Studies on Improving the Outcome for Molars with Hypomineralisation

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

Dedicated to my family

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Study 1 Abstract

Aims of the Research:

To establish the prevalence of self-reported sensitivity and pain in children who have Molar Incisor Hypomineralisation. To establish the relationship between the severity of the reported pain/sensitivity and the appearance of the hypomineralised molars clinically and the choice of treatment for the hypomineralised molars by Consultants in Paediatric Dentistry.

Materials and Methods:

The prevalence of pain and sensitivity in MIH hypomineralised teeth was investigated in patients who referred with Molar Incisor Hypomineralisation to Leeds Dental Hospital and Hull Community Dental Centre and who were invited to take part in the study. A questionnaire was developed to investigate and document pain severity from the various stimuli (cold, hot, brushing, and sweets) in MIH teeth and correlate the reported pain to the appearance of the teeth and the type of treatment planned. A validated face rating scale (Wong and Baker, 1988) was used to facilitate the pain rating by children. The questionnaire was piloted with a group of children before commencing the main study.

Results:

The total number of participants recruited was 200, with an age range of 6 to 10 years and a mean age of 8.29 years. The overall prevalence of pain reported was 91% (n= 182), while 9% (n=18) of participants said they did not experience pain from any of the stimuli mentioned (hot, cold, brushing or eating sweets). Children who presented with cuspal lesion involvement and post-eruptive breakdown (PEB) in their MIH teeth were more likely to report pain. The most significant treatment planned for hypomineralised first permanent molars by the assessing Consultants was extraction with varying percentages of 38% – 47% depending on which molar was planned for extraction. Children with teeth with PEB, cuspal involvement or brown colour

lesion were more likely to have extractions planned. The most likely other treatments included fissure sealing and composite restorations.

Conclusion:

This study showed that the majority of participants reported pain and this may reflect that they were being referred for tertiary care and are likely to have more severe forms of MIH. It appeared in this group that Consultants were most likely to make decisions based on the severity of the lesions and probably the pain history they elicited at the assessment visit.

Study 2 Abstract

Aim of the Research:

The research aims of the *in vitro* study were centred around comparing possible MIH enamel improvement (increased mineralisation) with different treatment regimens: Control = F-Free toothpaste, Control = F toothpaste, Control = F toothpaste + Novamin, and Test Treatment = F toothpaste + CPP-ACP.

Materials and Methods:

After obtaining ethical approval, eleven human MIH teeth were collected from the University of Leeds Human Tissue Bank and 20 slabs with MIH lesions were created. The 20 slabs were randomly allocated to one of the four groups: Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and Test F toothpaste + CPP-ACP. The study design included incubating the enamel slabs in artificial saliva and immersing them in the different materials twice a day for 28 days. Testing methods that were used to assess potential mineralisation involved measuring changes in surface hardness, changes in surface roughness, changes in mineralisation and changes in lesion colour of the lesions. This was carried out by microhardness testing, light profilometry, DIAGNOdent laser analysis and analysis of digital photographs before and after the test period of 28 days.

Results:

The test results showed statistically significant changes ($p < 0.05$) in the surface microhardness, surface roughness, and mineralisation levels in all groups. However, no statistically significant differences between the control groups and the test group were found.

Conclusion:

The use of F-Free toothpaste, F toothpaste, F toothpaste + Novamin or F toothpaste + CPP-ACP all resulted in improvement in the MIH enamel lesions. The addition of CCP-ACP or Novamin to the toothpaste did not have any further statistically significant benefit. This may suggest that

there is benefit in the regular use of toothpastestes containing calcium and phosphate as well as fluoride on the potential ongoing mineralisation of hypomineralised MIH lesions. Further research is needed to determine how other components in toothpaste might add benefit to this mineralisation process.

Chapter 1 **Introduction**

In 2000 at the European Academy of Paediatric Dentistry Congress, there were four presentations that described the same types of developmental defects of dental enamel affecting the first permanent molars (FPM). These reports called the condition hypomineralised first permanent molars (FPM) (Beentjes et al., 2000), idiopathic enamel hypomineralisation in first permanent molars (FPM) (Jälevik et al., 2000), non-fluoride hypomineralisation in first permanent molars (FPM) (Leppaniemi et al., 2000) and cheese molars (Weerheijm et al., 2000).

