sincerely,

p.p. 

Dr. Peter Lavin
Chairman, SJH/AMNCH Research Ethics Committee

The SJH/AMNCH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.

Appendix II: Published article in British Dental Journal 2018. How does the pulpal response to Biodentine and ProRoot mineral trioxide aggregate compare in the laboratory and clinic?

RESEARCH

How does the pulpal response to Biodentine and ProRoot mineral trioxide aggregate compare in the laboratory and clinic?

R. Careddu¹ and H. F. Duncan^{*1}

Key points

Represents a timely evidence-based analysis of whether Biodentine is more suitable for pulpal applications than the current 'gold-standard' ProRoot mineral trioxide aggregate (MTA).

Highlights that although there is a lack of clinical studies comparing the materials, Biodentine appears to perform well compared with ProRoot MTA.

Suggests that biologically the materials stimulate similar pulpal responses, however, ProRoot MTA has a better radioopacity, but longer setting time than Biodentine.

Suggests that Biodentine discolours teeth less than ProRoot MTA.

Introduction Vital-pulp-treatment (VPT) procedures are biologically-based therapies aimed at preserving pulp-vitality. Historically, calcium-hydroxide was the 'gold-standard' VPT-material, however, recently a tricalcium-silicate material, ProRoot-mineral-trioxide-aggregate (MTA) has emerged as the material of choice for pulpal-application in permanent teeth. ProRoot-MTA also has drawbacks, including long-setting time, handling-difficulties and post-application crown-discolouration. Relatively recently, another tricalcium-silicate material, Biodentine, has been indicated for VPT. **Objectives** The aim of this review was to compare from relevant laboratory, biological and clinical-studies whether Biodentine is a better material than ProRoot-MTA for use in pulpal applications in permanent teeth. **Methods** A comprehensive MEDLINE search was used to identify manuscripts, which directly compared the materials in relation to dental-pulp applications. **Results** Literature analysis demonstrated that most of the comparative research is *in vitro* cell-culture studies demonstrating a wide range of methodologies and conflicting findings. In contrast there is a paucity of clinical studies comparing the pulpal response to the materials in permanent teeth. Histological evidence suggests similar pulpal responses, while clinical outcome studies, although preliminary, show equally high material efficacy. Other physical characteristics diverge with Biodentine setting quicker and staining less than ProRoot-MTA, however, the radiopacity of Biodentine is below testing standards making identification difficult. **Conclusions** Biodentine does present an evidence-based biologically-based alternative VPT material to ProRoot-MTA. Future research should be directed at long-term clinical outcome studies and the interaction of Biodentine with the dentine matrix.

Introduction

Exposure of the pulp is a common clinical occurrence due to trauma, caries or iatrogenic reasons. The pulp is protected by an outer shell of enamel and dentine; when this protection is breached the pulp is colonised by microbes with the simulation of a pulpal inflammatory response.¹ If the pulpitis is untreated, ultimately, pulp necrosis occurs.² Pulp vitality can be maintained if the irritant stimulus is removed and the tooth restored.¹ The

importance of retaining all or part the pulp has been highlighted as it is more conservative, less technically demanding and more biologically-based compared with pulpectomy procedures.^{3,4}

The aim of the vital pulp treatment (VPT) is to preserve the vitality and function of the pulp, while stimulating hard tissue repair processes.^{5,6} VPT encompasses procedures with no pulp tissue removal, ie pulp capping (indirect and direct) as well as techniques with varying degrees of pulp excision, that is, pulpotomy (partial or complete).⁵ Several factors influence the success of VPT procedures including the level of pre-operative pulp inflammation,⁷ the post-operative prevention of further microbial insult⁸ and the material used to interface with the biological tissue.⁹ Historically, VPT was considered as an unpredictable procedure.¹⁰ Calcium hydroxide has

been the material of choice for many years,^{11,12} but is limited by poor mechanical properties, non-specific mechanism of action,¹³ absence of sealing properties¹⁴ and incomplete hard tissue formation over the pulp wound.^{8,9} Developments in our understanding of pulp defence mechanisms⁴ and the advent of tricalcium silicate materials have created new opportunities and enthusiasm for VPT.¹⁵ The tricalcium silicate, mineral trioxide aggregate (MTA), has demonstrated superior histological^{9,16} and clinical outcome compared with calcium hydroxide in VPT procedures.¹⁷

The original, most researched and, within the United Kingdom, the market leading commercial MTA product, ProRoot MTA (Dentsply Sirona, Ballaigues, Switzerland), is not an ideal material with several disadvantages highlighted including a long-setting time,

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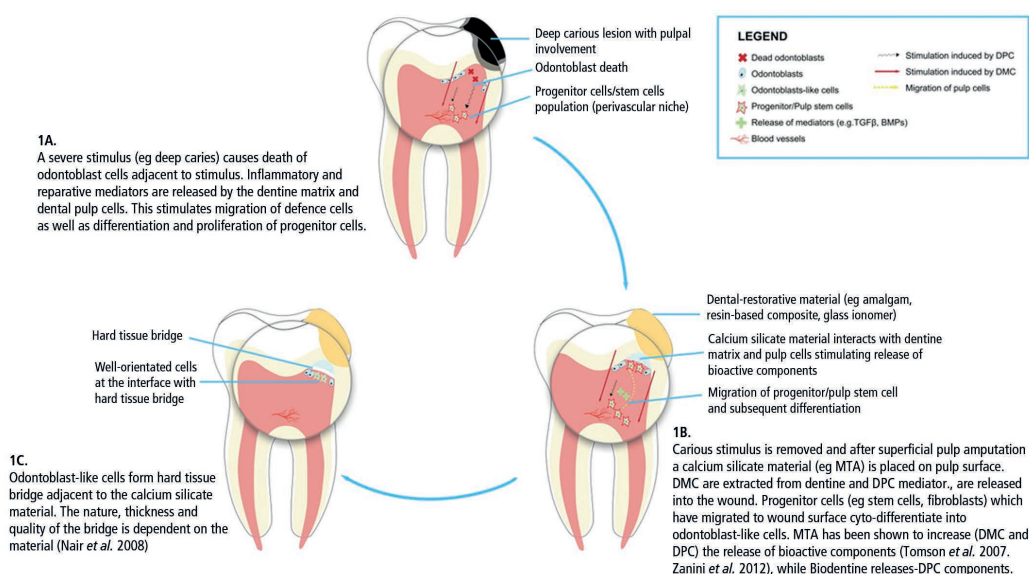


Fig. 1 Schematic theoretical representation of the process of reparative dentinogenesis after a VPT procedure using calcium silicate cement

tooth discolouration and high-cost.^{17–20} New generation tricalcium silicate-based cements have been introduced, which attempt to address many of the shortcomings of ProRoot MTA. Biodentine (Septodont, Saint-Maur-des-France Cedex, France) is a tricalcium silicate cement marketed specifically for use in VPT procedures. Publications have highlighted its positive effects on pulp mineralisation processes, shorter setting-time, improved mechanical resistance and ease of use compared with ProRoot MTA for use in VPT procedures.^{21–24}

ProRoot MTA is chemically composed of Portland cement with bismuth oxide added as a radiopacifier.²⁵ There are now a range of other commercial MTA products available, including Angelus MTA and Angelus HP (The Angelus, Londrina, Brazil), MM-MTA (Micro-Mega, Besancon, France), Retro/ Ortho MTA (BioMTA, Seoul, Republic of Korea) and MTA Plus (Avalon Biomed, Houston, USA), which share the presence of di and tricalcium silicates, as well as radiopacifiers.²⁶ These products vary in their radiopacifiers (bismuth oxide, zirconia, calcium tungstate and tantalite), but also have minor differences including the presence of calcium sulphate, calcium carbonate and phyllosilicates.²⁶ Biodentine is similar to ProRoot MTA, but does not contain calcium aluminate, calcium sulphate or bismuth oxide, while zirconium oxide is the radiopacifier.²⁶

The absence of bismuth oxide in Biodentine is significant not only for radiopacity, but also for other properties such as staining potential, setting time and micro-hardness.^{27,28} Due to differences in chemical formulation a recent review has suggested that these materials could be grouped as 'bioactive endodontic cements (BECs)' rather than calcium silicate materials.²⁹

From a reparative perspective, the role of the odontoblast cell is critical, forming the primary dentine during development, secondary dentine throughout the life of the tooth and when challenged the deposition of tertiary dentine in an attempt to 'wall off' the irritation.³⁰ Depending on the severity of the stimulus, tertiary dentine deposition can be reactionary or reparative.³¹ Reactionary dentine being formed by an upregulation of surviving post-mitotic odontoblasts exposed to the influence of relatively mild stimuli, while reparative dentine is formed generally after a stronger stimuli, which has led to death of the odontoblast cell.^{31,32} At a cellular level reparative dentine is believed to be produced following cyto-differentiation of pulpal progenitor cells and the formation of a new generation of odontoblast-like cells.^{31,32} Alternative theories suggest that other cells such as fibroblasts may cyto-differentiate to produce the mineralised tissue.³³ The cellular differentiation is likely to be guided by the influence of growth factors and bioactive molecules derived

from both the dentine and the pulp matrix.^{34,35} Although reactionary and reparative dentinogenesis are for didactic purposes considered separately, in the event of pulp exposure reparative dentine will form the mineralised bridge, while at the periphery of the cavity reactionary dentinogenesis will occur simultaneously.³

Inflammation is also an important stimulus to drive the reparative process with odontoblasts involved in initial sensory stimulus transmission from the dentine and also an immunocompetent role in cellular defence.^{36,37} Indeed the low level release of inflammatory mediators such as interleukins (for example, IL-1α, -1β, -2, -6) in mineralising cells in contact with MTA supports the need for a degree of inflammation in promoting regenerative processes.³⁸ A wide range of bioactive dentine matrix components are 'fossilised' in the mineralised tissue being released into pulp during caries or trauma.^{3,39,40} Demineralisation of dentine and indeed contact with materials such as Pro Root MTA, calcium hydroxide and other agents release a plethora of bioactive molecules including members of the TGF-β superfamily which can stimulate a complex cascade of molecular events that promote pulp repair.^{34,41–43} Notably, no evidence suggests that Biodentine is capable of sequestering dentine matrix components so comparative bioactivity analysis with ProRoot MTA is not possible.

Table 1 Biological laboratory *in vitro* studies comparing the efficacy of ProRoot MTA (PrMTA) and Biodentine (BD) within the same publication

Reference	Methodological approach and species	Materials investigated	Variables analysed	Principal results
Pérard <i>et al.</i> 2013 ⁹⁹	<i>In vitro</i> - Two murine pulpal cell-lines (MDPC-23, OD-21)	- BD - PrMTA	- Cell viability (acid phosphatase assay) - Gene expression (qRT-PCR) OP, ALP, Runx2, Col1a1 - Phenotypic analysis (SEM)	- MDPC-23 cells cultured with PrMTA superior viability to BD at 7d - PrMTA and BD reduced cell proliferation compared with control - Col1a1 expression reduced in PrMTA compared with BD
Corral Nunez <i>et al.</i> 2014 ¹⁰¹	<i>In vitro</i> - Murine embryo fibroblast cell line (3T3)	- BD - PrMTA - GIC	- Cell viability (Alamar blue assay) and proliferation - Cell morphology in response to dental material (SEM) - Cytokine expression (Semi Quantitative RT-PCR) for IL1β, IL6	- Similar cell viability for BD and PrMTA at 72h, reduced for GIC. - BD showed increased cell growth and development (extended cellular processes) in comparison with PrMTA and GIC - No difference in IL-1β expression, all groups. PrMTA showed increased IL6 expression at 3 hrs compared with BD, but not at 24 hrs
Widbiller <i>et al.</i> 2015 ¹⁰	<i>In vitro</i> - Human DPSCs	- BD - PrMTA - GIC	- Cell viability (MTT assay) - ALP activity - Gene expression (Quantitative RT-PCR) for ALP, Runx2, Col1a1, DSPP, RPS18	- BD showed higher cell viability than control. PrMTA had reduced viability at 7d; however, at 10d and 14d the difference not significant, instead GIC significantly lower cell viability - Reduction of ALP activity with PrMTA and BD compared to control - Gene expression of Col1a1 initially increased with PrMTA before decline. DSPP expression increased in BD and PrMTA. Expression of Runx2 down-regulated.
Poggio <i>et al.</i> 2015 ⁷²	<i>In vitro</i> - Murine pulpal cell-line (MDPC-23)	- Dycal - Calcicur - Calcimol - TheraCal - PrMTA - AMTA - BD	- Cytotoxicity - Cell viability (MTT, Alamar blue assay) - Cell damage/apoptosis (Confocal Laser Scanning Microscope)	- BD, AMTA, PrMTA not cytotoxic; Calcimol, Calcicur, TheraCal slightly more cytotoxic than control; Dycal high cytotoxicity. - BD cell viability remains stable, PrMTA and AMTA showed slightly decreased viability at 72h. Calcicur maintained discrete cell viability while Calcimol and TheraCal have important reduction at 72h. Dycal less cell viability
Margunato <i>et al.</i> 2015 ⁷¹	<i>In vitro</i> - Stem cells isolated from human mandibular bone marrow (hBMSCs)	- BD - PrMTA - MM-MTA	- Cell viability (ELISA) - ALP activity - Mineralisation (Alizarin Red) - Gene expression (qRT-PCR) for Col1a1, ON, Runx2	- Similar cell viability in all groups - ALP activity increased in both PrMTA species in relation to control group, BD decreased - Increased gene expression of Runx2 and ON compared to control group. - No significant differences in mineralisation between materials

Abbreviations: ALP = Alkaline phosphatase, AMTA = Angelus-MTA, Col1a1 = Collagen type I alpha 1 chain, DSPP = Dentin sialophosphoprotein, GIC = Glass ionomer, IL = Interleukin, IRM = Intermediate Restorative Material, PrMTA = ProRoot MTA, OP = Osteopontin, Runx2 = Runt-related transcription factor 2, RPS18 = Ribosomal protein S18

Using biologically-based dental materials that promote the healing process are paramount in vital pulp therapy and other irrigation strategies are being developed which enhance the release of bioactive molecules from dentine to improve the wound repair.^{44,45} Over the last ten years, tricalcium silicate materials, including ProRoot MTA and Biodentine, have demonstrated superior histological response compared with the gold standard material calcium hydroxide in VPT.^{9,24} It is assumed that tricalcium silicates work more efficiently than calcium hydroxide and in a similar way to each other (Fig. 1).^{13,24} ProRoot MTA and Biodentine have shown the capacity to promote the early synthesis of reparative dentine, perhaps due to enhanced regulation of TGF- β 1, osteopontin and other growth factors.^{23,24}

The aim of this review was to comprehensively analyse only the laboratory and clinical studies which compared the most commonly used and researched tricalcium silicate materials, ProRoot MTA and Biodentine, specifically in relation to the dental pulp.

Review

Search strategy

A comprehensive MEDLINE search up to November 2017 was conducted using medical subject headings (MeSH) in combination with 'and' or 'or'. The major MeSH terms searched were 'Endodontics', 'Dental Pulp Disease', 'Dental Pulp Test', 'Pulpotomy' and 'Dental Pulp Capping'. In addition, the following terms were added 'Biodentine', 'Mineral Trioxide Aggregate', 'MTA', 'calcium silicate cements', 'calcium silicate materials', 'calcium silicates' as well as 'di and tricalcium silicates'. Bibliographies of all relevant papers and previous review articles were hand-searched for further studies of relevance. Any relevant work published in the English language and presenting pertinent information related to this review was considered for inclusion. Titles were excluded if they were conference reports. Laboratory (biocompatibility, biological, material aspects) studies were considered if Biodentine and MTA were compared within

the same study. Clinical studies focused on comparative responses in permanent teeth.

How are the success of ProRoot MTA and Biodentine in VPT procedures compared clinically?

The aim of VPT is to maintain the vitality of the tooth in the absence of an apical radiolucency and, in case of immature teeth, achieve apexogenesis. Therefore, outcome should be assessed from both a clinical and radiographic perspective.^{5,46} Clinically, the treated tooth should respond positively to pulp sensibility testing (if possible to assess) and not present any sign or symptoms such as pain, tenderness to percussion or presence of a sinus tract.^{6,47} The radiographic examination should demonstrate no signs of apical periodontitis, no evidence of internal resorption, present evidence of mineralised bridge formation and continued root development in immature teeth.^{6,48,49} A follow-up appointment no longer than six months postoperatively is recommended and a recall of one year; long-term follow up is also

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Pérard <i>et al.</i> 2013 ⁶⁹	• <i>In vitro</i> • Two murine pulpal cell-lines (MDPC-23, OD-21)	• BD • PrMTA	• Cell viability (acid phosphatase assay) • Gene expression (qRT-PCR) OP, ALP, Runx2, Col1a1 • Phenotypic analysis (SEM)	• MDPC-23 cells cultured with PrMTA superior viability to BD at 7d • PrMTA and BD reduced cell proliferation compared with control • Col1a1 expression reduced in PrMTA compared with BD
Corral Nunez <i>et al.</i> 2014 ¹⁰¹	• <i>In vitro</i> • Murine embryo fibroblast cell line (3T3)	• BD • PrMTA • GIC	• Cell viability (Alamar blue assay) and proliferation • Cell morphology in response to dental material (SEM) • Cytokine expression (Semi Quantitative RT-PCR) for IL1β, IL6	• Similar cell viability for BD and PrMTA at 72h, reduced for GIC. • BD showed increased cell growth and development (extended cellular processes) in comparison with PrMTA and GIC • No difference in IL-1β expression, all groups. PrMTA showed increased IL6 expression at 3 hrs compared with BD, but not at 24 hrs
Widbiller <i>et al.</i> 2015 ⁷⁰	• <i>In vitro</i> • Human DPSCs	• BD • PrMTA • GIC	• Cell viability (MTT assay) • ALP activity • Gene expression (Quantitative RT-PCR) for ALP, Runx2, Col1a1, DSPP, RPS18	• BD showed higher cell viability than control. PrMTA had reduced viability at 7d; however, at 10d and 14d the difference not significant, instead GIC significantly lower cell viability • Reduction of ALP activity with PrMTA and BD compared to control • Gene expression of Col1a1 initially increased with PrMTA before decline. DSPP expression increased in BD and PrMTA. Expression of Runx2 down-regulated.
Poggio <i>et al.</i> 2015 ⁷²	• <i>In vitro</i> • Murine pulpal cell-line (MDPC-23)	• Dycal • Calcicur • Calcimol • TheraCal • PrMTA • AMTA • BD	• Cytotoxicity • Cell viability (MTT, Alamar blue assay) • Cell damage/apoptosis (Confocal Laser Scanning Microscope)	• BD, AMTA, PrMTA not cytotoxic; Calcimol, Calcicur, TheraCal slightly more cytotoxic than control; Dycal high cytotoxicity. • BD cell viability remains stable, PrMTA and AMTA showed slightly decreased viability at 72h, Calcicur maintained discrete cell viability while Calcimol and TheraCal have important reduction at 72h. Dycal less cell viability
Margunato <i>et al.</i> 2015 ⁷¹	• <i>In vitro</i> • Stem cells isolated from human mandibular bone marrow (hBMSCs)	• BD • PrMTA • MM-MTA	• Cell viability (ELISA) • ALP activity • Mineralisation (Alizarin Red) • Gene expression (qRT-PCR) for Col1a1, ON, Runx2	• Similar cell viability in all groups • ALP activity increased in both PrMTA species in relation to control group, BD decreased • Increased gene expression of Runx2 and ON compared to control group. • No significant differences in mineralisation between materials

Abbreviations: ALP = Alkaline phosphatase, AMTA = Angelus-MTA, Col1a1 = Collagen type I alpha 1 chain, DSPP = Dentin sialophosphoprotein, GIC = Glass ionomer, IL = Interleukin, IRM = Intermediate Restorative Material, PrMTA = ProRoot MTA, OP = Osteopontin, Runx2 = Runt-related transcription factor 2, RPS18 = Ribosomal protein S18

Using biologically-based dental materials that promote the healing process are paramount in vital pulp therapy and other irrigation strategies are being developed which enhance the release of bioactive molecules from dentine to improve the wound repair.^{44,45} Over the last ten years, tricalcium silicate materials, including ProRoot MTA and Biodentine, have demonstrated superior histological response compared with the gold standard material calcium hydroxide in VPT.^{9,24} It is assumed that tricalcium silicates work more efficiently than calcium hydroxide and in a similar way to each other (Fig. 1).^{13,24} ProRoot MTA and Biodentine have shown the capacity to promote the early synthesis of reparative dentine, perhaps due to enhanced regulation of TGF- β 1, osteopontin and other growth factors.^{23,24}

The aim of this review was to comprehensively analyse only the laboratory and clinical studies which compared the most commonly used and researched tricalcium silicate materials, ProRoot MTA and Biodentine, specifically in relation to the dental pulp.