Weerheijm et al. (2001a) stated that when discussing these developmental defects of dental enamel it would be desirable to use one name, one that made no reference to any other possible aetiology. They suggested to this end, the term Molar-Incisor Hypomineralisation, and developed a definition as MIH describes the clinical appearance of enamel hypomineralisation of systemic origin affecting one or more permanent first molars (PFMs) that are associated frequently with affected incisors (Weerheijm et al., 2001a). There are several criteria that can be used to describe and confirm a diagnosis of MIH: diagnosis of demarcated opacities in hypomineralised molars, posteruptive breakdown, atypical restorations, and cheesy looking appearance (Weerheijm, 2003a).

1.1 Literature Review

1.2 Tooth Development:

1.3 Dental Enamel Development (Amelogenesis)

Dental enamel is different from other mineralised tissues like (bone, cartilage, and dentine) because it is not collagenous and is of epithelial origin. Dental enamel forms the mineralised outer layer of the tooth, providing a hard surface essential for withstanding the forces of mastication. Dental enamel is the most highly mineralised structure in the body and is formed by the ameloblast cells of the inner enamel epithelium. The ameloblasts are responsible for the synthesis and secretion of proteins to form the enamel framework (Fincham et al., 1999).

Dental enamel development takes place in three functional stages: presecretory, secretory and maturation stages. The diversity of enamel malformations observed in Molar Incisor Hypomineralisation is believed to reflect the differences in the timing during amelogenesis when the disruptions occur. Proteins such as amelogenin, ameloblastin and enamelin are secreted to form a protein matrix during the initial stage of enamel formation.

Amelogenesis is unique in various ways:

- The secretory cell is an epithelial cell, whereas, in all other hard tissues, all secretory cells are ectomesenchymal.
- Mineralisation of enamel involves the Noncollagenous proteins, whereas in hard tissue collagen plays an important role.
- The enamel matrix doesn't contain collagen, but collagen plays an important role in other hard tissue formation and is the major protein. Partial mineralisation occurs in the enamel matrix, while its nonmineralised in other hard tissues. Therefore enamel lacks a distinct organic phase similar to predentin, cementoid or osteoid.
- There is no absorption of the secreted matrix in other hard tissues, but in enamel formation, 90% of the secreted matrix is absorbed, and this activity is done by ameloblasts themselves. After the formation of enamel, ameloblasts undergo apoptosis; thus, enamel formation does not occur later on. In other hard tissues in the body, formation occurs throughout life.

1.4 Dental Enamel Mineralisation

There are two stages that take place for the mineralisation of the enamel matrix (the mineralisation stage and the maturation stage), although there is a little time interval between the two. During the start of the first stage (mineralisation stage) immediate partial mineralisation occurs in the interprismatic substance and the matrix segment as they are laid down. The first mineral seen is actually in the form of crystalline apatite; this has been shown recently by electron microscopy and diffraction. However, in other studies, it has been shown that the initial mineral is octacalcium phosphate which may act as a template for hydroxyapatite. Nonetheless, because it's unstable, it converts into hydroxyapatite; one unit of the octacalcium forms two units of hydroxyapatite. The second stage is the maturation stage; it is characterised by the mineralisation being gradually completed. The maturation process starts from the height of the crown and progresses cervically. However, at each level, maturation seems to begin at the dentinal end of the rods. Thus there is an integration of two processes: each rod matures from the depth to the surface, and the sequence of maturing rods is from cusps or incisal edge toward the cervical line. Before the matrix has reached its full thickness, maturation begins. This is going on at the same time as the initial mineralisation is taking place in the outer recently formed matrix. The advancing frontal part is at first parallel to the dentinoenamel junction and later to the outer enamel surface. Based on this pattern, the occlusal and the incisal regions reach maturity before the cervical region. The growth of the crystals seen in the primary phase of the maturation stage is a characteristic of this stage. The crystal's original ribbon shape increases rapidly in thickness than in width. During the maturative stage, the crystals increase in size from 1.5 to 25 um. Enamel proteins have the function of regulating the enamel mineralisation and inhibiting the further deposition of crystals by binding to a specific surface of the crystals. The enamel rate of formation is 4 um/day; therefore, a 1 mm layer of enamel thickness would take about 240 days. Simultaneously the gradual thinning of the

organic matrix occurs and is widely spaced to create room for the growing crystals. The loss in volume of the organic matrix is shown by the chemical analysis, which reveals that a substantial amount of water and protein is withdrawn.