Review

Search strategy

A comprehensive MEDLINE search up to November 2017 was conducted using medical subject headings (MeSH) in combination with 'and' or 'or'. The major MeSH terms searched were 'Endodontics', 'Dental Pulp Disease', 'Dental Pulp Test', 'Pulpotomy' and 'Dental Pulp Capping'. In addition, the following terms were added 'Biodentine', 'Mineral Trioxide Aggregate', 'MTA', 'calcium silicate cements', 'calcium silicate materials', 'calcium silicates' as well as 'di and tricalcium silicates'. Bibliographies of all relevant papers and previous review articles were hand-searched for further studies of relevance. Any relevant work published in the English language and presenting pertinent information related to this review was considered for inclusion. Titles were excluded if they were conference reports. Laboratory (biocompatibility, biological, material aspects) studies were considered if Biodentine and MTA were compared within

the same study. Clinical studies focused on comparative responses in permanent teeth.

How are the success of ProRoot MTA and Biodentine in VPT procedures compared clinically?

The aim of VPT is to maintain the vitality of the tooth in the absence of an apical radiolucency and, in case of immature teeth, achieve apexogenesis. Therefore, outcome should be assessed from both a clinical and radiographic perspective.^{5,46} Clinically, the treated tooth should respond positively to pulp sensibility testing (if possible to assess) and not present any sign or symptoms such as pain, tenderness to percussion or presence of a sinus tract.^{6,47} The radiographic examination should demonstrate no signs of apical periodontitis, no evidence of internal resorption, present evidence of mineralised bridge formation and continued root development in immature teeth.^{6,48,49} A follow-up appointment no longer than six months postoperatively is recommended and a recall of one year; long-term follow up is also

Table 3 Comparison of practical issues and drawbacks of Biodentine (BD) and ProRoot MTA (PrMTA) investigated *in vivo* and *in vitro*

Issue	MTA	Biodentine	References
Discolouration	- PrMTA reported to cause crown discolouration. - Related to the presence of bismuth oxide	- No or less discolouration reported. - Generally less compared with Pr MTA. - Only one study reports more discolouration than PrMTA (Beatty & Svec 2015)⁹¹	Bortoluzzi <i>et al.</i> 2007, Lenherr <i>et al.</i> 2012, Felman & Parashos, 2013, Marciano <i>et al.</i> 2014, Camilleri 2015, Kohli <i>et al.</i> 2015, Vallés <i>et al.</i> 2015, Beatty & Svec 2015, Keskin <i>et al.</i> 2015, Yoldas <i>et al.</i> 2016 ^{8,20,27,85–91}
Setting time	Long setting time between 15 min (MTA Angelus) and >2 hours (ProRoot MTA)	- BD manufacturer setting time of 12 minutes - Reported up to to sevenfold longer - No significant difference between BD and MTA Angelus reported	Torabinejad <i>et al.</i> 1995, Kogan <i>et al.</i> 2006, Wiltbank <i>et al.</i> 2007, Grech <i>et al.</i> 2013, Kaup <i>et al.</i> 2015 ^{76,94,96–98}
Handling	- Handling is considered difficult because of formulation and difficulties in compacting - Can mix to desired consistency	- BD is generally considered easier to use and mix - Cannot mix to desired or bespoke consistency	Kogan <i>et al.</i> 2006, Wiltbank <i>et al.</i> 2007, Ma <i>et al.</i> 2011, Butt <i>et al.</i> 2014 ^{94,98–100}
Cost	High cost	High cost	Chin <i>et al.</i> 2016 ⁹
Radiopacity	Good radiopacity above ISO radiopacity standards	- BD difficult to visualise as radiopacity similar to dentine - Below ISO standards for radiopacity of dental materials	Tanalp <i>et al.</i> 2013, Kaup <i>et al.</i> 2015, Lucas <i>et al.</i> 2017 ^{76,92,93}

inadequate to potentially recognise any statistical difference between the materials.⁶¹

From the limited evidence available, the success rate for VPT procedures is high with both materials, however, existing studies are preliminary, limited by small numbers and short follow-up period, and only investigate pulp capping, not pulpotomy procedures. Other important clinical variables including calcium silicate-associated discolouration was only reported in one study.⁶¹

How are Biodentine and ProRoot MTA compared in the laboratory with respect to the pulp?

The majority of research comparing the histological response to VPT materials is carried out in sound teeth, which limits confounding factors and enables comparison, but decreases the clinical relevance of the study.^{33,62} Recruiting human subjects who are prepared to consent to a VPT procedure on a sound tooth before later scheduled extraction is challenging and as a result the majority of *ex vivo* studies are carried out using animal models, which have their own limitations.^{63–65} Several qualitative and quantitative *ex vivo* human studies comparing a range of VPT materials have been published.^{9,16,23,66,67} These studies should carefully evaluate histologically with serial sections the soft tissue response – inflammation, necrosis, odontoblast-like cell presence, bacterial infiltration, pulpal calcifications – as well as hard tissue bridge formation, recording its thickness, homogeneity and the cellular interface with the materials.^{9,23} Cone-beam computed tomography (CBCT) has been used to assess the hard tissue bridge before

the histological exam.⁶⁷ One recent histological study compared Biodentine and ProRoot MTA (as well as calcium hydroxide and composite resin) and concluded that both ProRoot MTA and Biodentine materials induced a similar mineralised bridge formation at the pulp-material interface. The bridges from Biodentine and ProRoot MTA were homogeneous and with minimal tunnel defects.⁶⁷

In addition to a limited number of *ex vivo* studies, other *in vitro* biological studies have compared Biodentine with ProRoot MTA within the same study (Table 1). There have been cell culture studies using primary cells (rodent or human dental pulp cells) or odontogenic cell lines (for example, MDPC–23), which analyse cellular processes such as cytotoxicity, migration, viability, differentiation, inflammation and mineralisation (Table 1).^{21,68–71} Although these laboratory studies represent the majority of research on this subject, unfortunately, it is not possible to conclude from these biological studies comparing Biodentine and ProRoot MTA that one material is consistently better than the other (Table 1).

How do Biodentine and ProRoot MTA compare to the criteria for an ideal VPT material?

If additional evidence is collated from clinical, *ex vivo* and *in vitro* studies and analysed with respect to Ingle's criteria, (Table 2) it is evident that Biodentine compares equally to ProRoot MTA for reparative dentine formation, maintaining pulp vitality, anti-bacterial properties, long-term insolubility and preventing coronal leakage.^{23,50,67,73–77} Notably, Biodentine

performed consistently better than ProRoot MTA in adherence to dentine measured by push-out testing, however, ProRoot MTA was more radiopaque than Biodentine, which consistently fell below ISO standards (Table 2).^{76,78,79} Certainly within these studies Biodentine compares favourably to ProRoot MTA and both tricalcium silicate materials are superior to other materials tested including glass ionomer cement (GIC), Dycal (calcium hydroxide), Theracal (resin-modified tricalcium silicate), Calcimol (calcium hydroxide), Calcicur (calcium hydroxide) and zinc-oxide eugenol cements such as IRM.

How are the practical aspects of Biodentine and MTA compared in the laboratory and clinic?

Crown discolouration as a result of VPT does not signify a failure of the procedure, however, patients are understandably concerned about potential shade change and staining issues as a result of any dental treatment.⁶ Comparative studies carried out on extracted teeth (animal and human) have concluded that Biodentine has less staining potential compared with ProRoot MTA (Table 3).^{80–83}

Initially, ProRoot MTA was only available in a grey formulation, which was later superseded by a white formula for use in aesthetic areas. White ProRoot MTA has been demonstrated to discolour teeth, particularly in the presence of sodium hypochlorite and blood.^{18,84,85} This discolouration has been attributed to the radiopacifier bismuth oxide.²⁷ The anaerobic environment of the root canal system and light irradiation have been reported to enhance

formation of stains, while presence of oxygen reduces discolouration.⁸⁰ Biodentine contains zirconium oxide rather than bismuth oxide, which has been attributed to reduced colour change in Biodentine treated teeth.^{86–88} While, it is acknowledged that tooth discolouration remains possible with all tricalcium silicate materials, particularly in the presence of blood products, in general Biodentine induced staining significantly less than ProRoot MTA or MTA Angelus.^{89,90} Indeed, only one study reported that Biodentine induced a higher incidence of stains than ProRoot MTA (Table 2).⁹¹ Although the absence of the radiopacifier bismuth oxide in Biodentine has been attributed to the lack of discolouration, the radiopacity of Biodentine is consistently reported as being below ISO 6876 standard of 3 mm of equivalent aluminium for a 1 mm thick sample, and indeed cannot be clearly visualised as distinct to dentine by the operator.^{76,92,93}

An important practical limitation of ProRoot MTA is the time taken to set.^{94,95} This process takes several hours. As a result new calcium silicates such as Biodentine were developed to reduce this time with a setting time of 12 minutes claimed by the manufacturers. This shortened setting time is not predictable, however, with times of up to 100 minutes reported.^{76,96} Direct comparison of setting times between studies is problematic to compare as the ISO methods require careful training and execution. For instance Biodentine may not set as quickly as the manufacturer claims, but it does set faster than ProRoot MTA.^{76,94,97,98} One study reported a similar setting time between that MTA Angelus and Biodentine.⁹⁹

ProRoot MTA has been reported to be difficult to handle, while Biodentine is considered easier to mix and use.^{94,98–100} Many dentists avoided using any calcium trisilicate materials in VPT, citing cost, lack of training and difficulties in handling.¹⁹

Conclusions

The choice of material for pulpal application is critical with tricalcium silicates established as the gold standard. Comparative analysis of Biodentine and ProRoot MTA within this review revealed a plethora of biological *in vitro* studies, with a range of methodologies and conflicting findings, however, both materials' pulpal interaction is consistently better than other materials investigated. There are a small number of short-term, but no long-term clinical studies comparing the

pulpal response to the materials in permanent teeth. Of the available evidence, histological evaluation highlights a similar response, while VPT outcome studies, although preliminary and under-powered, show high material-efficacy. Currently, practical issues consistently separate the materials, with ProRoot MTA causing more tooth staining, but having greater radiopacity than Biodentine. In the future, there is an urgent need for well-designed, adequately-powered, clinical trials in this area, as well as improved functional understanding of the interaction of Biodentine with the dentine-matrix.

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Appendix III: Published article in International Endodontic Journal 2020. The management of deep carious lesions and the exposed pulp amongst members of two European endodontic societies: a questionnaire-based study

The management of deep carious lesions and the exposed pulp amongst members of two European endodontic societies: a questionnaire-based study

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Abstract

Careddu R, Plotino G, Cotti E, Duncan HF. The management of deep carious lesions and the exposed pulp amongst members of two European endodontic societies: a questionnaire-based study. *International Endodontic Journal*, 54, 366–376, 2021.

Aims To investigate and compare views on management of deep caries and the exposed pulp by Endodontic Society members in Ireland (Irish Endodontic Society [IES]) and Italy (Accademia Italiana di Endodonzia [AIE]). Further aims were to investigate the influence of patient-related factors (age, symptoms) and operator-related factors (material choice, antibiotics) on management.

Methodology A structured online questionnaire containing two cases (an 18- and 45-year-old) and two scenarios ($\pm$ mild symptoms), including history and radiograph, was sent to IES and AIE members. The answers were analysed using chi-square and Fisher's exact test ($P < 0.05$).

Results In total, 120 dentists participated, representing 49% of the AIE and 48% of the IES membership. Age distribution was similar between the societies; however, most AIE members had no further qualifications (63%), while IES respondents generally had a postgraduate endodontic qualification (71%). AIE respondents carried out a larger volume of vital pulp treatment (VPT) per month, with 69% carrying out over five cases, compared with 22% of IES respondents. The presence of patient symptoms significantly altered treatment planning decisions ($P < 0.001$) with

root canal treatment (RCT) more frequently selected in both societies. Patient age significantly influenced treatment choice in the absence ($P = 0.043$) or presence ($P = 0.012$) of symptoms with less VPT advocated in older patients. There were no significant differences in the treatment of a young patient in the presence ($P = 0.302$) or absence of symptoms ($P = 0.297$); however, older patient management differed between the societies for symptomatic ($P = 0.041$) and asymptomatic scenarios ($P = 0.044$) with significantly more RCTs carried out in the AIE than the IES. Hydraulic calcium silicate materials were commonly selected, accounting for 81% of IES and 69% of AIE VPT materials, although younger members of the AIE preferred calcium hydroxide materials. Younger dentists in both societies prescribed less RCT than older age groups.

Conclusions Although VPT is carried out by members of both societies there was no consistency regarding the most appropriate management for the exposed pulp or the VPT material of choice. Patient symptoms and age significantly influence the decision-making process and invasiveness of treatment. Hydraulic calcium silicate materials were the most commonly advocated material in all groups except young AIE members who preferred calcium hydroxide.

Keywords: deep caries, exposed pulp, hydraulic calcium silicate cement, pulp capping, pulpitis, questionnaire, vital pulp treatment.

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radiographs (Fig. 1a,b). In order to confirm that the questionnaire could be completed within 10 min and that the questions and flow of the survey were coherent and logical, a small pilot study was organized and evaluated using three endodontists in Dublin. This was carried out in paper format, with the average time to complete the survey 5 min. The evaluators were encouraged to be critical; however, the feedback was positive. In order to avoid possible misunderstandings and longer time to complete the survey by AIE members, it was translated in Italian by the principle researcher (RCD), who speaks both languages fluently. There was no difference in content between the two versions of the questionnaire.

After completion of the pilot study, and subsequent minor modifications, an online version was created using 'Google Docs' (<https://docs.google.com/forms>). The investigators elected to use this platform as it was familiar and data could be collected without asking the participants to download software or other required actions. This online version was created using RCD's account and verified through a 'test

link'. After confirming that the survey displayed correctly and that the sample answers were coherent, the survey was deemed ready for distribution.

Initially, the principle researcher (RCD) contacted the Presidents of the two endodontic societies, the AIE in Italy and the IES in Ireland, in order to confirm that they were willing to participate in an online research study, which asked their members to discuss management of deep carious lesions. After both societies agreed to engage in the study, a final copy of the questionnaire was sent to both the AIE and IES committees for their formal approval. The questionnaire was appraised by the committee of the AIE and IES who agreed with the format of the questionnaire. Finally, on 19 April 2018 they distributed the survey via email to all members of the AIE (197 members) and the IES (50 members), who had provided their email address to the society with a deadline of completion of 3 weeks. Subsequently, an invitation reminder was sent on the 3 May 2018 asking participants to complete the survey was sent to the secretary of both Societies.

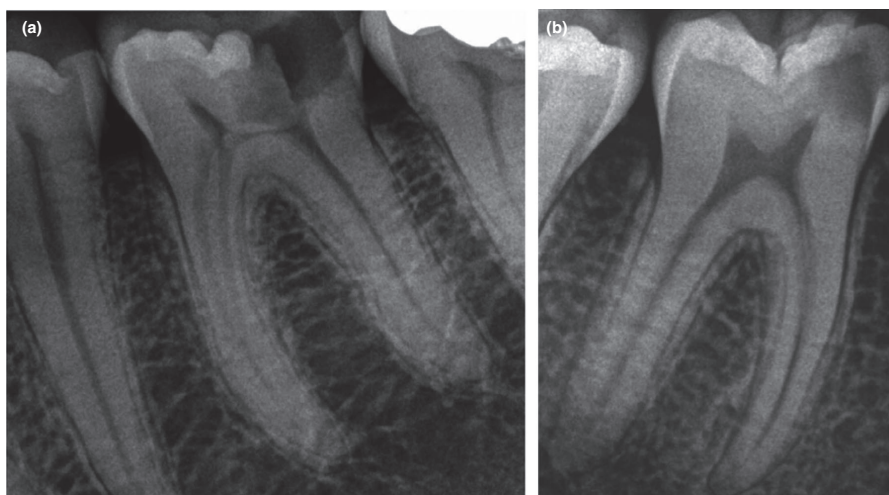


Figure 1 (a) Case 1: An 18-year-old patient presenting with a deep carious lesion on 36. Tooth remained vital, and the pulp was accidentally exposed during nonselective carious tissue excavation. There were two scenarios; in scenario one there were no symptoms, while in scenario two there were mild symptoms triggered by cold/sweet and tenderness when biting. (b) Case 2: A 45-year-old patient presenting with a deep carious lesion on 46. The tooth remained vital, and the pulp was accidentally exposed during non-selective carious tissue excavation. There were two scenarios; in scenario one there were no symptoms, while in scenario two there were mild symptoms triggered by cold/sweet and tenderness when biting.

Demographic component of questionnaire

Within the questionnaire format, a series of general demographic questions were asked in order to collect as much information as possible, related to age, experience and training (Table 1). Qualifications and numbers of years since obtaining the primary degree were recorded, as well as the nation where the participant obtained the primary degree and currently based their practice. Finally, in order to quantify the experience of the participant, the number of VPT procedures carried out every month was requested (Table 1).

Clinical component of questionnaire

Periapical radiographs from two separate patients demonstrating deep carious lesions were used as the basis of the scenarios (Case 1 and 2). For the purposes of this study, deep caries was defined as 'Caries reaching the inner quarter of dentine, but with a zone of hard or firm dentine between the caries and the pulp, which is radiographically detectable when located on an interproximal or occlusal surface. There is a risk of pulp exposure during operative treatment' (Duncan *et al.* 2019). Case 1 was based on a radiograph demonstrating a radiolucency indicative of a deep carious lesion in the 36 (lower left molar) in 18-year-old patient (Fig. 1a), while case two (Fig. 1b) was based on a radiograph showing a similar radiolucency of the 46 (lower right first molar) in a 45-year-old patient. In both case 1 and 2, the clinical examination described the pulp as being 'exposed during complete carious tissue excavation'. Within both case 1 and 2, there were two additional scenarios, which differed in their description of the presence or the

absence of symptoms. In scenario 1, there was a complete absence of any pulpal symptoms, while in scenario 2, the patient reported mild symptoms triggered by cold and sweet substances and on examination the tooth was tender to percussion (TTP).

Four further multiple-choice questions were asked about each of the scenarios (Fig. 2). The first question related to the treatment of choice for each scenario, with a range of answers from minimally invasive techniques to RCT (patient-related question). The second question related to the material of choice for VPT, while the third question asked for the specific reason behind the choice of material choice, again selecting from a list of options (operator-related). A fourth and final question was asked whether the use of an antibiotic, either topical or systemic would be considered (operator-related; Fig. 2).

Data collection and statistical analysis

The participants received the survey as a link, specific to the AIE (<https://forms.gle/qDVkUD1NEeQujUj28>) and specific to the IES (<https://forms.gle/tzZov6Etzzfw7Mow8>). After accessing the link, the participant was redirected to a webpage to complete the questionnaire. At the end of the questionnaire, the information was automatically and anonymously forwarded to a 'Google Doc' account of the principal researcher (RCD). The responses were individually analysed initially for completeness and then transferred and collated into an electronic database for analysis. The chi-square and Fisher's exact tests were used for evaluation of the results. The *P*-value < 0.05 was considered significant.