1.5 Disturbances in Enamel Development

Epigenetic and environmental disturbances (systemic illnesses, chemical poisons, radiation, fluoride and strontium, or trauma) could occur during the formation of enamel. This may result in the damage of ameloblasts during the process of enamel formation (Seow, 1991). Damage to the ameloblasts results in a reduction in the quantity of enamel, presenting as pits, grooves, or thin or missing enamel which is known as enamel hypoplasia; or reduced mineralisation presenting as soft enamel which is known as enamel hypomineralisation with altered translucency affecting the entire tooth, or in localised areas as opacity or diffuse patches. These defects may be described as hypomineralisation or hypomaturation depending on their presentation. (Clarkson, 1992, Crawford et al., 2007, Gadhia et al., 2012):

- Pre-secretory stage (matrix formation): Ameloblasts acquire their characteristics and develop an extensive protein synthetic apparatus to secrete the organic matrix of enamel. Disruption of ameloblasts at this stage will cause hypoplastic enamel defects. Quantitative defects are observed at this phase.

- Secretory stage: Ameloblasts secrete the entire thickness of the enamel. At this stage, the hydroxyapatite crystals are separated by organic material and water. Disruption of ameloblasts at this stage will cause hypoplastic enamel defects.

- Maturation stage: Ameloblasts allow inorganic ions to be secreted and exchanged for the water and organic contents, resulting in an increase in the inorganic content and the length and width of the enamel prisms. Disruption of ameloblasts at this stage will cause hypomaturation or hypomineralisation enamel defects. Qualitative defects are observed during calcification and the maturation stage; these are defined as enamel hypomineralisation.

1.6 Molar Incisor Hypomineralisation

1.7 Definition

The term Molar-incisor Hypomineralization was first proposed by (Weerheijm et al., 2001a) to describe a condition where the permanent molars and incisors have demarcated areas of hypomineralisation or opacities which may be coloured yellow or brownish. It has also been defined as the systemic origin of one or four first permanent molars that are frequently associated with affected incisors. Later descriptions have noted that primary second molars may also be involved (Weerheijm et al., 2003).

According to Weerheijm et al. (2003), they explained that any examination for MIH should be undertaken on clean wet teeth and at the optimal age of 8 years old because at this age all first permanent molars and most of the incisors will have erupted.

1.8 Prevalence

In the late 1970s, the condition seems to have been first recognised by Swedish dentists working within the Public Dental Services. They reported an increasing number of patients that

were presenting with hypomineralisation of the enamel of the incisors and FPM for which they could not find an aetiology. It is may have been unknown and unrecognised for considerably longer. An archaeological study by Ogden et al. (2007), felt that in the past it may have been masked by large carious cavities and is only becoming more visible because of a reduction of caries prevalence. They pointed out that MIH may turn out to be related to a condition they termed cuspal enamel hypoplasia in children found in teeth of skulls from a 16th -18th century London graveyard. Koch et al. (1987) investigated the prevalence of idiopathic enamel hypomineralisation in permanent teeth. It is important to note that this was undertaken before the definition of MIH by (Weerheijm et al., 2001a). However, it is possible to extract approximate prevalence figures for MIH from their collected data, and these were from about 3.6% to about 21.5% depending on the year that the child was born. Other studies collected data from 1987 to 2001 (the publication date of the definition) but are difficult to interpret, as they were not working using the definition of MIH and some projects did not have the prevalence as an aim of their study; it was a secondary outcome. However, they were included in the study as they do seem to be describing MIH. Reported prevalence figures range from 2.8% - 25% (Alaluusua et al., 1996a, Alaluusua et al., 1996b, Arrow, 2008, Calderara et al., 2005, CHO et al., 2008, Dietrich et al., 2003, Fteita et al., 2006, Jälevik et al., 2001a, Jasulaityte et al., 2007, Kosem et al., 2004, Leppaniemi et al., 2001, Lygidakis et al., 2004, Lygidakis et al., 2008a, Muratbegovic et al., 2007, Preusser et al., 2007, Weerheijm et al., 2001b). It has to be pointed out that the prevalence figure of 25% (Alaluusua et al., 1996b) was before 2001. It was from a sample of 97 children, and the study was investigating dental defects associated with long-term breastfeeding; the prevalence figure was also not the aim of that study.

Several studies have reported the prevalence of MIH, the vast majority of which have been conducted in Northern Europe. The reported rates in Europe vary from 3.6% to 37.5%. Fewer studies have been carried out outside of Europe. In Hong Kong a study by (CHO et al., 2008)

showed the prevalence of MIH in children was 2.8%; another study conducted in Brazil by (Soviero et al., 2009), had a prevalence of 40% of MIH. Comparing the results of various studies is difficult because of the use of different indices and criteria, examination variability, methods of recording and different age groups.

1.9 Indices and diagnostic criteria

Most of the studies have used (directly or with some local modifications) one of three indices. The Developmental Defects of Enamel (DDE) was developed in 1982 for recording all types of enamel defects for epidemiological surveys. But in 1992 the (DDE) index was considered to be not adequate and too time-consuming for use in MIH prevalence studies.

Another index was introduced by Brook et al. (2001). The Enamel Defect Index (EDI) included the score for Posteruptive Enamel Breakdown (PEB) which is a sign often seen in molar incisor hypomineralisation. This index was not considered useful in the case of MIH, because the first level of the EDI included one score for opacities to indicate demarcated as well as diffuse opacities. For this reason, the demarcated opacities related to MIH are put together with the diffuse opacities on the same score, and these might be caused by high fluoride intake.

An examination for molar hypomineralisation should be performed on wet teeth after cleaning. Eight years of age is considered the best time for any examination for this condition. At this age, all the first permanent molars should have erupted, and signs of MH will still be present before caries are established. A study by Leppaniemi et al. (2001) discussed if it is believed that MIH is a progressive condition with continuous enamel breakdown compared to healthy permanent first molar teeth without excessive post eruptive breakdown.

If the EAPD Index Guideline (2003) is used, teeth should be recorded for the following:

- Absence or presence of demarcated opacities
- Posteruptive Enamel Breakdown
- Atypical restorations
- Extraction of molars due to MH
- Failure of eruption of a molar or an incisor.

Lately, a new Molar Hypomineralisation severity index was created and trialled by (Oliver et al., 2014) Table 1:

Table 1 Characteristics of molar hypomineralisation defects

Characteristics of molar hypomineralisation defects	**Severity of Characteristic**	**Weighting assigned**
Eruption status	Unerupted	0
	Erupted	1
Colour of most severe defect	None	0
	White/cream	1
	Yellow	2
	Brown	3
Location of most severe defect	None	0
	Smooth surface	1
	Occlusal surface (FPMs)	2
	Incisal edge (PIs)	2
	Cuspal involvement (FPMs)	3
Restorations placed/replaced (prior to study entry)	None	0
	One	1
	Two or more	2
Atypical restorations (prior to study entry)	None	0
	Present	1

Post eruptive enamel breakdown (PEB)	None	0
	Present	1
Sensitive to temperature (child report)	None	0
	Sensitive	1
Sensitive to tooth brushing (child report)	None	0
	Sensitive	1

1.10 Aetiology of MIH

The timing of enamel formation is very important to understand when a developmental defect is likely to have occurred. The first permanent molars (FPM) start to develop during the fourth month of gestation. Hess et al. (1932) and Logan and Kronfeld (1933) discovered that the first signs of histological and radiographic mineralisation of the FPM are seen in the cusp tips around or soon after birth and the four cusps become united by mineralisation around the age of six months. Deposition of the enamel matrix is completed in the occlusal half of the crown and maturation is ongoing at the end of the first year. Complete enamel formation takes just under three years (Reid and Dean, 2006).