Results

Demographic results

From a total of 197 AIE members, 96 responded and from a total of 50 IES members, 24 responded (total 120). This represents a response rate of 49% for the AIE and 48% for the IES. Demographic data revealed a broadly similar age distribution between the two societies (Table 2). In terms of further education in Endodontics, the majority of AIE members had no further qualifications (63%), while the majority of IES respondents had a masters in endodontics (71%). Five AIE, but no IES respondents had a PhD (Table 3). The reported volume of VPT carried out per month differed between the two societies. AIE respondents

Table 1 Questions related to demographic information and volume of VPT carried out per month

Demographic data collected
Age
Country where you work
Country of primary qualification
Number of years after obtaining primary dental qualification
Qualifications
List any university-accredited postgraduate qualifications you have in endodontics (e.g. certificate, diploma, masters, PhD, Royal College of Surgeons examination)
How many VPTs would you carry out per month? (0, 0–5, 5–10, 10+)

1. HOW WOULD YOU TREAT THIS TOOTH?

- ☐ Pulp Capping
☐ Partial Pulpotomy (or Cvek pulpotomy)
☐ Full coronal pulpotomy
☐ Pulpectomy and root canal treatment

2. IF PULPOTOMY IS PERFORMED WHAT MATERIAL WOULD YOU CHOOSE?

- ☐ Hard setting calcium hydroxide
☐ MTA
☐ Biodentine
☐ Non-setting calcium hydroxide
☐ Ledermix/ Odontopaste
☐ Other _____

3. WHY?

- ☐ It gives me predictable outcomes
☐ I was trained to use it at college
☐ I am not familiar with the others
☐ It is easiest to handle
☐ Expense
 Other _____

4. WOULD YOU USE ANTIBIOTICS (EITHER TOPICAL [E.G. LEDERMIX] OR SYSTEMIC)

Y

N

Figure 2 Multiple-choice questions asked about each of four scenarios in the questionnaire. The same template was used for both cases and scenarios.

Table 2 Age distribution between the two societies

AGE (years)	25–35 (%)	35–45 (%)	45–55 (%)	55+ (%)
AIE	26.3	30.5	22.1	21.1
IES	25.1	20.8	33.3	20.8

Table 3 Postgraduate Endodontic education reported by participants

	PhD	MSc	Diploma	None
AIE (96)	5 (5.21%)	20 (20.83%)	11 (11.46%)	60 (62.50%)
IES (24)	0	17 (70.83%)	2 (8.33%)	5 (20.83%)

Figure in brackets = number of participants.

reported a range of VPTs per month, with 1–5 cases completed in 32%, 5–10 in 33% and over 10 completed in 36%, while in comparison IES respondents

reported completing less VPT with 1–5 cases in 61%, 5–10 in 13%, over 10 in 9%, with 18% not carrying out any VPT.

Patient-related results

Do patient symptoms alter the decision-making process?

After pooling the results of both societies, the presence or absence of symptoms significantly altered treatment planning decisions ($P < 0.001$). The younger asymptomatic patient described in Case 1 Scenario 1 resulted in more conservative-treatment options, while there was a significant increase in the selection of RCT when symptoms were added to the history (Case 1 Scenario 2; Table 4). Symptoms had a similar impact on older patients, with the absence of symptoms (Case 2 Scenario 1) resulting in more VPT

Table 4 Pooled results of AIE and IES (total 120) with regard to symptoms

	Pulp Capping	Partial Pulpotomy	Full Pulpotomy	RCT
C1 – S1	64 (53%)	22 (18%)	9 (8%)	25 (21%)
C1 – S2	19 (16%)	29 (24%)	14 (12%)	58 (48%)
C2 – S1	52 (43.5%)	22 (18.5%)	4 (3%)	42 (35%)
C2 – S2	17 (14%)	18 (15%)	5 (4%)	80 (67%)

Difference in treatment planning in absence (Scenario 1 – S1) or presence (Scenario 2 – S2) of symptoms for younger (Case 1 – C1) and older patient (Case 2 – C2).

than if symptoms were present (Case 2 Scenario 2; $P < 0.0001$; Table 4). The presence of symptoms led to more invasive treatment choices, with a 27% increase in RCTs in the young (Case 1) and 32% in the older patient (Case 2). Pulpotomies were selected more often in both societies for young symptomatic patients, compared with asymptomatic cases (partial pulpotomy 24% vs. 18%; full pulpotomy 12% vs. 8%); however, this pattern was not evident in the older patient. Pulp capping was commonly selected treatment modality for asymptomatic carious exposure regardless of age (Table 4).

Does the patient's age alter the decision-making process?

Patient age significantly influenced the treatment choice. There was a significant difference between younger (Case 1) and older patient's (Case 2) treatment plans in both absence ($P = 0.043$) or presence ($P = 0.012$) of symptoms when the results were pooled (Table 4). In the older patient, participants tended to carry out less VPT and are more invasive selecting RCT 14% more often for asymptomatic older patients and 19% for the older symptomatic cases compared with younger cohorts (Table 4).

If the overall responses of the AIE and IES are compared, there were no significant differences in the treatment of a young patient (Case 1) in the presence ($P = 0.302$) or absence of symptoms ($P = 0.297$). Treatment decisions for an older patient (Case 2), demonstrated a significant difference between the two

Societies for both symptomatic ($P = 0.041$) and asymptomatic scenarios ($P = 0.044$) with significantly more RCTS carried out in the AIE than the IES (Table 5).

Operator-related results

What VPT materials were most commonly chosen?

Hydraulic calcium silicate materials (HCSMs) were the most commonly selected (Fig. 3), with Biodentine and MTA combined accounting 81% of IES and 69% of AIE selected VPT materials. A smaller percentage of IES members choose Biodentine (17%) over MTA (64%), compared with AIE respondents (Biodentine 28% and MTA 31%). Both hard and non-setting Ca (OH_2) materials were also commonly used (40% of AIE, 19% of IES), with hard setting materials more common in both societies (Fig. 4). No other materials were commonly used by either society.

Did the age/experience of the operator make a difference to treatment plan?

To gauge the influence of experience on the treatment plan, respondents from both societies were categorized by age group. In general, younger dentists (25–35 and 35–45 years) prescribe less RCT than the older groups (45–55 and 55+ years; Table 6). When the youngest age group (25–35 years) was compared with older dentists (45–55 and 55+ years), the older cohorts selected RCT as the most appropriate choice in all Cases and Scenarios in both societies. Overall, the most invasive cohort for both societies was the 45–55-year group, while for all age groups, symptomatology increased invasiveness in all groups (Table 6).

Did the age/experience of the operator alter material choice?

In the AIE, Ca(OH_2) (hard and non-setting) was chosen as the most suitable material by 60% of the 25- to 35-year-olds, while HCSMs (Biodentine and MTA) were the first choice in all other age groups (35–

Table 5 Separated results of AIE (96 respondents) and IES (24 respondents) with regard to older patient (Case 2)

Case 2	Society	Pulp Capping	Partial Pulpotomy	Full Pulpotomy	RCT
S1	AIE	44 (46%)	13 (13.5%)	2 (2%)	37 (38.5%)
S1	IES	8 (33.5%)	9 (37.5%)	1 (4%)	6 (25%)
S2	AIE	15 (15.5%)	12 (12.5%)	2 (2%)	67 (70%)
S2	IES	2 (8.5%)	6 (25%)	3 (12.5%)	13 (54%)

Difference in treatment planning in absence (Scenario 1 – S1) or presence (Scenario 2 – S2) of symptoms illustrated.

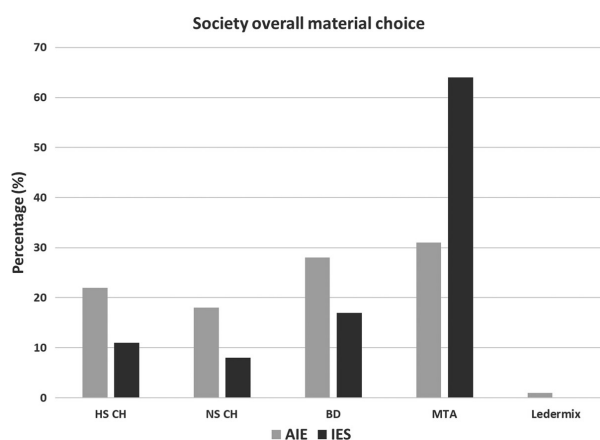


Figure 3 Overall VPT material choice in both societies. HS CH = Hard setting calcium hydroxide; NS CH = Non-setting calcium hydroxide; BD = Biodentine; MTA = Mineral trioxide aggregate.

45 years 63%; 45–55 years 62%; 55+ years 76%; Fig. 4). Biodentine and MTA were chosen similarly within all age groups (25–35 years BD 19%, MTA 21%; 35–45 years BD 37%, MTA 32%; 45–55 years BD 27%, MTA 33%; 55+ years BD 24%, MTA 36%).

For the IES, the age group 25–35 years selected Ca (OH)₂ as material of choice in 45% of cases and a HCSCM in 55%, while in every other age group choose calcium silicates was the overwhelming choice (35–34 years 83%; 45–55 years 79%; 55+ years 78%; Fig. 4). For Irish respondents, MTA was selected more than Biodentine in all age groups with 83% of 35- to 45-year-olds, 63% of 45- to 55-year-olds and 56% of 55 years + selecting MTA over Biodentine.

What was the reason for choosing a material?

The dominant factor for the material choice was the predictability of outcome (AIE 59%, IES 86%), followed by the handling of the product (AIE 17%, IES 5%). Cost was only considered an issue by less than 5% of both society members.

Were antibiotics prescribed?

The use of antibiotics was considered unnecessary by almost all the respondents. Only two respondents indicated using antibiotics, both for case 2 (older patient, with or without symptoms). Both respondents were members of the AIE.

Discussion

The questionnaire was designed to be easy to read and straightforward to complete. The survey was carried out online, avoiding the need for member addresses, postage, printing and stamped envelopes for subsequent return of the questionnaire. It was hoped that this would increase engagement with the study. Indeed, the response rate of nearly 50% is good in comparison with similar recent postal studies carried out in this subject area (Chin *et al.* 2016, Ha *et al.* 2016, Schwendicke *et al.* 2017). Although effectively half of the membership of the two societies responded, the IES and AIE differ markedly in their member numbers, so it is accepted that the study is limited by the fact that many more views were analysed from the Italian cohort. Notably, more respondents from the IES reported having a taught Master's degree in Endodontics, while the majority of AIE members have no structured postgraduate education. By contrast, five AIE members had a PhD, while no IES respondents possessed a doctorate. This is all likelihood reflects different traditions in the two jurisdictions as there is no history of structured endodontic training in Italy and equally no history of postgraduate research activity by endodontists in Ireland. A potential limitation of this study is the representativeness of the sample chosen, which is not a general dentist population. This was by design as although

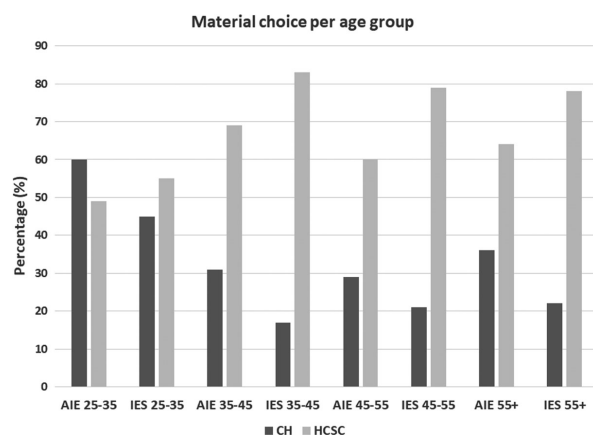


Figure 4 Choice of VPT materials in the AIE and IES by age group.

Table 6 Percentage RCT proposed for Case 1 (younger patient) and Case 2 (older patient) in the absence (Scenario 1) and presence of symptoms (Scenario 2)

	Case 1 Scenario 1		Case 1 Scenario 2		Case 2 Scenario 1		Case 2 Scenario 2	
	AIE (%)	IES (%)	AIE (%)	IES (%)	AIE (%)	IES (%)	AIE (%)	IES (%)
AIE IES 25–35 years	16	0	43	33	27	0	60	17
AIE IES 35–45 years	21	0	58	25	24	50	72	75
AIE IES 45–55 years	25	37	47	50	60	37	80	75
AIE IES 55+ years	29	20	44	40	43	20	70	40

several studies have analysed views of general dental practitioners in VPT (Oen *et al.* 2007, Stangvaltaite *et al.* 2017, Schwendicke *et al.* 2017), there is an absence of studies analysing the view of endodontists. As neither Italy or Ireland officially recognize the speciality of Endodontics, members of the endodontic societies represented a group aligned to the subject in those countries. As endodontists have recently become the principle group driving minimally invasive VPT for the exposed pulp (Bjørndal *et al.* 2019, Duncan *et al.* 2019), this group view on VPT was of particular interest.

Over the last 10–15 years, MTA has become the international 'gold standard' material for use in VPT (Damaschke 2008, Nair *et al.* 2008, Ricucci *et al.* 2020). As a result, it was hypothesized during the planning stages of this study that MTA and other HCSMs (e.g. Biodentine) would be the most popular material choices, particularly amongst younger respondents. Particularly within Italy,

calcium hydroxide materials remained as popular as MTA and Biodentine, and perhaps more surprisingly younger AIE and IES members preferred Ca(OH)₂ over HCSMs, compared with older respondents. The reasons for this are unclear but are likely to relate at least in part to undergraduate teaching and training, where the influence is still stronger for those just qualified than for those who have been qualified for a longer period. Older practitioners may be more influenced by experts speaking at conferences and practical courses, as there has been much evolution in the area of VPT since they qualified. MTA and Biodentine are significantly more expensive than Ca(OH)₂; however, this was considered a minor reason not to use the material being highlighted by less than 4% of respondents in both territories. Of course, younger dentists in reality are more likely to be working for someone else and are often not in the position to select the materials they would prefer. HCSMs are awkward to handle

clinically, and therefore, we were interested in whether this was a factor determining material choice. Although, it was noted to be important by 17% of AIE respondents, it was only a minor concern for IES respondents with only 5% suggesting it influenced their choice of material. It may be a reason as to why younger people are using the more recently introduced Biodentine, which has shown to be easier to handle (Careddu & Duncan 2018, Rajasekharan *et al.* 2018).

Biodentine was used relatively more by AIE members than IES members in all except the youngest age group. Again, this may reflect differing teaching practices in the two jurisdictions, but it may also reflect the impact of the marketing on material choice and it is possible that in Italy the distribution and advertisement for Biodentine have been more consistent and aggressive than in Ireland. Septodont as a company has traditionally been involved in the sale of dental anaesthetic products rather than dental materials in Ireland, although a recent change in direction has started the promotion of biomaterials such as Biodentine and Bioroot RCS in dental schools.

In the AIE, VPT techniques were more common carried out with an almost equal number of responses for the three groups (1–5, 5–10, 10+ VPT in a month). On the contrary, Irish respondents were less inclined to perform VPT with the majority of them performing not more than 5 treatments in a month and many respondents reporting that they did not to carry out these procedures at all. This could be related to a difference in teaching received at University as well as the fact that in Italy, Endodontists often perform some general practice while in Ireland there is a stronger referral-based system in place.

In general, the presence of pulpal symptoms increased the number of respondents who would perform a RCT, rather than VPT both in AIE and IES. This is a clear indication that, even mild/moderate symptoms are considered a characteristic that may lead to failure of a conservative approach. This is in light of the fact that there is not a good correlation between diagnosis based on clinical symptoms and actual status of the pulp (Mejäre *et al.* 2012). This has been challenged recently when a good correspondence between clinical findings, and the reversible/irreversible pulpitis histologically was demonstrated (Ricucci *et al.* 2014). In this study, the cases highlighted the presence of mild symptoms, more indicative of reversible than irreversible pulpitis; however, this was sufficient for respondents to select

pulpectomy more often than a VPT procedure if symptoms were present.

Patient age appeared to have a considerable influence on the decision-making process in both societies. In both the presence of absence of pulpal symptoms, participants adopted a less conservative approach in older patients, which cannot be attributed to the level of root formation as in both cases, as root formation was complete. This supports other questionnaire-based studies examining operator choice in a range of scenarios, which have highlighted more invasive choices in older patients (Frisk *et al.* 2013). When considering why patient age is considered important, it should be noted that there is no firm clinical evidence to suggest that age is a significant factor for the success of VPT. Although, a recent prospective pulp capping study compared results in patients above and below 40 years and concluded that treatment was less successful in older patients (Marques *et al.* 2015); other studies with a similar age ranges have showed no difference (Matsuo *et al.* 1996, Mente *et al.* 2014). There is certainly a perception that young people and indeed pulpal healing occurs more predictably with prospective studies analysing the histological response to VPT materials invariably involving young patients (Hörsted-Bindslev *et al.* 2003, Accorinte *et al.* 2008, Nair *et al.* 2008) as do clinical pulp capping outcome studies in carious teeth (Farsi *et al.* 2006, Taha & Khazali 2017). Younger patients, under the age of 20 years, are selected due to the greater blood supply, the potential for open root apices and increased cellularity of their pulps; it has been suggested that these characteristics should result in more predictable healing (Massler 1972).

A further point of interest was that when we analysed the age since qualification older groups were more inclined to perform RCT than the younger, especially in absence of symptoms. This may be due to younger dentists being more aware of the latest developments in VPT (Björndal *et al.* 2010, Wolters *et al.* 2017), or it may reflect experience dictating that RCT was more predictable in their 'hands' than VPT. On a positive note, it appears that the need to reduce antibiotic prescribing and the lack of rationale for using antibiotics for 'toothache' has been effective at least for this cohort of participants (Agnihotry *et al.* 2019).

Conclusions

Within the limitations of this study, it is clear that VPT is carried out frequently by both members of the

IES and AIE. There is no consistency in approach about the most appropriate management for the exposed pulp or the VPT material of choice. Patient symptoms and age influence the decision-making process. Ca(OH₂) was the most commonly used material only in the youngest AIE respondents, while MTA was the most commonly selected material from all ages of IES respondents. Members of both societies suggested that the predictability of the results, rather than cost or handling was the principle reason behind material choice.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Appendix IV: Published article in International Endodontic Journal 2021. A prospective clinical study investigating the effectiveness of partial pulpotomy after relating preoperative symptoms to a new and established classification of pulpitis



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ORIGINAL ARTICLE

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A prospective clinical study investigating the effectiveness of partial pulpotomy after relating preoperative symptoms to a new and established classification of pulpitis

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Abstract

Aim: To prospectively investigate the outcome of partial pulpotomy after 1 year, using a hydraulic calcium silicate cement (HCSC) on symptomatic cariously exposed pulps in adult teeth. To compare the traditional American Association of Endodontists (AAE) pulpitis classification with the recently proposed Wolters classification system in predicting the likelihood of treatment failure.

Methodology: Sixty-two symptomatic adult teeth with deep and extremely deep carious lesions were classified according to the Wolters (mild/moderate/severe pulpitis) and the traditional pulpitis classification (reversible/irreversible pulpitis). Eleven teeth were excluded intraoperatively as there was no pulp exposure after non-selective caries removal. The remaining 51 teeth, regardless of diagnosis, were treated by partial pulpotomy, pulpal lavage with 2.5% sodium hypochlorite solution, haemostasis and HCSC application (Biodentine™) as a pulp capping material. A permanent restoration was placed during a second appointment 1–2 weeks later. Preoperative tenderness to percussion (TTP), bleeding time and material setting time were recorded as was preoperative and postoperative tooth colour under standardized conditions. Clinical review occurred at regular intervals with clinical/radiographic analysis at 12 months. Chi-square analysis and Fisher's exact test assessed different outcomes amongst the diagnostic categories; the Kruskal–Wallis and Wilcoxon rank-sum test assessed influence of pulp bleeding time, TTP or variation in setting time ($p < .05$).

Results: Ten cases were lost to review, and a total of 41 teeth were reviewed at 1 year and classified as either “success,” “successful but unresponsive to sensibility testing” or “failed.” This included five severe, 17 moderate and 19 mild pulpitis according to Wolters classification or 23 reversible pulpitis and 18 irreversible pulpitis cases by the AAE classification. The majority of the 62 enrolled cases were “extremely deep” ($n = 50$), rather than “deep” ($n = 12$) caries with all failures occurring in the extremely deep group. Partial pulpotomy was 90% successful (100% reversible, 78% irreversible or 100% mild, 88% moderate, 60% severe pulpitis) with a significant difference in outcome between mild and severe pulpitis groups ($p = .04$). Only one, severe pulpitis/irreversible pulpitis, case failed painfully prior to the 1-year review

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appointment. Bleeding time ($p = .26$) and TTP ($p = .61$) did not influence treatment outcome, whilst Biodentine™ setting time was significantly longer than manufacturers' claim ($p < .05$). No teeth discoloured.

Conclusions: Partial pulpotomy using Biodentine™ was successful for treating symptomatic carious pulpal exposures after 1 year, but included cases where pulp vitality could not be confirmed. Within the limitations of this study, cases with signs and symptoms indicative of irreversible pulpitis were not less successful; however, Wolters classification highlighted severe pulpitis to be less successful than mild pulpitis, thereby providing a potential prognostic benefit in diagnostically subdividing pulpitis. Caries depth was an indicator of failure, whilst bleeding time and preoperative tenderness to percussion were not.

KEYWORDS

deep caries, exposed pulp, hydraulic calcium silicate cement, partial pulpotomy, pulpitis, vital pulp treatment

INTRODUCTION

The dental pulp can be irritated by a range of chemical, thermal and traumatic stimuli; however, microbial challenge represents the principal threat to pulp vitality (Möller et al., 1981). In response to this threat, the dentine–pulp complex mounts a complex immune response triggered initially via biosensing by odontoblast cells and thereafter by an orchestrated inflammatory process by dental pulp cells and circulating immune cells recruited locally to the injury site (Duncan & Cooper, 2020; Duncan et al., 2019; Farges et al., 2015). The early stages of pulpal inflammation are generally limited to the coronal pulp tissue with other areas of the pulp relatively healthy (Ricucci et al., 2014); however, current ways to categorize pulpitis are absolute considering the pulp to be either salvageable or not (American Association of Endodontists [AAE], 2013).

Inflammation of the dental pulp is classically categorized as being either reversible and irreversible pulpitis based on the relationship of signs and symptoms to management (AAE, 2013). Irreversible pulpitis indicates that the pulp tissue is so damaged that it is impossible to maintain vitality; thus, root canal treatment (RCT) is necessary. On the contrary, reversible pulpitis is a condition in which the pulp, even if damaged, is capable of recovery if the irritant stimulus is removed and the tooth adequately restored (Tronstad & Mjör, 1972). Unfortunately, this classification simplifies the complex and often unpredictable nature of pulpitis and fails to reflect the recently reported success of partial and full pulpotomy in cases with signs and symptoms indicative of irreversible pulpitis (Özyürek & Demiryürek, 2016; Taha & Khazali, 2017; Uesrichai et al., 2019). As a result, there has recently been a call for new classifications of pulpal status to be proposed in an

attempt to link the signs and symptoms of pulpitis to vital pulp management strategies (Wolters et al., 2017) and to reflect the localized nature of pulp damage (Rechenberg & Zehnder, 2020; Ricucci et al., 2014). Recently, Wolters et al. (2017) proposed a system based on symptoms with four different categories of pulpitis, initial, mild, moderate and severe, with pulpal exposure and pulpotomy only indicated in selected categories. Notably, the word “irreversible” has been removed from all diagnoses in this proposed classification (Wolters et al., 2017). Although this represents a potentially more relevant diagnostic system, at present, there are no studies investigating its usefulness in determining the predictability of the vital pulp treatment (VPT) procedures.

Hydraulic calcium silicate cements (HCSCs) are recommended as the “gold standard” material for conservative treatment of the exposed pulp (ESE, 2019). HCSCs are characterized by high biocompatibility and hydrophilic nature, which assists their use in VPT procedures; however, mineral trioxide materials (MTA) have been shown to be associated with extended setting and tooth discolouration (Careddu & Duncan, 2018). HCSCs, such as ProRoot® MTA (Dentsply Sirona), modulate the pulp response reducing pulpal inflammation and increasing the quality of hard tissue bridge formation over the exposed pulp, compared with hard setting calcium hydroxide materials (Nair et al., 2008). A recently introduced HCSC, Biodentine™ (Septodont) is specifically marketed for VPT with manufacturer claims that it sets in 12 min, do not cause tooth discolouration with clinical research indicating good success in pulp capping over 2–3 years (Harms et al., 2019) and also pulpotomy (Taha & Abdelkader, 2018).

The overarching aim of this study was to prospectively investigate the predictability of partial pulpotomy using the

HCSC, Biodentine™, in adult teeth presenting with symptomatic deep and extremely carious lesions and classified according to the AAE and Wolters diagnostic systems (AAE, 2013; Wolters et al., 2017). The following objectives were studied: (1) primary objective—the outcome clinically and radiographically of symptomatic cariously exposed pulps treated using a partial pulpotomy technique and capped with Biodentine™; (2) primary—the practicality and relevance of Wolters (Wolters et al., 2017) and the AAE (AAE, 2013) classification for diagnosis of pulpitis, management and prediction of VPT outcomes; (3) secondary—the association of bleeding time and tenderness to percussion (TTP) with reversible, irreversible pulpitis and mild/moderate and severe pulpitis; (4) secondary—practical aspects of Biodentine™ use, including discolouration, setting time and postoperative symptoms.

MATERIALS AND METHODS

Experimental observational study

The proposed study was a longitudinal prospective single-arm clinical study investigating the reliability of Biodentine™ in the treatment of adult teeth presenting with deep/extremely caries and symptoms of pulpitis that related the outcome to an established and new classification of pulpitis. The study was carried out according to the STROBE checklist for observational studies. For the purposes of this study, “deep caries” was defined as reaching the inner quarter of dentine, but with a zone of hard or firm dentine between the caries and the pulp, which was radiographically detectable when located on an interproximal or occlusal surface, whilst “extremely deep caries” was defined as caries penetrating the entire thickness of the dentine, radiographically detectable when located on an interproximal or occlusal surface in which pulp exposure was unavoidable during operative treatment (ESE, 2019). The clinical component was carried out by one operator in dental practice in Dublin (RCD) linked to one senior principal investigator (HD) in the Division of Restorative Dentistry, Dublin Dental University Hospital (DDUH), Ireland.

Ethical approval

As the nature of this study is a clinical intervention on human subjects, an ethical approval was necessary. An application was submitted to the Research Ethics Committee at St James's Hospital (JREC), Dublin, and Ethical Approval (REC ref: 2017/04/01) was obtained on 25 April 2017.

Size of patient cohort

It was not possible to design this study as a randomized clinical trial comparing two diagnoses, but rather as a practicality-based prospective clinical study investigating the usefulness of a new classification system in general dental practice and relating it to outcome. Although the sample size was based on the numbers included in several studies investigating both the outcome of partial and full pulpotomy in teeth exhibiting reversible and irreversible pulpitis (Taha & Abdelkader, 2018; Taha & Abdelkader, 2018 [reviewed in] Cushley et al., 2019), as well as outcome studies demonstrating the success rate of partial pulpotomy in teeth with reversible pulpitis to be 98% (Chailertvanitkul et al., 2014) and approximately 68% with irreversible pulpitis (Elmsmari et al., 2019; Taha & Khazali, 2017) after 1 year. As the aim of this study was exploratory to investigate the usefulness of two pulp diagnosis systems a convenience sample size was used.

Patient sample

The sample group consisted of patients with a deep or extremely deep carious lesion and associated symptoms (ESE, 2019). Symptomatology was recorded and used to categorize the clinical features into mild, moderate or severe pulpitis (Wolters et al., 2017). In order to compare the results with a more established classification, the teeth were also categorized as either reversible or symptomatic irreversible pulpitis (AAE, 2013). Although “Wolters” classification (Wolters et al., 2017) was used to categorize pulpal condition, the proposed management strategies also advocated by Wolters were not rigidly followed and all cases were treated by non-selective caries removal and partial pulpotomy if the pulp was exposed after non-selective removal. This was carried out in order to standardize treatment and to assess the validity of Wolters' diagnosis as well as evaluating the efficacy of partial pulpotomy after non-selective caries removal in deep and extremely deep carious lesions with associated symptoms. The material chosen as pulp capping agent was Biodentine™ a HCSC marketed for VPT procedures.

Inclusion and exclusion criteria

The sample group consisted of patients between 14 and 60 years old, presence of deep/extremely carious lesion (evident from periapical ± bitewing radiographs), pulpal symptoms ranging from mild-to-severe pain, positive response to cold pulp sensibility test, and pulpal exposure during non-selective caries removal.

Pregnant women, teeth that were giving mild-to-severe pain and the patient preferred RCT, teeth not responsive to pulp sensibility testing (cold) and teeth that were not exposed after non-selective removal of caries were excluded (Table 1). In addition, teeth in which the pulpal haemorrhage could not be arrested intraoperatively were also excluded.

Patients were recruited by the operator (RCD). After taking a detailed pain history, and clinical/radiographic (periapical) examination, if a case was considered suitable for the study, he/she was informed about the possibility of being involved in this research project and a full explanation about the procedure, its risks and benefits were discussed. A clinical information leaflet was also provided for the patient.

Consent and pseudo-anonymous data storage

After a patient was considered potentially suitable for the study and expressed an interest in participating, an informed consent was obtained. During the consent procedure, it was stressed that the current “gold standard” for the treatment for the cariously exposed pulp in mature adult teeth was RCT. Due to the emergency nature of “toothache,” it was not possible in the bulk of cases to allow a “cooling off” period as the patient was in pain and required immediate treatment. For this reason, the operator (RCD) carefully discussed consent with the patient on the day of presentation, clarifying doubts and explaining aspects of the procedure that the patient was unclear about. Afterwards, if the patient remained willing to participate, he/she was asked to sign the form. Within the consent procedure, it was further explained that the patient would only be enrolled into the study if the pulp was exposed after non-selective caries removal. If a pulp was not exposed, an indirect pulp cap would be carried out, and equally if on entry to the pulp, there

were clear signs of pulp necrosis a RCT would be performed. The number of these cases was recorded in the experiment flow chart (Figure 1). Three copies of the consent were signed: one for the patient, one for the patient’s dental chart and one for the researcher’s own study record. Any data collected by RCD collected were stored on an encrypted computer. The key to the code was kept on a separate encrypted computer held by the principal investigator (HD), hence keeping any data in a pseudo-anonymized state. All the images (excluding radiographs) taken before, during and after the procedure were not associated to names (only codes) and did not include the face of the patient and did not have any identifiable features. This material was stored under the same code as described above. All data will be deleted after 5 years.

Patient assessment and operative procedure

Before beginning the procedure, the patient was assessed and the tooth categorized according to the classification of Wolters et al. (2017); “Mild Pulpitis” if the tooth was asymptomatic until triggered by cold stimuli or percussion, “Moderate Pulpitis” if the tooth demonstrated clear symptoms triggered by cold stimuli or percussion that lasted for minutes, but the pain could be stopped using medications; or “Severe Pulpitis” if the tooth displayed severe spontaneous pain and clear strong reaction to thermal stimuli as well as pain to percussion. The tooth was also categorized as having either reversible or symptomatic irreversible pulpitis based on strict definitions (AAE 2013) (Table 2). Further analysis of an intraoral periapical radiograph was carried out by the operator and later by a second evaluator to ascertain the depth of the lesion as being either “deep” or “extremely” deep caries. Although this did not alter the operative procedure, as all cases were treated by non-selective caries removal, this subdivision

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
- Patient aged 14–60 years	- Patient aged <14 or >60 years
- One tooth per patient	- Incisors or canines
- Premolar or molar tooth	- Absence of deep carious lesions radiographically
- Presence of a deep and extremely deep carious lesions on radiograph	- Preference: patient has moderate-severe pain and preferred root canal treatment
- Symptoms: range from mild, non-spontaneous pain to spontaneous severe pain	- Pregnant women
- Positive response to pulp sensibility test	- Negative response to vitality test
- Pulp exposure after non-selective caries removal	- Presence of “sound” dentine over pulp
	- Pulp not exposed intraoperatively
	- Pulpal haemorrhage could not be arrested
	- Pulp necrosis evident on exposure

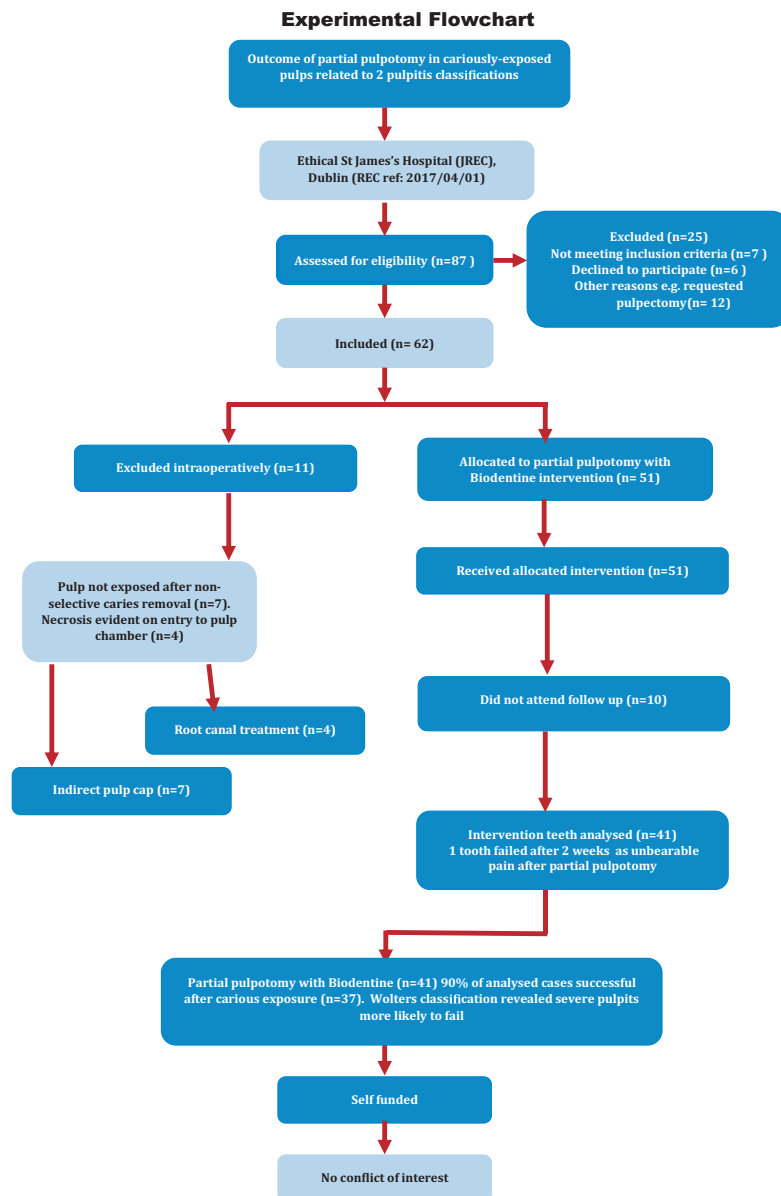


FIGURE 1 Experimental flow chart

facilitated later subanalysis of the results according to caries depth (Tables 3 and 4).

In order to assess preoperative pulp vitality, the teeth were isolated with cotton wool and dried prior to a -50°C

thermal test (Endofrost; Roeko) being applied. After administration of the local anaesthetic (2% lignocaine hydrochloride with adrenaline 1:80 000), the tooth was isolated with rubber dam and liquid dam (Ena-dam; Micerium).

TABLE 2 Pulpal disease classification systems used

Wolters classification Wolters et al. (2017)	Standard classification AAE Endodontic diagnosis (2013)
Mild pulpitis: Heightened and lengthened reaction to cold, warm and sweet stimuli that can last up to 20 s, but then subsides. Tooth may be percussion sensitive.	Reversible pulpitis: Discomfort triggered by cold/sweet stimuli, which relieves within seconds after the removal of the stimulus. The pain is non-spontaneous
Moderate pulpitis: Clear symptoms, strong, heightened and prolonged reaction to cold, which can last for minutes, possibly percussion sensitive and spontaneous dull pain that can be suppressed with pain medication.	Symptomatic irreversible pulpitis: Sharp and lingering pain triggered by thermal stimulus (often 30 s or longer after stimulus removal), spontaneous pain and often referred pain. The pain can be accentuated by postural changes such as lying down or bending over and over-the-counter analgesics are typically ineffective.
Severe pulpitis: Severe spontaneous pain and clear pain reaction to warm and cold stimuli, often, sharp to dull throbbing pain, patients have trouble sleeping because of the pain (worsens when lying down). Tooth is sensitive to touch and percussion.	

Thereafter, the isolated area was cleaned using a cotton pellet soaked in 3% hydrogen peroxide followed by 0.2% chlorhexidine (Hörsted-Bindslev et al., 2003). Using an operative microscope (Labomed Prima; Labomed), the gross caries was non-selectively removed, initially using a diamond bur in a high-speed handpiece under water coolant, prior to using a sterile round steel bur in a slow handpiece. The absence of carious dentine was confirmed using FIND (Fluorescence Inspect and Determine system) present in Ledex WL90+ (DentMate). The area in closest proximity to the pulp was cleaned manually with a sharp hand excavator. If after non-selective caries removal, the pulp was exposed, a further 2–3 mm of pulp was removed with a fresh sterile carbide bur in a high-speed handpiece under abundant water coolant (Figure 2). After rinsing the pulp with NaOCl 2.5% solution, a cotton pellet soaked in a 2.5% NaOCl was pressed against the exposed pulp and haemostasis was checked every minute up to 6 min (Figure 2c). The time required for the pulpal tissue to stop bleeding was recorded.

Biodentine™ was mixed according to the manufacturer instructions and applied with gentle pressure directly onto the pulpal wound surface (Figure 2d) and used to fill the entire cavity and setting time of the material was recorded. Setting time was defined, as the time taken for the material to harden to the extent that light pressure with an amalgam plugger did not penetrate more than 1 mm. The patient was scheduled to return after 1 week, when 2–3 mm of the Biodentine™ restoration was removed and a permanent resin-based composite (RBC) restoration was placed (Figure 2e,f). Pulp sensibility (Endofrost) was checked at 1 week, 3 months, 6 months, 1 year and was considered positive if the patient consistently reported even mild sensitivity to cold. After 1 year, a periapical radiograph was taken with a 6-month radiograph taken in selected cases.

Preoperatively and post-restoration of the tooth, the colour of the crown was recorded on both the buccal and lingual aspect of the tooth. In order to standardize the conditions of light and visibility, the shade of the tooth was taken under magnification 10× and maximum

light intensity using a microscope with LED white light (Labomed Prima) and the Vita Classic shading guide (VITA). During the subsequent follow-up appointments, discoloration was further monitored and recorded.

Outcome evaluation

At every follow-up appointment (1 week, 3, 6 and 12 months), the teeth were assessed for the presence of signs and symptoms of pulpitis, whilst pulp vitality was verified and recorded. This involved carrying out a percussion test and a cold test at –50°C (Endofrost). The integrity of the coronal restoration was verified visually under the microscope. After 12 months, a periapical radiograph supplemented the history and clinical examination. For practical reasons, the pulp sensibility testing was carried out by the practitioner; however, the 12-month radiographs were assessed blindly by a second evaluator (HD) not involved in the provision of treatment. At all review points, the teeth were categorized as either “successful,” “unresponsive but successful” or “failed.” Treatment was considered “successful” when the tooth responded positively (including a consistently reduced response) to cold test, the response did not linger, was symptom-free and not tender to percussion, and there was no evidence of an apical radiolucency. Unresponsive teeth that did not demonstrate any apical radiolucency and were symptom-free but did not respond to the cold tests were noted as “unresponsive but successful.” A treatment failure was noted if either the patient reported pain, the tooth was tender to percussion and/or a periapical radiolucency was present.