The disturbances that can occur during the periods (prenatal, perinatal, and postnatal) of enamel formation are discussed below:

1.10.1 Prenatal:

Medical problems during the later stages of pregnancy could be associated with MIH. In one particular study during the last trimester, a urinary infection in the mother was associated with MIH-like lesions (Fredén and Grönvik, 1980). But based on the findings of Whatling and Fearne (2008) and Lygidakis et al. (2008a), specific diseases were not able to be associated with MIH. Several studies have also reported that medical problems were more common in mothers of

MIH children than in those mothers whose children did not have MIH (Lygidakis et al., 2008a, Whatling and Fearne, 2008).

1.10.2 Perinatal:

The most common perinatal problems reported where MIH was associated are; emergency Caesarian section, prolonged delivery, premature birth and being pregnant with twins (Brogårdh-Roth et al., 2011, Lygidakis et al., 2008a). On the other hand, other studies have not been able to link perinatal problems with MIH (Dietrich et al., 2003, Whatling and Fearne, 2008). Hypoxia could be associated with medical problems related to birth such as prematurity, respiratory stress and excessively prolonged duration of birth. However during pregnancy, the occurrence of maternal health, medicine use, duration of breastfeeding and childhood illnesses are less frequently remembered resulting in recall bias (Silva et al., 2016). Lack of oxygen in active ameloblasts has been suggested as a causative factor of MIH or opacities in molars and incisors, (Aine et al., 2000, Lygidakis et al., 2008b, Seow, 1996, Van Amerongen and Kreulen, 1995). Hypocalcaemia in the child is another condition that could occur during the perinatal period but also in prenatal and postnatal periods. The finding that calcium levels were very low in MIH lesions, suggests that they may have been caused by impaired calcium metabolism of the ameloblasts (Jälevik et al., 2001b).

1.10.3 Postnatal:

Children with MIH have been shown to have more postnatal problems during the first year of their lives (Lygidakis et al., 2008b). Another study showed that children with MIH had a history of illness during the first three years of their lives (Kusku et al., 2008). On the other hand, children who were exposed to lower levels of dioxins, did not have MIH therefore there was no correlation (Laisi et al., 2008). A Turkish study showed a similar prevalence of MIH in children living in an urban area polluted by dioxins and in those living in an area with low pollution (Kuscu et al., 2009). Although the previously mentioned studies have found factors that could contribute to the occurrence of MIH, there have been no statistically significant differences

when compared with control groups. In all studies, some children did not have MIH even while they had the same problems that happened during prenatal, perinatal and postnatal periods as children with MIH. Childhood illness, medication and environmental toxins that have been identified as possibly causing MIH are summed up in the following Table 2:

Table 2 Factors that could cause MIH

Authors	Childhood illness, medications, environmental toxicants
Beentjes et al., 2002	Otitis media
Beentjes et al., 2002. Jälevik et al., 2001	Pneumonia
Jälevik et al., 2001	Asthma
Jalevik et al., 2001b	chicken pox, fever
Tapias-Ledesma et al., 2003	Urinary tract infections
Whatling and Fearne, 2008	Chickenpox
Laisi et al., 2009. Hong et al., 2005. Whatling and Fearne, 2008	Amoxicillin
Laisi et al., 2009	Erythromycin
Tapias-Ledesma et al., 2003 Abe et al., 2003 (in rats)	Macrolides
Alaluusua et al., 1996b	Dioxins (in mothers milk)
Jan and Vrbič, 2000	polychlorinated biphenyls (PCBs)
Alaluusua et al., 2004. Jan et al., 2007	Dioxins or polychlorinated biphenyls (PCBs)