Statistical analysis

Arrest of bleeding and Biodentine™ setting time were submitted to a Shapiro–Wilk test of normality ($p < .05$ samples are not normally distributed). A non-parametric

TABLE 3 Record of clinical features and 1-year success rate

Classification (number of cases)	Subdivision (number)	Bleeding time (average)	Tenderness to percussion (%)	Success at 1 year (%)	Success at 1 year (%) If unresponsive classed as failure
AAE (2013) (41)	Reversible pulpitis (18)	1 min 32 s	7/18 (39%)	13 Success 5 Unresponsive success (100%)	13 Success 5 Unresponsive failure (72%)
	Irreversible pulpitis (23)	1 min 37 s	19/23 (83%)	14 Success 5 Unresponsive success 4 Failures (83%)	14 Success 5 Unresponsive failure 4 Failures (61%)
Wolters et al. (2017) (41)	Mild (19)	1 min 9 s	7/19 (37%)	14 Success 5 Unresponsive success (100%)	14 Success 5 Unresponsive failure (74%)
	Moderate (17)	1 min 8 s	14/17 (82%)	11 Success 4 Unresponsive success 2 Failures (88%)	11 Success 4 Unresponsive failure 2 Failures (65%)
	Severe (5)	2 min 6 s	5/5 (100%)	2 Success 1 Unresponsive success 2 Failures (60%)	2 Success 1 Unresponsive failure 2 Failures (40%)

Kruskal–Wallis test was used to analyse differences in bleeding time between Wolter's diagnostic groups and Mann–Whitney *U*-tests were used to analyse bleeding time between reversible and irreversible pulpitis. The Wilcoxon rank-sum test was used for differences in setting time. Differences in the outcome of partial pulpotomy with each diagnosis of pulpitis were tested and cross-tabulated with contingency tables, chi-square analysis and Fisher's exact test. Data analysis used SPSS (v25; IBM) ($p < .05$). For radiographic caries, depth assessment interobserver

TABLE 4 Cross-tabulation of pulpal classifications according to preoperative symptoms and tests

Pulpitis	Mild	Moderate	Severe	Total
Reversible	18	1	0	19
Irreversible	1	17	5	23
Total	19	18	5	

Note: This table highlights that by symptoms moderate and severe form effectively a sub-classification of irreversible pulpitis. $p < .001$ significant correlation between the two classification systems.

agreement was calculated using Cohen's κ test (K) according to the scale for strength of agreement $K < 0.20$: poor, $K = 0.21$ – 0.40 : fair, $K = 0.41$ – 0.60 : moderate, $K = 0.61$ – 0.80 : good, $K = 0.81$ – 1.00 : very good (Altman, 1990).

RESULTS

Sample characteristics

Eighty-seven patients were assessed for eligibility having presented with a symptomatic carious lesion in a premolar or molar tooth. Of those patients, 25 were excluded due to preferring RCT/extraction (12 patients), refusing to participate (6 patients) and not meeting inclusion criteria for deep/extremely caries (7 patients) (Figure 1). Sixty-two teeth were enrolled for this study and 11 were subsequently excluded intraoperatively. Of those 11 cases, seven (one with severe, two with moderate and four with mild pulpitis) did not have carious pulp exposure after non-selective removal of caries and they were treated either with RBC restorations or with an indirect pulp capping and a calcium hydroxide lining (Figure 3a,b). Subsequently, one of the teeth without pulp exposure but with a diagnosis of moderate pulpitis required RCT after 5 months as it displayed signs of pulpal necrosis and apical radiolucency. Of the remaining 4 cases, one case provisionally diagnosed with mild pulpitis and three cases with severe pulpitis were treated immediately with RCT

as after access to the pulp chamber partial pulpal necrosis was diagnosed (Figure 3c,d).

Fifty-one teeth were finally included and treated within this study, with 41 teeth reviewed after 1 year (Figure 1). The diagnostic breakdown of the initial sample by Wolters criteria (Wolters et al., 2017) includes five cases of severe pulpitis, 22 of moderate pulpitis and 24 with mild pulpitis. By the traditional AAE pulpal diagnostic system, there were 23 reversible pulpitis and 28 irreversible pulpitis (AAE, 2013). All the cases were treated with partial pulpotomy (removal of 3–4 mm pulp tissue), rinsed with 2.5% NaOCl and capped with Biodentine™. All treated patients reported mild postoperative discomfort on the day of the treatment, which was managed with “over the counter” analgesics as required. Postoperative pain generally disappeared within 24 h; however, in the 7 days following the procedure more than 50% of the sample reported an increased sensitivity to cold stimuli that gradually subsided during the following 3 months. One patient with irreversible/ severe pulpitis developed unbearable pain within a week and had a pulpectomy carried out which was categorized as failure; this was the only painful failure within the 1-year period. Ten patients did not re-attend for 1-year review and were excluded from the study (5 reversible, 5 irreversible pulpitis; 5 moderate, 5 mild pulpitis) (Figure 1). Specifically, four of the ten patients did not attend the second appointment for permanent filling placement; however, they subsequently attended the clinic as an emergency presenting with pain and sensitivity due to dislodgement of the Biodentine™ restoration. All four teeth had subsequent RCT. The remaining 6 patients returned to get the permanent restoration placed, but not the subsequent 1-year review.

Generally, the response to pulp sensibility testing was less intense after treatment. As a result, in four cases because of lack of response at the second appointment, the permanent filling was performed without administering local anaesthetic and the cold test was repeated after removing part of the Biodentine™; the response was positive in all four cases. Three patients were initially classified as unresponsive, but at the subsequent review appointment at 6 months demonstrated a mild response to a cold test. The visualization of a hard tissue bridge radiographically was a variable finding being present on only 12 postoperative review radiographs at 1 year (Figure 4). Two consecutive patients, one 15-year-old and one 16-year-old patient presented with deep carious lesions in two mandibular second molar teeth (Figure 5), which after completion of partial pulpotomy remained responsive to pulp sensibility testing at review. The mandibular left second molar in the 15 years old, although remaining positive to sensibility testing developed internal resorption in the middle third

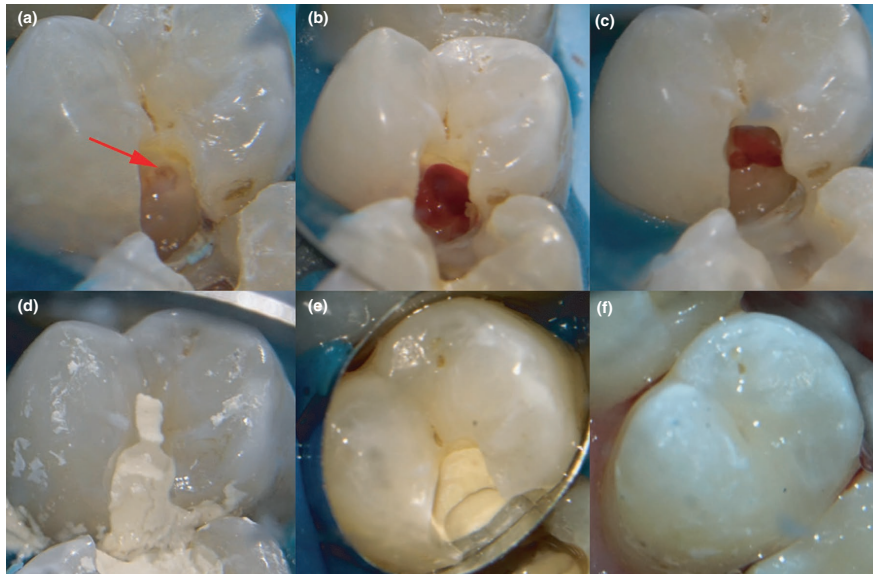


FIGURE 2 Partial pulpotomy clinical protocol. (a) Pulp exposure after non-selective caries removal (red arrow). (b) Superficial pulp tissue removed (to depth of 2–3 mm). Pulpal haemorrhage evident. (c) Haemostasis obtained after several minutes by placing cotton pellet soaked in 2.5% NaOCl, pressed against the exposed pulp tissue. (d) Biodentine™ used to restore the entire cavity. (e) Superficial removal of Biodentine™ at the second visit 1 week later. At least 3 mm of material is left above the exposure site before restoration. (f) Tooth permanently restored with resin-based composite material

of both the mesial and distal roots and was classified as a treatment failure (Figure 5f).

Outcome

For the 41 patients attending at 1-year review, of the 19 mild pulpitis cases treated, 14 were deemed successful and five unresponsive but successful, whilst of the 17 cases of moderate pulpitis 12 were considered successful, three unresponsive but successful with two failures. For teeth presenting with severe pulpitis, two cases were considered as success, one was unresponsive but successful and two failed (one within a week) (Table 3). Grouping success and unresponsive but successful, the outcome for partial pulpotomy at 1 year was 90%. Relating outcome to preoperative diagnosis, revealed that teeth with reversible pulpitis and pulp exposure treated by partial pulpotomy were 100% successful and irreversible pulpitis 78% successful at 1 year. Using Wolters classification, a preoperative diagnosis of mild pulpitis was 100% successful, 88% moderate pulpitis successful and severe pulpitis 60% successful at 1 year. If the results are reconsidered and unresponsive is a failure, reversible pulpitis were 72%

successful and irreversible pulpitis cases 61% successful at 1 year, whilst using Wolters classification mild pulpitis was 74%, moderate pulpitis 65% and severe pulpitis 40% successful (Table 3).

The 1-year outcome with unresponsive considered a success was compared between groups classified as being reversibly and irreversibly inflamed, with no significant difference being reported ($p = .32$). Outcomes at 1 year were also compared using the three Wolters diagnostic groups with no significant difference between “mild” and “moderate” pulpitis ($p = .49$) and “moderate” and “severe” pulpitis groups ($p = .19$). When “mild” and “severe” pulpitis success rates were compared at 1 year, the “mild” group had a significantly better outcome than the “severe” pulpitis group ($p = .04$). When unresponsive was a reconsidered, a failure overall success dropped to 66% at 1 year; however, there were no significant differences between reversible and irreversible cases ($p = .52$) and any of the “mild,” “moderate” or “severe” groups ($p > .28$) (Table 3). Notably, when the two classification systems were cross-tabulated according to symptoms (Table 4), it can be seen that whilst there was good correlation between the classification systems ($p < .001$), moderate and severe pulpitis essentially represented a subdivision of irreversible pulpitis;

FIGURE 3 Deep carious lesions that were excluded from the study. (a) Preoperative view of an upper left second premolar with a deep carious lesion, which after non-selective removal did not have a pulp exposure. (b) Postoperative view of another upper left second premolar in which the pulp was not exposed after non-selective caries removal and was treated with indirect pulp capping. (c) Tooth 46 responsive to vitality test presenting a deep carious lesion. (d) Same tooth in figure c treated with RCT and temporary restored with glass-ionomer as presenting clear signs of necrosis were evident in the exposed pulp tissue on visual inspection

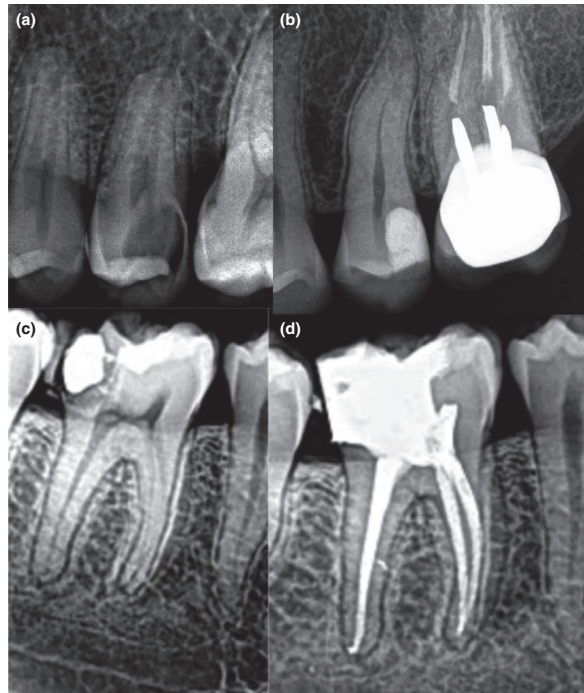
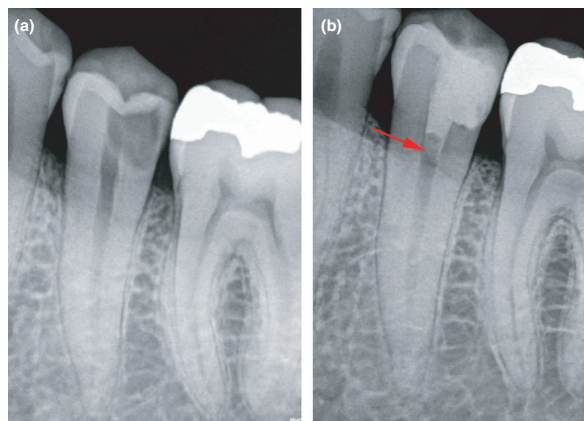


FIGURE 4 Lower left second premolar presented with moderate (irreversible) pulpitis treated with partial pulpotomy using Biodentine™. (a) Preoperative periapical radiograph of the tooth with coronal radiolucency indicative of extremely deep caries. (b) Twelve-month radiographic evaluation showing hard tissue bridge formation (red arrow). The tooth responded positively to pulp sensibility testing and was asymptomatic



with the subdivision related to treatment outcome with 12% failure in the moderate pulpitis group and 40% in the severe pulpitis group at 1 year (Table 3).

The time taken for pulpal haemostasis ranged from 1 to 4 min. In all cases, pulpal haemorrhage could be arrested. Notably, there was only a small difference between the average duration of bleeding for mild and moderate pulpitis (mild

pulpitis—average 1 min 9 s; moderate pulpitis—average 1 min 8 s); however, bleeding time was increased in the cases with severe pulpitis to an average of 2 min 6 s (Table 3). This difference between the groups was not significant ($p = .26$). There was also no significant association between outcome and time to achieve haemostasis ($p = .6$). Although all severe pulpitis cases were tender to percussion, compared with

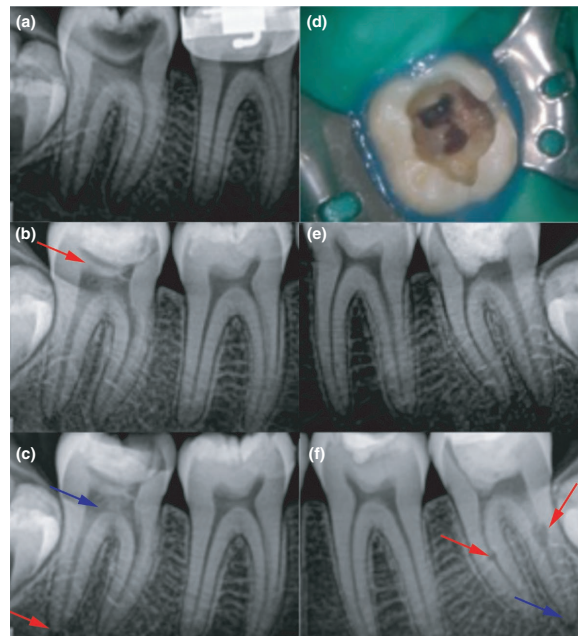


FIGURE 5 Two separate patients presented with extremely deep carious lesions on teeth 47 and 37, respectively. Both teeth treated with partial pulpotomy using Biodentine™. (a) Deep carious lesion on tooth 47 presented with discomfort. (b) Six months after partial pulpotomy hard tissue bridge evident (red arrow). Pulp sensibility testing confirmed a positive response. (c) Twelve months after partial pulpotomy, there is no evidence of an emerging radiolucency (red arrow). Presence of radiopaque material indicative of ectopic hard tissue deposition in pulp chamber (blue arrow). (d) Deep carious lesion removal and partial pulpotomy procedure on tooth 37. (e) At 3 months, sensibility was maintained at a positive reading and there are no adverse signs or symptoms. (f) Twelve months after partial pulpotomy, the tooth responded to pulp sensibility tests, no evidence of an emerging radiolucency (blue arrow), but areas of internal resorption were evident in the 37 (red arrow) denoting treatment failure

82% of samples with moderate pulpitis and 37% of cases with mild pulpitis (Table 3), there was no significant correlation between tenderness and increased failure rate ($p = .61$).

The results were subdivided by preoperative caries depth (judged radiographically) into “deep” and “extremely” deep caries where it can be seen the majority of cases included in the study were “extremely deep” ($n = 50$), rather than “deep” ($n = 12$) caries (Table 5). Of the 12 “deep” cases, 7 were later excluded as the pulp was not exposed after non-selective caries removal, whilst all the “extremely” deep cases were exposed; 4 were excluded as the pulp was necrotic on entry to the pulp chamber. The 4 deep “caries” cases were all successful, albeit one case with irreversible/moderate pulpitis was unresponsive to sensibility testing, whilst all 5 failures presented within the “extremely” deep caries group (Table 5). The two “blinded” evaluators demonstrated a moderate level of agreement in assessing caries depth from an intraoral periapical radiograph ($\kappa = 0.57$).

This study was designed as a single-visit partial pulpotomy procedure with a second visit scheduled 1–2 weeks later for permanent restoration placement. Restoration of the entire cavity with Biodentine™ is recommended by the manufacturers (Septodont); however, this is reliant on the material setting. In only two cases did the material set within the manufacturer's stated time of 12 min, with one case taking 45 min to set. The average setting time for Biodentine™ was 22 min. A Wilcoxon rank-sum test revealed between the average setting time to be significantly different from the manufacturer's claim ($p < .05$). In all 41 cases reviewed at 1 year, no visual discolouration of tooth substance was detected.

DISCUSSION

Partial pulpotomy is defined as the removal of a small portion of coronal pulp tissue after exposure, followed by

TABLE 5 Cases subdivided on caries depth (deep or extremely deep)

Cases meeting inclusion criteria	Subdivision due to caries depth (number)	Exclusions (reason)	Number enrolled	Number reviewed (reason for drop out)	Preoperative diagnosis	Outcome (success %)
62	Deep (12)	7 (non-selective caries removal not exposed)	5	4 (1 did not attend follow-up)	Reversible: 3 Irreversible: 1	Reversible: 3 Success (100%) Irreversible: 1 Unresponsive success (100%)
					Mild: 3 Moderate: 1 Severe: 0	Mild: 3 Success (100%) Moderate: 1 Unresponsive success (100%) Severe: 0
	Extremely deep (50)	4 (necrotic on entry to pulp)	46	37 (9 did not attend follow-up)	Reversible: 15 Irreversible: 22	Reversible: 10 Success; 5 Unresponsive success (100%) Irreversible: 14 Success; 4 Unresponsive success; 4 Failures (82%)
					Mild: 16 Moderate: 16 Severe: 5	Mild: 11 Success; 5 Unresponsive success (100%) Moderate: 11 Success; 3 Unresponsive success, 2 Failures (87%) Severe: 2 Success, 1 Unresponsive success, 2 Failures (60%)

Note: Unresponsive cases were classed as a success.

application of a biomaterial directly onto the remaining pulp tissue prior to placement of a permanent restoration (ESE, 2019). Partial pulpotomy has been advocated as the preferred treatment for cariously exposed pulp tissue as it removes both the biofilm on the surface of the pulp tissue and the superficially inflamed pulp and it has been recognized as suitable treatment for permanent teeth with signs of pulpitis and even irreversible pulpitis (Massler, 1959; Taha & Khazali, 2017). It has been advocated as a possible management strategy for young cariously exposed teeth with no clinical symptoms, with success in excess of 90% reported at 1 year for cases treated with partial pulpotomy and capped with calcium hydroxide (Mejäre & Cvek, 1993; Zilberman et al., 1989). Indeed, the results of this study using HCSC in a range of ages are in accordance with the outcome of these studies (Mejäre & Cvek, 1993; Zilberman et al., 1989) highlighting an overall success of 90% for partial pulpotomy after carious exposure, but with associated symptoms. Indeed, the high rate of success in the current study may be partly attributed to the use of an enhanced technique using an operating microscope, disinfection of the pulpal wound, strict asepsis using rubber dam and placement a HCSC in what has been described as a class II VPT procedure (Bjørndal et al., 2019).

A principal problem of treating particularly deep, and to a lesser extent, extremely deep caries is the decision as to whether carious dentine should be left in a one visit or stepwise excavation technique with the avoidance of pulp exposure (Bjørndal et al., 2010, 2019); or whether the carious dentine should be completely removed and the pulp exposure examined and managed (Ricucci et al., 2020). In this study, all teeth presented with symptoms associated with a deep or extremely carious lesion, so a decision was made to non-selectively remove the carious tissue in all cases. Although this could be considered overtreatment by some (Schwendicke et al., 2016), others have reported excellent outcome by non-selective carious removal and careful restoration of the exposed pulp (Marques et al., 2015). Notably, in the current study all teeth were symptomatic so partial pulpotomy was selected and both deep and extremely deep caries groups were included. Indeed, only cases that were not exposed after non-selective removal were treated by indirect pulp capping and excluded from the study even though they were symptomatic. This was carried out for methodological reasons as within the design it was critical that “sound” dentine was not breached in order to expose the pulp. Interestingly, 2 moderate and 1 severe pulpitis (or 3 irreversible pulpitic teeth) were contained within this group and although one case of moderate pulpitis failed, the other two cases did not fail within the designated review period. It may be stressed, however, that these cases were not included within the review protocol and the outcome of indirect

pulp capping in symptomatic pulpitis was not an aim of this study. Nonetheless, it is an interesting observation that warrants future study. Further subanalysis of the results highlights that the majority of the included cases were extremely deep (90%) rather than deep caries. This likely represents an increased chance of extremely deep caries being symptomatic, which was an inclusion criterion for this study. Notably, all extremely deep cases were exposed, whilst only 42% of the deep cases were exposed after non-selective removal and all failure cases were in the extremely deep caries group highlighting the importance of caries depth in the outcome of VPT (Bjørndal et al., 2019). Two experienced dentists, independently and blinded to outcome evaluated the radiograph dividing them into deep and extremely deep caries with a moderate level of inter-operative agreement ($\kappa = 0.57$). This agreement may have been expected to be higher, but perhaps reflects the difficulty (and arbitrary nature) of objectively analysing a two-dimensional radiograph and separating into distinct categories, particularly for borderline cases as well as periapical radiograph distortion. In this study, discussion afterwards resulted in consensus regarding the depth of caries. Finally, all the failure cases occurred in the extremely deep caries group, which highlights the potential prognostic benefit of establishing and recording radiographic caries depth preoperatively.

As a result of an improved understanding of the pulp's response to injury (Smith et al., 2016) and the advent of HCSCs such as MTA and Biodentine™, there has been renewed interest and improved outcomes in the area of conservative treatment of pulp exposure (Aeinehchi et al., 2003; Nair et al., 2008; Parirokh et al., 2017). However, a continual challenge for strategies aimed at preserving the pulp is the establishment of an accurate assessment of the clinical features that reflect the “true” histological condition of the pulp, as it has been shown that infection and inflammation reduce the outcome of the therapy (Al-Hiyasat et al., 2006). It has long been considered that the correlation between signs and symptoms of pulpitis and histological cell infiltrate was poor (Dummer et al., 1980); however, a recent study highlighted a good association between symptoms and histological status of pulpitis (Ricucci et al., 2014). The finding that inflammation is generally localized to the area sub-adjacent to the carious lesion stimulated new research into the preservation of pulp tissue and conservative management in cases with reversible and irreversible pulpitis (Simon et al., 2013). Indeed, even more recent data on the histological and bacterial profile of the carious teeth in advance stages of disease could also support the data that inflammation is localized to the pulp chamber and relates to the carious lesion penetration, whilst underlining the importance of caries depth (Demant et al., 2021).

In the current pragmatic study carried out in one general dental practice, partial pulpotomy was generally effective with an overall success rate of 90% with only one painful failure occurring within the year-long review. These results differ from a recent study investigating pulp capping of asymptomatic carious exposures with HCSC, which reported a high level of early failure (Ballal et al., 2020). This can perhaps be explained by the fact that all pulpal wounds within this study were washed with NaOCl, which has been shown to reduce painful failure in the study (Ballal et al., 2020) and also that partial pulpotomy was used rather than simply pulp capping, a technique that removes the superficially inflamed and infected dentine and pulp tissue and is recommended for procedures with increased pulpal damage (ESE, 2019).

As a result of the success of VPT procedures being advocated in cases of irreversible pulpitis, a new classification system has been proposed and linked to treatment indications (Wolters et al., 2017). The diagnostic system proposed by Wolters and co-workers was used in this study as a way of investigating how the nature and severity of symptoms affected outcome. The influence of exposure on the outcome of VPT remains controversial with multi-centre trial evidence reporting a negative prognostic effect (Bjørndal et al., 2010), whilst several principally single-arm studies carried out in Endodontic practice reporting high VPT success after carious exposure (Bogen et al., 2008; Marques et al., 2015; Taha & Khazali, 2017). In this study of symptomatic teeth, it was elected to non-selectively remove all carious dentine in every case and if the pulp was exposed carry out a partial pulpotomy. This approach was previously adopted when treating both symptomatic and asymptomatic teeth with partial pulpotomy (Mejäre & Cvek, 1993).

Any classification of pulpitis should ideally guide a subsequent decision as to whether the pulp can be maintained; however, the task of accurately ascertaining whether the pulp is actually irreversibly inflamed remains a significant challenge (Bjørndal et al., 2019; Mejäre et al., 2012). Multiple recent reports highlighting high success rates for partial and full pulpotomy in teeth with signs and symptoms of irreversible pulpitis (Simon et al., 2013; Taha & Khazali, 2017; Uesrichai et al., 2019) underline the need for a more sensitive classification of pulpal disease. This need has stimulated research groups to suggest new classifications or subdivisions of pulpitis that may be more reflective of the likelihood of the pulp to respond to conservative treatment (Hashem et al., 2015; Wolters et al., 2017). One such classification (Wolters et al., 2017) divided pulpitis into four practical categories, three of which were associated with symptoms. On closer examination (Table 2), two of Wolters proposed groups moderate and severe pulpitis simulate a division of the AAE

irreversible pulpitis group (AAE, 2013; Wolters et al., 2017). The current study demonstrates that such a division may be helpful in predicting which cases are likely to fail after treatment as the severe group had significantly more failure (60% successful) than the mild group; however, the moderate group was not significantly different from the mild group. Notably, these differences were lost when the broader descriptor irreversible pulpitis was employed. This offers the potential for symptoms and the use of Wolters subdivisions to offer a prognostic guide for clinicians when deciding whether to embark on VPT. Indeed, within the limitations of this work subdivision of patient preoperative symptoms was a more useful indicator of success than preoperative features such as TTP or the intraoperative assessment of pulpal haemostasis. Furthermore, the increased complexity by adding more divisions to the new classification system was considered simple to use and in practical terms only involved small extensions to existing pain history relating to the nature and duration of the pain (Table 2).

Bleeding has long been considered important to both the success and practicality of VPT (Accorinte et al., 2008; Matsuo et al., 1996). Recently, time to arrest haemorrhage was highlighted as a potential prognostic indicator in primary teeth (Mutluay et al., 2018). In the current study, variation in bleeding was evident with copious haemorrhage evident in teeth displaying very mild symptoms, and alternatively symptomatic teeth displaying only a small amount of bleeding. Although bleeding time was increased in severe and irreversible pulpitis categories (Table 3), there was no significant correlation between bleeding time and outcome. Clearly uncontrolled haemorrhage can indicate a severely damaged pulp as well as influencing the placement of certain restorative materials, for example resin-based materials (Accorinte et al., 2008); however, this may not be so critical for hydrophilic materials such as HCSCs. Notably, in this study there were no cases in which the bleeding could not be arrested prior to material placement.

When considering the clinical features to assess pulpitis, it was decided to use the most reliable findings in order to label the teeth. Indeed, pain and response to vitality test were the principal characteristics assessed. It is difficult to predictably quantify the level of TTP, so this variable was dichotomized into TTP positive or negative. Surprisingly, TTP was frequently evident in mild and moderate pulpitis, whilst it was present in all severe pulpitis cases; however, its preoperative presence was not significantly linked to failure in this study. An unusual finding in this study was the fact that 10 patients (1 deep and 9 extremely deep caries [Table 5]) were unresponsive to pulp sensibility testing after 1 year, which is at odds with previous partial pulpotomy studies using cold

testing (Taha & Khazali, 2017), which did not highlight this as a feature in their results. Within this study, these cases were grouped as a success as there was no sign of an emerging apical radiolucency or other sign of failure, which in line with a recent ESE position statement (ESE, 2019). Interestingly, pulp sensibility responses were temporarily lost in several cases, which is relatively common after dental trauma (Bastos et al., 2014). Longer term follow-up demonstrated recovery in the majority of cases, highlighting the necessity for continuing review (ESE, 2019). However, it is conceded that tooth sensibility tests may have been restored in more cases if the follow-up had been extended to 2 years. Success in this study was a composite measure of patient-reported and clinician-reported outcomes (Duncan et al., 2021), that included signs and symptoms of failure, response to pulp sensibility testing (responsive/ unresponsive) and radiographic analysis. It is possible that even in the absence of signs, symptoms and radiographic apical breakdown that the pulp may be necrotic and for this reason the results were recalculated as a “worst-case” scenario with all “non-responsive” cases having failed (Table 3). If unresponsive cases are considered as a failure, the overall success of partial pulpotomy after 1 year drops to 66%; however, the same trends related to pulp classification are evident with mild pulpitis 84%, moderate pulpitis 71% and severe pulpitis 40% successful, respectively. The problem with reliable sensibility testing after VPT although, not previously highlighted in partial pulpotomy studies of symptomatic cases (Taha & Khazali, 2017), has been highlighted as a concern after complete pulpotomy in the recent deep caries position statement, which suggested that a positive responsive to sensibility testing was unlikely in these cases (ESE, 2019).

Ten of the 51 included teeth were excluded because they failed to attend recall appointments or have the definite restoration placed (19.5% drop out). Of the 10 exclusions, four attended the clinic in pain within 6 months and were managed with RCT. In all four cases, the patient did not return for the second visit as planned, and after several months, the pulp was necrotic and the Biodentine™ was dislodged. These anecdotal data question the manufacturer's claims that the material is suitable as a long-term temporary restorative material. In one molar, internal resorption was evident in both roots, which although the tooth was otherwise a success this is still classed as an unusual type of failure (ESE, 2006).

Biodentine™ is marketed as a single-visit VPT material with a setting time that should be 12 min. Mixing and dispensing the material according to the manufacturer instructions did not prevent differences in the consistency of the Biodentine™ once the mixing process was complete. Some samples demonstrated a “creamy” aspect whilst some others had a dry and brittle consistency. Once

placed in the cavity, Biodentine™ assumed a soft and flowable consistency. The material generally took significantly longer to set than would be practical in one session with an average setting time of 22 min, which is almost double the manufacturers indicated time. As VPT is often carried out as an emergency procedure, the potential attractiveness of a single-visit treatment is considerable; however, a longer setting time may lead to the dentist to opt for a two-stage procedure, reducing the advantage of Biodentine™ over the classic formulation of MTA (which sets in several hours). Notably, Biodentine™ was not associated with any postoperative discolouration or staining which have been a feature of other MTA materials (Camilleri, 2015). With the limitations of this study, the results confirm the studies carried out on Biodentine™ discolouration (Camilleri, 2015; Kohli et al., 2015; Yoldaş et al., 2016).

Although this work is the first to analyse the effectiveness of a new classification of pulp condition, it has certain limitations. The study had only one operator, which although reducing intraoperative variability and increasing practicality, as it was set in primary care, does reduce generalizability of the data as only one individual carried out the operator elements of the work. In order to reduce potential bias, the radiographs were blinded and assessed by a second operator; however, for practical reasons the review appointments were carried out by the operator. It is accepted that larger studies with multiple operators will be required in the future to verify the results of this study. Furthermore, there was no control group in the study as the study was designed as an outcome study to assess the relative effectiveness of a new pulp classification to which the patients could not be randomized. Finally, Wolters and co-workers designed a new classification system as part of an overall strategy to guide subsequent endodontic management (Wolters et al., 2017). In the current study, the management recommendations were not followed and every pulp that was exposed after non-selective caries removal was treated by partial pulpotomy. Although this limits the applicability of the management arms of that study, it was carried out to limit bias and allow comparison of the management of the exposed pulp using two differ pulpal diagnosis classifications.

CONCLUSIONS

Partial pulpotomy using Biodentine™ was successful in 90% of analysed cases for the treatment of symptomatic carious exposures of the pulp after 1 year, but included cases where pulp vitality could not be confirmed. Within the limitations of this study, it can be concluded that cases with signs and symptoms indicative of irreversible pulpitis were as successful as cases with reversible pulpitis;

however, using Wolters classification cases with severe pulpitis all presented with extremely deep caries and were significantly less successful than cases with mild pulpitis, which highlights a potential prognostic benefit in diagnostically subdividing cases with more severe symptoms. Bleeding time and tenderness to percussion were not significant indicators of the outcome of partial pulpotomy procedure and although Biodentine™ did not induce discolouration after 1 year, the setting time was significantly longer than the manufacturers claim.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTIONS

RC carried out all experimental work and review of patients as well as drafting parts of the manuscript. HD planned study, assess radiographs, drafted and edited sections of the manuscript.

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Appendix V: Published article in Giornale Italiano di endodonzia 2020. COVID-19 and dental practice: overview and protocols during pandemic



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MINI REVIEW ARTICLE/MINI REVIEW DELLA LETTERATURA

COVID-19 and dental practice: overview and protocols during pandemic

COVID-19 e odontoiatria: generalità e protocolli durante la pandemia

KEYWORDS

SARS-CoV-2, COVID-19, Dental Practice, Infection Control, Pandemic

PAROLE CHIAVE

SARS-CoV-2, COVID-19, Odontoiatria, Controllo dell'infezione, Pandemia

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Abstract

In December 2019, in Wuhan (China), there were described the first cases of a Severe Acute Respiratory Syndrome caused by SARS-CoV-2 and named COVID-19. Since then the disease has spread in several countries and in March 2020 the WHO declared it pandemic.

COVID-19 is associated with a wide range of manifestations from no symptoms to temperature cough, dyspnea, need for artificial ventilation and eventually death. Mortality has been reported to be around 3%. Cases of spreading from asymptomatic infected individuals have been documented.

It has become clear that among healthcare professionals, dentists are the most exposed category to the risk of such infection as the routes of transmission are contact, droplets and aerosol, therefore the necessity of providing clear guidelines has suddenly arisen.

This article is aimed at analysing the available literature about "SARS-CoV-2" and "COVID-19", and comparing it with the guidelines for other coronavirus infections and dental practice with a view to providing clinical recommendations about prevention and infection control in the dental environment.

Nel dicembre 2019 sono stati descritti a Wuhan (Cina) i primi casi di Severe Acute Respiratory Syndrome causata dal virus SARS-CoV-2 e chiamata COVID-19. La patologia si è successivamente diffusa in diversi Stati fino a che nel marzo 2020 l'OMS ha dichiarato lo stato di pandemia. COVID-19 ha diverse possibili manifestazioni: può essere completamente asintomatica o presentarsi con febbre, tosse, dispnea e talvolta necessità di ventilazione assistita con rischio di morte. La mortalità si attesta attorno al 3%. Sono stati documentati casi di contagio avvenuto mediante portatori sani.

Dato che il virus si trasmette mediante contatto, droplet e aerosol, i dentisti risultano la categoria più a rischio di contrarre la malattia rispetto agli altri operatori sanitari. Per questo motivo delle linee guida sulla gestione della pratica odontoiatrica durante la pandemia sono quanto mai necessarie.

Lo scopo di questo articolo è di analizzare la letteratura disponibile riguardo "SARS-CoV-2" e "COVID-19", comparandola con le linee guida esistenti per gestire i coronavirus nell'ambiente odontoiatrico, al fine di poter dare delle raccomandazioni inerenti prevenzione e controllo dell'infezione da SARS-CoV-2 nello studio odontoiatrico.

Introduction

In December 2019, several cases of viral pneumonia were discovered in Wuhan, Hubei, China. A novel coronavirus was recognised as the pathogen responsible for the infection and named 2019 Novel Coronavirus

(2019-nCoV), then changed in SARS-CoV-2 (1, 2). Since then the virus has spread in 177 countries.

On the 12th of February 2020, the World Health Organisation (WHO) named COVID-19 the disease caused by SARS-CoV-2 and in March 11th, declared it a pandemic (WHO Feb 2020; WHO Mar 2020).

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Healthcare professionals are exposed to this virus that can be spread through contact, droplets and airborne and indeed dentists are one of the most exposed category (Gamio 2020; Peng et al. 2020).

The aim of this article is to collect the available literature and provide guidelines based on literature and guidelines about SARS and MERS epidemic events and from the information so far available on SARS-CoV-2.

A comprehensive MEDLINE search up to 22th March 2020 was conducted using medical subject headings (MeSH) in combination with 'and' or 'or'. The MeSH terms searched were 'SARS-CoV-2', 'COVID-19', 'Dental' and 'Dental Practice'. In addition, the following terms were added, 'Dental Surgery', '2019 -nCoV'.

Because of the lack of scientific papers a comprehensive MEDLINE search up to 22th March 2020 using MeSH 'SARS-CoV-2', 'COVID-19', in combination with 'and' or 'or' was carried out. All the relevant papers regarding infection control and prevention were hand-searched in order to find all possible information that may apply to the dental field.

Review

General features

Coronavirus were isolated for the first time in 1966 from patients with common cold: they are a class of enveloped positive sense RNA viruses with diameter between 60 and 140 nm whose name come from the presence of spike-like projections that may resemble a crown (7, 8).

Coronaviruses are divided in four subfamilies a, b, g, d. SARS-CoV-2 belongs to the B lineage of the b-coronaviruses. It is most likely to have in bats its natural host and appears to have many similarities with the virus SARS-CoV (9, 10).

All ages and genders are potentially affected by COVID-19 even though males seem to be more susceptible to the infection. The range of symptoms goes from a completely asymptomatic state to Acute Respiratory Distress Syndrome (ARDS) and possible death (11). Common clinical features are quite not specific including

high temperature, cough, myalgia, dyspnea, sore throat and conjunctivitis but the virus can be carried by asymptomatic people that are able to infect others (Pan et al. 2020; Rodriguez-Morales; Rothe et al. 2020; Singhal 2020).

Individuals with underlying medical conditions are more susceptible to an unfavourable outcome. The risk of death of patients presenting comorbidities like cardiovascular disease, diabetes, chronic respiratory disease, cancer and COVID-19 is statistically higher (Coronavirus Outbreak 2020).

Intensive care is needed in 25-30% of the cases, with an average hospital stay of ten days. Fatality ranges between 2 and 3 % and it is more common in the elderly and people who suffer from co morbidities (15). Three routes of transmission have been found (table 1).

1) Contact transmission: when someone touches an infected object and subsequently touches his mouth, nose or eyes. 2) Droplets transmission: when the droplets generated by cough and sneezes are ingested or inhaled. 3) Airborne transmission: when droplets mix with the air creating aerosols that may cause infection if inhaled (11, 17). A feco-oral transmission is considered possible as well (18).

Human coronaviruses can stay active on certain surfaces from hours up to few days, but are inactivated within one minute after the disinfection with 0.1% sodium hypochlorite or 62-71% ethanol (19). On the other hand the virus has demonstrated to be viable in aerosols for at least 3 hours (20).

SARS-CoV-2 and dentistry

Possible transmission

Due to the nature of SARS-Cov-2 and its routes of transmission, it is clear that dentists, dental staff and patients are at risk of infection when dental treatments are provided (6).

Contamination can happen directly between operators and patients due to droplets during pre-clinical assessment or discussion of the treatment plan but contamination of air and surfaces plays a strategic role in the diffusion of the virus.

**Table 1****Possible transmission of SARS-CoV2**

Transmission route	Method of transmission	References
Contact	Direct or Indirect contact with mucosae	Kampf 2020
Droplets	Droplets of infected saliva ejected with cough or sneezes	Peng 2020
Airborne	Mix of air and viruses that can be inhaled	Peng 2020
Feco-Oral	Possible transmission through digestive tract	Zhang 2020

It has been demonstrated that dental treatments and in particular dental hygiene procedures produce aerosol and splatters with presence of microorganisms, causing contamination of tools, equipment, gowns, surfaces and air (21, 22).

Contamination of surfaces is unavoidable when aerosol is produced and SARS-CoV-2 can resist several days on surfaces, putting the operators at risk of infection (19). Apparently the virus persists better at room's humidity 50% than 30% (6).

Infection Control

Standard precautions used in dental practices are able to prevent cross-infections originated by direct contacts with body fluids and contaminated surfaces. SARS-CoV-2 though, presenting three routes of transmission including the airborne needs to be dealt with Transmission-Based Precautions (17).

Patient Screening

During the outbreak of SARS-COVs it is not recommended to perform routine dental treatments as the risk of contributing to the spread of the virus is high. Dental care should be provided just for dental emergencies or urgent dental care (6). Dental emergencies are considered potentially life threatening conditions such as uncontrolled bleeding, cellulitis with swelling that could compromise the airways, facial trauma with possible airways involvement. Hospital emergency departments are overloaded during a pandemic and urgent dental care service should be provided for those patients that otherwise would have to use hospital services. Severe dental pain and conditions like pericoronitis of third molars/surgical

post extractive osteitis should be considered urgent (23).

It is therefore important to perform a telephone triage in order to assess the risks and the severity of the condition. An interview over the phone is then necessary to prevent to bring infected patients into the surgery as much as possible.

The following questions should be asked (6, 24):

1. Have you experienced fever or symptoms like cough, shortness of breath or other respiratory problems in the past 14 days?
2. Have you been in contact with someone affected by COVID-19 in the past 14 days?
3. Have you been in contact with someone reporting fever or respiratory problems in the past 14 days?
4. Did you participate in gatherings, meetings or had contact with many unacquainted people in the past 14 days?
5. Did you travel from areas with Level 3 Travel Health Notice for COVID-19? (this question can be omitted when the virus is declared at community level)

If the patient answers yes to any of these questions the treatment should be postponed and the patient invited to contact his physician if the answer to questions number one or two is positive.

Patient Management

When emergency care must be provided, preventive measure should be taken in order to decrease the possible viral load (25). Chlorhexidine is often used in dental practice as an antiseptic, but this will not probably be effective against SARS-CoV2. The virus appears to be vulnerable to oxidation, thus a pre-operative rinse with



1% hydrogen peroxide should reduce the viral load in the oral cavity (6, 19, 23). Other than hydrogen peroxide, Povidone 0.2% has been recommended as rinse, as these two antiseptics have been demonstrated effective to reduce salivary viral load with low risk of causing secondary complications in the oral cavity (26).

Personal Protective Equipment

SARS-CoV-2 infection may occur through direct or indirect contact and aerosol, therefore when the outbreak is at community level Transmission-Based Precautions for contact, droplets and airborne must be taken for all patients. Standard surgical masks are effective against splashes and large-sized droplets but not effective against small airborne particles thus fit-tested N-95 respirators or superior ones are required, even more when aerosol-producing treatments are performed (28, 17, 29, 27).

Protective disposable impermeable gowns and caps are recommended as well as gloves and eye protection. These medical devices should be worn before coming in contact with the patient and discarded safely before leaving the room (28, 17). It is paramount that all the staff is adequately instructed on the use and doffing of Personal Protective Equipment (PPE) as deviation from standard procedures will increase the risk of infection (30).

Air Supply

Airborne Transmission Based Precautions treatments performing aerosol should be treated in Airborne Infection Isolation Room (AIIR) that is a single-patient room designed to maximise the infection control, equipped with special air handling and ventilation capacity compliant with AIIR standards (29). A COVID-19 positive or suspected patient should not be treated in a dental surgery

Table 2

Dental Emergency and Urgent Dental Care (ADA 2020)

	Description	Conditions
Dental Emergency	Potentially life threatening conditions that require immediate care	• Uncontrolled bleeding • Diffuse bacterial infection with intra-oral or extra-oral swelling that potentially compromise the patient's airway • Trauma involving facial bones, potentially compromising the patient's airway
Urgent Dental Care	Conditions that require immediate attention to relieve severe pain/infections and to avoid patients to seek for treatment in hospital emergency departments	• Severe dental pain from pulpal inflammation • Pericoronitis or third-molar pain • Surgical post-operative osteitis, dry socket dressing changes • Abscess, or localized bacterial infection resulting in localized pain and swelling • Tooth fracture resulting in pain or causing soft tissue trauma • Dental trauma with avulsion/luxation • Dental treatment required prior to critical medical procedures • Final crown/bridge cementation if the temporary restoration is lost, broken or causing gingival irritation • Biopsy of abnormal tissue

**Table 3****Recommended Precautions for SARS-CoV-2**

Transmission	PPE	Indications	Environment	Patient Management	References
Contact	- Gloves (Latex or Nitrile) - Isolation Gowns - Disposable cap	Wear these PPE before any contact with the patient and dispose them safely at the room entrance. Doffing of gowns and gloves must be done following existing guidelines.	- Eliminate all unnecessary equipment from the room - Be extremely careful in the disposal of contaminated equipment	- Ask the patient to use hand sanitiser when arrives - Ask the patient to wear a mask until his treatment	6, 11, 12, 17, 29, 30, 31, 32, 33.
Droplets	- Goggles - Face Shields	Goggles with antifog system have to be preferred. Face shields are more protective, especially if extended from chin to crown	Disposable covers help avoiding surface contamination	- Ask the patient to follow respiratory hygiene and cough etiquette - Use Rubber dam	
Airborne	N95 Respirators (FFP2)	N95 Respirators are recommended to avoid airborne transmission. Face fitting is mandatory	- Avoid as much as possible to create aerosol - Avoid the use of rotary handpieces and minimise the use of 3 in 1 syringe - If available, patient should be treated in AIIR	Ask the patient to rinse his mouth with 1% hydrogen peroxide solution	

that has not the above mentioned equipment as it would be impossible to use airborne precautions. Since infection from SARS-CoV-2 is proven to be possible from asymptomatic patients it is recommended not to treat patients unless it is a dental emergency and, even in case of asymptomatic patients, all PPE for airborne precaution should be used, all possible care has to be used to reduce the amount of aerosol produced and the single patient room has to have the door closed and adequate ventilation to dilute the infected air. Table 2 reports what is recognised as dental emergency and urgent dental care according to the American Dental Association (23). Recommended precautions are summarised in table 3. If the patient is symptomatic or at

high risk to be infected he should attend the practice and, if he does, be asked to wait in a closed room with a surgical mask covering nose and mouth, then the patient should be referred to the hospital or dealt with as regulated by the local health authority (17, 31).

Disinfection

Alcohol based or sodium hypochlorite based disinfectants are active against coronaviruses and they should be used to disinfect not only every component of the dental chair but the surfaces that can be in contact with aerosol spray as well. Removing from the room every not necessary equipment and covering all possible surfaces with disposable covers may help in improving the contact infection control.



Conclusions

Sars-CoV-2 is a very infective virus that causes COVID-19, a disease with a very broad range of manifestations, from lack of symptoms to ARDS and eventually death. Because of the routes of transmission of this pathogen dentists are among the health professionals who are exposed to high risk of infection.

When the disease is at community level, it is paramount that dental care professionals protect themselves, their staff and patients, avoiding any risk of spreading the virus. It is important to remember that completely asymptomatic patients are carriers of the infection.

In this view it is mandatory to postpone any elective treatments and dentists should treat only emergencies or provide care for those whom, if not treated, would need hospital care (table 2). In any case risk assessment over the phone is important to avoid high risk patients to attend the surgery if this is not equipped for proper airborne infection control.

In the dental environment infection may

occur because of direct or indirect contacts with body fluids of an infected person or touching contaminated surfaces and then touching eyes or face. Droplets are a possible route of transmission as well as aerosol generated from dental treatments (table 1). When treating a dental emergency during the outbreak of an airborne transmitted pathogen, Standard Precautions for infection control are not sufficient and Transmission Based Precautions must be applied.

Ideally treatments should be provided in AIIR when treating patients that have possibly come in contact with the virus. Because dental practices are not usually equipped with an AIIR, if an emergency arises it is important to use a single patient room with closed door, ensure adequate ventilation to the room, remove all unnecessary equipment and cover all surfaces that can be contaminated.

Because of the aerosol produced, operator and assistant must wear face fitting N95 respirators, goggles/face shields, isolation gowns, gloves and disposable caps. These PPE must be worn before any contact with the patient and disposed safely before leav-

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ing the room. It is important that dental staff is correctly instructed on the correct use and disposal of protective equipment as incorrect doffing has been linked with increased risk of infection.

Patients should be requested to disinfect their hands on the arrival with an alcohol based hand sanitiser, to follow respiratory hygiene and cough etiquette and to wear a surgical mask until the treatment commences. A rinse with 1% hydrogen peroxide may be helpful to reduce the amount of SARS-CoV-2 in the saliva.

Dental aerosol should be avoided: use of ultrasonic scaler or high speed handpieces is not recommended and the use of the 3 in 1 syringe should be reduced. Rubber dam must be used when possible and should cover mouth and nose.

Dental surgery decontamination has to be carefully performed. The virus is rapidly inactivated by disinfectant containing 0.1% sodium hypochlorite or 62-71% ethanol. Because of the aerosols the room has to be adequately ventilated to reduce the amount of virus present in the air.

SARS-CoV-2 is a challenge for healthcare

professionals. During the outbreak of the disease only emergency treatments are recommended and special precautions and PPE must be used when providing dental care. Training and correct information about prevention and control of airborne infections should be provided to health-care workers.

Clinical Relevance

COVID-19 is a major concern for public health and dentists are among the health-care professionals facing the greatest risks of infection.

This article provides an overview on the disease and its routes of transmissions and gives indications for prevention and infection control in the dental environment.

Conflict of Interest

The Authors deny any conflict of interest

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Appendix VI: Published article in Frontiers in Dentistry 2020. Dental Practice after COVID-19 Pandemic: Analysis of Italian Guidelines and Current Literature to Propose Appropriate Protocols for Dental Practices



Frontiers in Dentistry

Dental Practice after COVID-19 Pandemic: Analysis of Italian Guidelines and Current Literature to Propose Appropriate Protocols for Dental Practices

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ABSTRACT

On March 11th, 2020, WHO declared COVID-19 a pandemic. This disease is caused by SARS-CoV-2, a virus that can be spread by symptomatic or asymptomatic carriers through contact, droplets and airborne transmission. Because of the transmission routes of this virus, dental treatments are considered "high risk" procedures, both for patients and operators. Italy was one of the most affected European countries and experienced a long lockdown period. Guidelines were provided by the Italian Health Ministry for a safe reopening of dental practices. In this article those guidelines are reported and compared with the available literature. The Italian model for reopening dental practice appears to be coherent with the indications provided by WHO and is supported by several studies.

Keywords: COVID-19; SARS-CoV-2; Dentistry

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INTRODUCTION

COVID-19 is a severe acute respiratory disease caused by the coronavirus SARS-CoV-2. The first cases of this disease were reported in December 2019 in Wuhan (China), but the virus rapidly spread and on March 11th, 2020, WHO declared it a pandemic. COVID-19 presents various manifestations, from a condition of absence of symptoms to acute respiratory distress syndrome that can lead to death [1]. Manifestations can be quite varied like high temperature, cough, dyspnea, and conjunctivitis. The infection can be spread by asymptomatic carriers [2] and may be transmitted through contact, droplets and

airborne routes and can have an asymptomatic incubation period of 14 days [3].

Dental professionals are constantly exposed to aerosols rich in infective agents and because of the abovementioned infection routes, they are considered a high-risk category [4]. The usual standard of disinfection and infection-prevention in dental practice is extremely efficient in protecting against other pathogens. However, it appears not to be sufficient to ensure safety for dental staff and patients and therefore, "Transmission Based Precautions" should be put in place [5]. Italy was one of the first European Countries to be hit by the pandemic and one of the most affected ones.

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After an initial strict lockdown, a lot of restrictions are still in place. Indications for dental practice have been issued by the Health Ministry [6]. The aim of this article is to report the Italian guidelines in view of the currently available literature as they may serve as a model for dental practice during COVID-19 pandemic.

Protocol Description

Triage

In order to avoid contact with possibly affected people as much as possible, an extremely accurate over the phone triage is mandatory. It should be done the day before the scheduled appointment, so as to obtain the most accurate information. The aim of the questionnaire is to evaluate the risk of infection and to investigate possible symptoms or contacts that may indicate that the person could be a carrier.

Receptionists must ask six questions before confirming any appointment and, if *even one* of the items is not completely satisfied, the appointment must be postponed.

- 1- Are you affected or do you suspect to be affected by COVID-19?
- 2- Have you been positive for COVID-19 and declared to be non-infectious? If yes, was this confirmed with a lab test?
- 3- Did you have contact with any positive person in the last three weeks?
- 4- Do you have or have you had any of these symptoms:

- Increased temperature
- Cough
- Dyspnea
- Conjunctivitis
- Diarrhea
- Sore throat
- Dysgeusia or altered sense of smell
- Headache
- Myalgia
- Fatigue
- Skin rash (children)

- 5- Did you have any contact with someone who is self-isolating or was sent to quarantine by the authority in the last four weeks?

- 6- Did you have contacts with COVID-19 positive patients in your workplace?

The whole dental practice has to be organised in order to decrease the risk of spreading the virus. Table 1 summarises the protocols that should be followed in both operative and non-operative areas. It is paramount to carefully follow every instruction and that personal protective equipment (PPE) donning and doffing is correctly carried out.

DISCUSSION

During the COVID-19 pandemic, researchers have focused their efforts on discovering the features of the virus and on gathering information on how to prevent or stop the spread. Dentistry has been one of the most affected professions due to the nature of the work itself.

The main problem regarding dentistry and COVID-19 are transmission pathways as this virus can spread through contact, droplets and airborne routes; therefore, transmission-based precautions have to be put in place [4,5].

Dental workers are well used to dealing with transmissible disease and cross-infection; hence, disinfection protocols and PPE have been already present in the dental environment. Standard dental sanitization protocols are more than sufficient to eliminate SARS-CoV-2 from instruments and surfaces, as the virus is not very resistant to disinfectants [11].

The reasons why this pathogen needs a different approach is its transmission via aerosol. Dentistry by nature is a high aerosol-generating profession and the fact that SARS-CoV-2 remains viable for hours in aerosol suspension causes an increased risk of spread [15].

Table 1 shows that Italian guidelines are supported by the current literature and they are quite comprehensive. Because the virus can be transmitted by asymptomatic individuals, every patient is considered a potential source of infection [2,6].

While some countries like Italy and Spain have very similar guidelines, other European States chose different ways to deal with the reopening of dental surgical clinics.

Table 1. Management of non-operative and operative rooms and personal protective equipment recommendations according to the Italian Guidelines

ACTION	
NON-OPERATIVE AREAS [7-13]	
Reception	Temperature check upon arrival. The patient is asked to: - Sanitise hands - Fill a COVID-19 survey and consent
Waiting room	- Remove all unnecessary objects. - Reduce the number of people present. When possible, the patient should come alone. - Face mask must be worn inside the waiting room. - Access to toilets should be regulated.
OPERATIVE AREA [3, 12-14]	
Surgery	- Prepare the operative area with all the necessary equipment/materials at hand and remove/cover everything that will not be used during the session. - Get all the relevant chartings/documents/x-rays ready before the patient enters the room. - Flush every instrument that generates water-spray for 20 seconds. - After the procedure, it is recommended to ventilate the room for 15 min. If air conditioner or mechanical ventilation is present, accurate handling and maintenance of the filters are paramount. - The operative area must be disinfected with either sodium hypochlorite (0.1-0.5%), ethanol (62-71%) or hydrogen peroxide (0.5%); all handpieces must be removed.
Patient	- Before treatments, the patient is asked to rinse with 1% hydrogen peroxide or 0.2% povidone-iodine or 0.05-0.1% cetylpyridinium chloride solution for one minute and then with a 0.2-0.3% chlorhexidine solution.
Protocols	- Use rubber dam whenever possible. - Prefer hand scaling to ultrasonic instruments to avoid aerosol. - Prefer extra-oral to intra-oral x-rays, whenever possible. - Use handpieces with anti-reflux designs.
Personal protective equipment [3,4,12-14]	
Masks	- Surgical masks are recommended for administration or sanitization procedures. - FFP2 masks/respirators are needed for every clinical procedure.
Face-shields/Goggles	- Goggles are less protective and should be used for procedures like consultation or sanitization. - Whenever aerosol is produced, face-shields should be used.
Gowns	- Single-use TNT gowns compliant with UNI EN 14126 must be used. - TTR gowns compliant with UNI EN 13795 can be used and sterilised up to 80 times.
Gloves	- Gloves must be worn for every procedure.
Caps	- A disposable cap must be associated to the gown.

The overall guidelines about patient triage, hygiene, and organisation of the workflow are similar, but some aspects differ from one country to the other.

CONCLUSION

During COVID-19 pandemic, performing or receiving dental treatments increases the risks for both patients and dental staff; therefore, it is important to adhere to strict protocols during the acute phase of the pandemic and its remission. Italian guidelines appear to be coherent with the literature and could be used as a model during the COVID-19 pandemic.

CONFLICT OF INTEREST STATEMENT

None declared.

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Appendix VII: Published article in Australian Endodontic Journal 2023. Efficacy of different irrigant activation devices in removing dentin debris from an artificial isthmus connecting curved canals

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ORIGINAL RESEARCH



Efficacy of different irrigant activation devices in removing dentin debris from an artificial isthmus connecting curved canals

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Abstract

The aim of this study was to evaluate the debridement efficacy of sonic irrigation (EDDY; VDW, Munich, Germany and EndoActivator; Dentsply-Sirona, Ballaigues, Switzerland) and ultrasonically activated irrigation (Irrisafe; Satelec Acteon, Merignac, France) in a simulated canal isthmus connecting curved canals. Transparent resin blocks were produced containing two curved canals connected with an isthmus. The isthmus was then filled with dentin debris. Three irrigant activation cycles were performed and the amount of remaining debris was compared analysing pictures taken after each activation cycle. Data were statistically analysed using one-way ANOVA and post hoc Tukey tests at a significance level of $p < 0.05$. EDDY showed greater efficiency in removing dentin debris from the simulated isthmus than the other techniques tested. An increase in the activation time enhanced the efficiency of both EDDY and ultrasonically activated irrigation in debris removal.

KEYWORDS

debris, irrigation, isthmus, sonic activation, ultrasonically activated irrigation

INTRODUCTION

Correct disinfection of the root canal system is paramount for success in endodontics; however complex anatomies represent one of the biggest challenges in achieving this goal [1]. An isthmus has been defined as a ribbon-shaped narrow connection containing pulp tissue that extends between two canals [2]. When present, this area is extremely difficult to reach with mechanical instrumentation and

can be easily blocked by debris resulting from the instrument action, thus representing a reservoir for microorganisms. These deposits can also limit the accessibility and effectiveness of irrigating solutions in these spaces [3, 4] and the persistence of bacteria in unprepared areas may adversely affect the success of surgical and non-surgical endodontic therapy [5–7]. The apical third of mandibular and maxillary molars presents the highest incidence of this type of anatomy, therefore it is important that

irrigant solutions may be physically and chemically effective in this region in order to disinfect the isthmus [8–11]. Conventional needle irrigation method (CNI) is considered to be insufficient due to the limited amount of fluid delivered in the apical third and the difficulties in effectively cleaning the dentin walls in anatomically complex areas [12, 13].

Many techniques, including sonic and ultrasonic devices, have been developed to overcome the drawbacks of CNI. Irrigant solution activation with ultrasonic devices (ultrasonically activated irrigation, UAI) is performed by placing a non-cutting ultrasonically oscillating (25–30 kHz) tip in the centre of the prepared canal filled with the solution [14]. The effects of ultrasonic activation are both cavitation and acoustic streaming. Acoustic streaming appears to be the one that enhances the cleaning effect by removing debris left in the canal after preparation through the shear forces that it produces [15, 16]. The superiority of UAI compared to CNI in cleaning root canal walls and removing organic tissue and debris has been demonstrated in many studies [17–19]. EndoActivator (EA; Dentsply Sirona, Ballaigues, Switzerland) and EDDY (VDW, Munich, Germany), which were more recently introduced in the market, are two popular sonic devices. EA has a flat and highly flexible polymer tip that works at low frequencies (<1 kHz) and creates less shear forces than UAI, while EDDY is a high-power sonic activation device that has a smooth and flat polymer tip and is activated by a sonic air-driven handpiece at 6 kHz [20, 21].

Several studies examined the debris removal efficacy of sonic and ultrasonic activation devices, which were generally tested with 3 activation cycles of the irrigant inside the canal [7, 19, 21]. However, in the available literature, there is no study investigating the debris removal efficacy of sonic or ultrasonic activation devices in an artificial isthmus model with curved canals at different activation times. Therefore, the aims of this study were to evaluate the debridement efficacy of EDDY, EA and UAI activation devices in a simulated isthmus connecting curved canals and the influence of different activation times on isthmus cleaning. The null hypotheses tested in the study were that there is no difference among the various activation devices tested in terms of debris removal from an artificial isthmus in the curved canal and among the different activation intervals tested.

MATERIALS AND METHODS

Root canal model

Polymethylmethacrylate (PMMA) root canal models, specifically designed and produced using a 3D printer (Ay Design, Ankara, Turkey) were used for the present study (Figure 1). The model consisted of a coronal reservoir (height 4 mm, length 5 mm, width 4 mm) and two root canals (length 16 mm, apical diameter 0.25 mm, taper 0.06) simulating two mesial root canals of a lower molar,

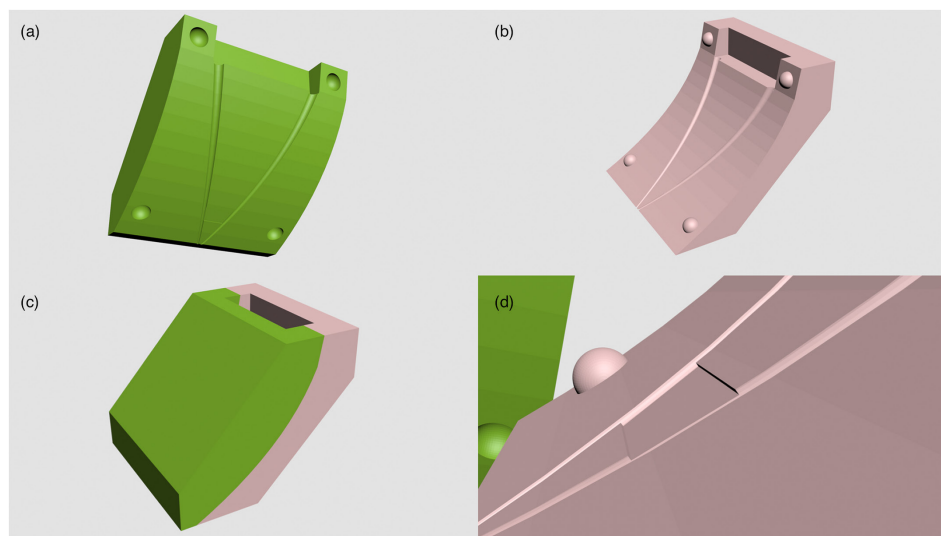


FIGURE 1 Three-dimensional rendering of the simulated canals used in the present study. The two pieces of the device are shown in a and b when separate and in c when assembled; a particular of the isthmus is shown in d.

both with a 45° mesiodistal and 15° buccolingual angle of curvature. The canals were connected by an isthmus (medio-distal depth 0.15 mm, corono-apical length 2 mm, buccolingual length 3 mm), located 2 mm from the apical terminus. This PMMA model was divided into two halves, with the isthmus located into one of these parts, such that the two halves can be assembled creating a closed canal system, as performed by Swimberghe et al. [21].

Simulated dentin debris

Simulated dentin debris was obtained from extracted teeth using a 320 GRIT coarse-grained sandpaper that produced dentine particles with a size of $71 \pm 23 \mu\text{m}$ measured with a non-contact optical profiler (NANOVEA ST400 3D, Tribotron AG, Switzerland). Simulated dentin debris was weighted with an electronic scale and mixed with distilled water in a standardised ratio to achieve a wet sand-like consistency (100 mg dentin chips with 0.1 mL distilled water). Then, simulated dentin debris was dried for 5 s with an absorbent paper to obtain a compound similar to wet sand [19]. Directly after applying the simulated dentin debris in the isthmus, the two halves of the model were re-assembled and tightened by means of screws and bolts.

Irrigation protocols

The same model was used for each experimental group and the test was repeated 20 times for each group. The canals and the coronal reservoir were filled entirely with distilled water which was used as an irrigating solution.

In Group 1, activation was performed with an EDDY tip driven by a high-power sonic scaler at 6000 Hz (Proxeo, W&H, Bürmoos, Austria). The EDDY tip was moved up and down over a distance of 4 mm, starting 1 mm from the apical terminus according to the manufacturer's recommendations.

In Group 2, activation was performed with EA equipped with a medium activator tip (size 25, 0.04 taper) and operated at the highest speed. The tip was moved up and down over a distance of 4 mm, starting at 1 mm from the apical terminus.

In Group 3, activation was performed with UAI using a non-cutting size 25 file (Irrisafe; Satelec Acteon, Merignac, France) driven by an ultrasonic device (P5 Newton; Satelec Acteon) at power setting 7. The ultrasonic file was pre-curved according to the canal curvature, positioned centrally in the canal 1 mm from the apical terminus and, while activated, moved up and down over a distance of 4 mm.

Twenty-second activation cycles were performed three times for each of the above groups. In between

each activation cycle, the canal was flushed with 5 mL of irrigating solution using a 30-gauge endodontic needle (Navitip; Ultradent, South Jordan, UT, USA).

In Group 4, as a control group, CNI was performed in each artificial canal using a 5-mL syringe with a 30-gauge endodontic needle for 60 s at a flow rate of 5 mL/20 s. The needle was moved up and down over a distance of 4 mm, starting 1 mm short of the apical terminus. After irrigation, the canals were dried with size 25.06 sterile paper points.

Images and image analysis

To determine the debris removal efficiency for each irrigation activation method, a standard amount of wet dentin was placed in the isthmus and the isthmus was photographed using a digital camera (EOS 200D; Canon, Tokyo, Japan). After each irrigation activation cycles (20/40/60 s), the two halves of the model were separated and the isthmus on a single half was photographed again. The amount of debris removed was calculated by superimposing the pre- and post-activation images with AUTOCAD software (AutoCAD 2012, Autodesk, San Rafael, USA). A total of 80 pictures for each activation method were obtained and the percentage of debris removal for every stage was obtained as follows: percentage of removed debris = (area before irrigation - area after irrigation / area before irrigation) $\times 100$.

Statistical analysis

To verify the assumption of normality, the data were first analysed using Shapiro-Wilk test. The normality test showed that the persistence of removed debris data had a normal distribution. Thus, a one-way analysis of variance and post hoc Tamhane tests were performed for intergroup comparisons. All the statistical analyses were performed using SPSS 21.0 (IBM - SPSS Inc., Chicago, IL, USA) software. The statistical significance level was set at $p < 0.05$.

RESULTS

Different percentages of removed debris after 20, 40 and 60 s were found according to the irrigation protocol used (Table 1). EDDY removed the biggest amount of debris from the simulated isthmus at each activation time ($p < 0.05$). UAI was the second most effective device ($p < 0.05$), while CNI and EA removed statistically less debris from the isthmus without any statistical difference between them ($p > 0.05$).

TABLE 1 Means and standard deviations of removed debris percentage after 20, 40, and 60 s after different irrigation protocols.

	Group 1 (EDDY)	Group 2 (EA)	Group 3 (UAI)	Group 4 (CNI)	p-Value
20 s	72.08 ± 5.64 ^{bx}	24.11 ± 2.64 ^{axe}	35.05 ± 4.90 ^{cx}	20.08 ± 3.87 ^{axe}	<0.05
40 s	80.86 ± 6.63 ^{by}	25.09 ± 4.12 ^{axe}	57.77 ± 8.55 ^{cy}	22.92 ± 2.12 ^{axe}	<0.05
60 s	91.55 ± 8.69 ^{bz}	27.35 ± 3.24 ^{axe}	73.66 ± 11.62 ^{cz}	25.46 ± 4.70 ^{axe}	<0.05
p-Value	<0.05	>0.05	<0.05	>0.05	

Note: Different superscript letters indicate statistically significant difference (a, b, c for rows; x, y, z for rows) ($p < 0.05$).

In EDDY and UAI groups, every 20 s increase in activation time significantly increased the amount of debris removed ($p < 0.05$). On the contrary, as the activation time increased, the amount of debris removed did not change in the CNI and EA groups ($p > 0.05$).

DISCUSSION

Despite the development of many new endodontic instruments, most of the root canal system might not be treated by mechanical instrumentation due to variability in the root canal anatomy, including isthmuses and recesses. Activation of the irrigant solution provides better cleaning of the endodontic system and its use is paramount to achieve the best disinfection possible of anatomically complex areas [20]. The aim of this study was to investigate the efficacy of sonic and ultrasonic activation methods and different activation times for removing dentin debris from an artificially created isthmus in models with curved canals. The null hypotheses tested in the study were rejected due to the differences in debris removal among the different irrigant activation techniques and times of activation.

Artificial acrylic models have been used to provide high levels of standardisation in examining the debris removal activities of different activation methods. In this way, variables such as canal sizes, dentin structure, root canal geometry and isthmus dimensions that may result from extracted natural teeth or bovine teeth have been minimised [19, 21, 22]. This study differs from many previous ones regarding artificial models because a curved canal model was used, reproducing better the root canal anatomy and generating more accurate results [19, 21, 22]. The acrylic transparent model made possible to see and evaluate the isthmus directly without any manipulation, as necessary in SEM studies [20, 23].

Ultrasonically activated irrigation tips have shown the best effect when placed 1 mm from the apex [24]. Therefore, the tips of the irrigation activation devices used in this study and the CNI needle tip were initially placed at a distance of 1 mm from the apical terminus. The goal of this study was to only evaluate the mechanical action of different activation devices in debris removal and not

assess the chemical effect of the irrigating solution used. Hence, plain distilled water was used as irrigant to eliminate any possible confounding factor in debris removal evaluation.

According to the current results, EDDY tip was the irrigant activation method that removed statistically more debris from the artificial isthmus in all activation intervals (20, 40, 60 sec). Although the operating frequency of EDDY is lower than UAI, it has removed more debris than UAI in the present study. Studies reported that EDDY removed less, equal or more debris than UAI [20, 21, 23, 25]. The cleaning effect of UAI has been linked with the file-induced microstreaming and cavitation generated by an instrument oscillating at ultrasonic frequency inside an irrigant-filled root canal [26]. EDDY produced a continuous and steady flow with revolving currents (eddies) in the isthmus, whose location and number differed from the isthmus type [27]. The fluid dynamic could explain the benefits provided by EDDY. Further investigations are required to confirm these findings.

The stiffness of the ultrasonic tip can damage the canal surface and limit the debris output [12]. For this reason, the ultrasonic tip was pre-curved in order not to dampen its energy in contact with the root canal walls. On the contrary, the flexibility of EDDY polymer tip permits a bigger lateral excursion while vibrating and this may have contributed to its increased effectiveness in cleaning the lateral isthmus.

Swimberghe et al. mixed debris with hydrogel-mimicking biofilm, placed it in an isthmus and examined the debris removal efficacy of sonic, ultrasonic and laser activation techniques [21]. Despite a different protocol, their results were similar to those from the present study, as they found that EDDY removed more hydrogel than UAI and EA and no differences between the CNI and EA groups. In the present study dentin debris obtained from extracted teeth was used and packed into the artificial isthmus, as previously reported [19, 21, 22, 28, 29].

In the present study, the activation of the sonic and ultrasonic tips was repeated 3 times, each activation lasting 20 s, and the amount of debris removed with each activation cycle was examined [4, 30]. According to the results obtained, increasing the irrigation activation time (20 to 40 and then 60 s) significantly enhanced the

efficiency of EDDY and UAI in removing debris from the artificial isthmus. With the increase in activation time, the percentage of debris removed by EDDY tip grew from 70% to 90% and by UAI from 35% to 70%. The current results are in agreement with those obtained in a previous study, which reported in all groups an increased debris removal efficiency as the activation time increased [19]. Similarly to our results, the authors reported that EDDY removed more debris than ultrasonic tips [19].

Duque et al. examined the debris removal effectiveness from the mesial root canals of mandibular molar teeth and isthmuses after a first, second and third activation period [30]. They reported that all the activation methods, including UAI, EA and CNI, removed more debris after three activation periods, regardless of the distance from the apex they were placed (2, 4 and 6 mm). On the contrary, in the present study, the increase in activation time did not change the debris removal efficiency of EA and CNI, being the groups with the lowest amount of debris removed at all the activation times. The polymer tip of EA operates at lower frequencies (33–167 Hz) compared to UAI and EDDY, thus producing a lower irrigation flow and this may explain why it removed less debris and smear layer than UAI and EDDY in the present and other studies [19, 20]. The lower debris removal rate of CNI compared to the other activation techniques has been reported in many previous studies and systematic review and meta-analysis [8, 20, 22, 23, 25, 31]. The limited flow given by CNI to the irrigating solution may explain the lower rate of debris removal of this conventional irrigation method [30], which appears to be insufficient for the correct cleaning of the apical part of the root canal and the isthmuses [30]. De Gregorio et al. examined the solution penetration of irrigant activation methods with fluid dynamics and reported that conventional needle irrigation provides less penetration than sonic and ultrasonic methods [10].

The main limitations of the present study are the use of 2D images as an evaluation method and the testing model employed. Indeed, the model differed from real teeth in the smoothness of the root canal surfaces, so that the flat walls of the isthmus may have facilitated debris removal. Furthermore, the present study was the first one examining debris removal from an isthmus that connects two curved artificial canals. Further clinical and laboratory studies are needed to compare and improve the effectiveness of irrigation protocols in order to set the gold standard for root canal cleaning and disinfection procedures.

Laboratory results should be interpreted with caution in clinical practice. Indeed, several factors including anatomical configuration, irrigation protocol and operator ability act simultaneously in vivo conditions and could affect the results obtained. In addition, it is important to

underline that statistically significant differences could not be clinically relevant.

In conclusion, within the limitations of this in vitro study, EDDY removed more debris from a simulated isthmus connecting two curved canals compared with the other techniques investigated and appears to be the best irrigant activation method available. An increase in activation time enhances EDDY and UAI debris removal efficiency but not the one of EA and CNI.

AUTHOR CONTRIBUTIONS

All authors have contributed significantly and are in agreement with the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors deny any conflicts of interest related to this study.

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Appendix VIII Non-thesis related activities, publication, presentations and positions of responsibility

Non-thesis learning activities completed:

Course/Activity	Date	Venue	Certificate (Yes/No)	Facilitator's Name	Hours
Post graduate Diploma in Micro-Endodontics	Academic year 2016-2017	University of Valencia, Spain	Yes	Prof. L. Forner	48
Ace Young: Implantology hands on course	7-9.04.2016	Pesaro, Italy	Yes	Dr S. Gori	16
Irish Endodontic Society: Annual scientific meeting	27-28.01.2017	Dublin, Ireland	Yes	Dr S. Lennon	8
Irish Endodontic Society: Case night	23.03.2017	Dublin, Ireland	No	Dr S. Lennon	2

Irish Endodontic Society: Annual scientific meeting	26-27.01.2018	Dublin, Ireland	Yes	Dr S. Lennon	8
Endodontic Hands on course	15-16.03.2018 29-30.03.2018 19-20.04.2018	Rome, Italy	Yes	Prof NM Grande, Dr G Plotino	48
Postgraduate Research Seminar Presentation	25.04.2018	Dublin, Ireland	No	Prof M. O'Sullivan	3
One to one endodontic course	7-12.05.2018	Rome, Italy	No	Prof NM Grande, Dr G Plotino	24
European Society of Endodontology: Research Meeting	26-27.10.2018	Amsterdam, The Netherlands	Yes	Prof H. Duncan	14
Irish Endodontic Society: Annual scientific meeting	24-25.01.2019	Dublin, Ireland	Yes	Dr S. Lennon	8

European Society of Endodontology: General Meeting	10-14.09.2019	Vienna, Austria	Yes	Dr P. Dummer	18
Irish Endodontic Society: Annual scientific meeting	23-24.01.2020	Dublin, Ireland	Yes	Dr S. Lennon	9
Level 1 Training in Dental CBCT	20.07.2021	Online	Yes	Dr S Ellis	7
Dublin Dental Academy- Restorative on Implants	03.09.2021	Dublin Ireland	Yes	Dr F. Molina	4
Adhesive prosthodontics hands on course	29-30.11.2021	Dublin, Ireland	Yes	Dr F. Andrian	16
Hands on Course on Endodontic Surgery	21-22.1.2022	Conversano, Italy	No	Dr G. Carrieri	16
Irish Endodontic Society: annual scientific meeting	27-28.01.2022	Dublin, Ireland	Yes	Dr M. Egan	8
European Society of Endodontology General Meeting	7-10.09.2022	Budapest, Hungary	Yes	Prof J. Withworth	18
Biomimetic aesthetic dentistry course	11-12.11.2022	London, UK	Yes	Dr Bill Gergis	12

Irish Endodontic Society: Annual scientific meeting	20.01.2023	Dublin, Ireland	Yes	Dr M. Eagan	6
Accademia Italiana di Endodonzia: Annual Meeting	23-25.02.2023	Bologna, Italy	Yes	Prof. NM Grande	16
European Society of Endodontology: Biennial meeting	7-10.09.2023	Helsinki, Finland	Yes	Prof J. Withworth	18

Oral Presentations and workshops:

- 22.5.2018 Oral Presentation at Dublin Dental University Hospital, Postgraduate Research Seminar Presentation
- 21.10.2019 Lecture on VPT at Master's program in Endodontology, University of Cagliari, Italy
- 23.11.2019 Lecture: "Modern endodontics, new materials to achieve excellence." 4th dental conference Pristina, Kosovo
- 21.04.2021 Endodontic webinar: "Modern Endodontics". Online event
- 08.10.2021 Oral Presentation at IADR meeting Belfast, Northern Ireland
- 23.11.2021 Orodeka endodontic hands on workshop Dublin, Ireland
- 26.11.2021 Orodeka endodontic hands on workshop Cork, Ireland
- 28.01.2022 Lecture on VPT at Irish endodontic society meeting, Dublin, Ireland
- 27.03.22 Oral presentation and scientific article discussion for Asian Pacific Endodontic Confederation. Online event
- 14.4.2022 Lecture on VPT at Master's program in Endodontology, University of Cagliari, Italy
- 9.9.2022 Oral research presentation on VPT at European Society of Endodontology, Budapest, Hungary
- 8.12.2022 Lecture on the evolution in Endodontics at Asian Pacific Association for Dental and Oral Health. Online Event.
- 23-25.2.2023 Accademia Italiana Endodonzia General Meeting, hands on course on Pre-mixed bioceramics.
- 18.2.2023 Lecture at Vital Pulp Therapy Meeting, Cluj Romania
- 11.5.2023 Hands On endo course for the Irish Dental association Kilkenny Ireland
- 24.5.2023 Odontocaffe event by Dentalworld (online Event)

Positions of responsibility:

- Committee Member of the Irish Endodontic Society since 2017
- ESE Representative for the Irish Endodontic Society since 2019
- Post-graduate member of the European Society of Endodontology
- Clinical Supervisor for undergraduate dental students in the Dublin Dental University Hospital Academic years 2021/22- 2022/23
- Key Opinion Leader Dentalworld
- Opinion Leader Orodeka
- Member of the Accademia Italiana di Endodonzia
- Chaired scientific session- European Society of Endodontology Biennial Meeting 2022

Publications:

Careddu R, Duncan HF (2018) How does the pulpal response to Biodentine and ProRoot mineral trioxide aggregate compare in the laboratory and clinic? *British Dental Journal*, **225**, 743–749.

Careddu R, Plotino G, Cotti E, Duncan HF (2021) The management of deep carious lesions and the exposed pulp amongst members of two European endodontic societies: a questionnaire-based study. *International Endodontic Journal*, **54**, 366–376.

Careddu R, Duncan HF. (2021) A prospective clinical study investigating the effectiveness of partial pulpotomy after relating preoperative symptoms to a new and established classification of pulpitis. *International Endodontic Journal*, **54**, 2156–2172.

Careddu R, Ciaschetti M, Creavin G, Molina F, Plotino G (2020) COVID-19 and dental practice: overview and protocols during pandemic. *Giornale Italiano Di Endodonzia*, **34**, 1.

Careddu R, Creavin G, Plotino G (2021) Dental Practice after COVID-19 Pandemic: Analysis of Italian Guidelines and Current Literature to Propose Appropriate Protocols for Dental Practices. *Frontiers in Dentistry*, **18**, 16

Plotino G, Özyürek T, Gündoğar M, Uslu G, Pedullà E, Careddu R, Franco V (2023) Efficacy of different irrigant activation devices in removing dentin debris from an artificial isthmus connecting curved canals. *Australian Endodontic Journal*, **24**. doi: 10.1111/aej.12748. Epub ahead of print.

Careddu R, (2021) Restoring endodontically treated teeth. *The Dentist*, **7** 76-78