It has been suggested by (Brook and Smith, 1998) that the risk of developing MIH in some cases could be underlined by a predisposing genetic factor. It has been hypothesised by (Marthu-Muju and Wright, 2006) that not all MIH defects in the same individual are equally vulnerable to development disturbances; therefore, they have suggested genetic factors in the pathogenesis. (Marthu-Muju and Wright, 2006) have also stated that the majority of previous

studies proposed that the aetiology of MIH is complex with unknown genetic and systematic factors that disrupt the amelogenesis in the affected MIH teeth. (Lygidakis et al., 2010) have found that the results of their study support the theory that MIH is considered a multifactorial aetiology and that there is an association between genetics and the development of MIH. A study on twins by (Teixeira et al., 2018) found that there was a greater agreement in the occurrence of MIH in monozygotic twins compared to dizygotic. In addition, genetic research has expanded the possibility, and the likelihood of determining the effect of genes variants related to amelogenesis on MIH development (Jeremias et al., 2016) furthermore the variants of the immune response genes have been recently suggested to affect the amelogenesis (Bussaneli et al., 2019).

Tooth enamel development and the variety of genes involved are considered complex processes in the development of MIH. The gene mutation, functional changes and spatial and temporal expression could determine the enamel phenotype (Wright et al., 2015). changes in the molecules could interfere with or alter the gene expression and the development of enamel (Wright et al., 2015). The increase of ameloblastin expression in the enamel matrix will result in the development of disorganised and shorter hydroxyapatite crystals (Lu et al., 2011). The gene that causes the expression of the protein in the enamel matrix is Alleles; it has been found to be associated with the development of MIH in children in the Brazilian population (Jeremias et al., 2013).

To summarise, various evaluations of the aetiology factors have not revealed a clear link between environmental factors and MIH. It has been suggested by (Vieira and Kup, 2016) that the genetic origin is displayed in the “geographically specific” MIH prevalence because MIH morbidity differs among regions. Although the MIH aetiology remains unclear, both genetic and environmental factors are presumed to play a role in causing MIH (Teixeira et al., 2018).

1.11 Clinical presentation of MIH

Clinically, the lesions of MIH mostly are demarcated opacities, whitish-yellow or yellowish-brown in colour depending on the severity of the case; they may or may not be associated with post eruptive enamel breakdown. Affected incisors rarely exhibit post eruptive enamel breakdown (Muratbegovic et al., 2007, Weerheijm, 2003b, Fayle, 2003) and it is believed that this is due to the lack of occlusal forces on the opacities, as happens in the FPM. It is important to realise that the enamel breakdown is post-eruptive and should not be mistaken for hypoplasia, which is a quantitative developmental defect of enamel that results in deficient enamel matrix formation. In cases of hypoplasia clinically, it has been pointed out that the margins are mainly smooth, whereas in MIH the margins of the post-eruptive breakdown of enamel are irregular (Weerheijm, 2003a). The hypomineralised areas have been classified, by Alaluusua et al. (1996a), as mild (colour change: white, yellow or brown), moderate (loss of enamel only) and severe (loss of enamel in association with affected dentine). MIH affected FPM are more susceptible to dental caries due to the porous enamel that facilitates plaque accumulation compared to a normal tooth with good enamel quality, therefore caries may progress very rapidly, and the presence of caries can mask the true diagnosis of MIH. The reason for caries progression in MIH teeth could be that children may avoid brushing because of sensitivity issues (Beentjes et al., 2002, Weerheijm, 2003a). The severity of MIH varies between individuals but it can also vary within the mouth of a single individual, such that not all molars will be affected to the same extent; some molars may not be affected in some cases. However, it has been pointed out that where a severe defect is found in one FPM it is likely that the contralateral tooth will also be affected (Weerheijm, 2003b). Also, as more FPM are affected, then the defects are more severe (Jälevik et al., 2001a, Jasulaityte et al., 2007) and 70% of children with severe defects have affected incisors (Jälevik et al., 2001a). Finally, as the

number of affected FPM increases the chances of having affected incisors also increase (Preusser et al., 2007, Weerheijm et al., 2001b).

A number of researchers have investigated the average number of affected teeth per child, finding that this could vary from 2 to 5.7, of which 1.6-3.16 were FPM (Calderara et al., 2005, CHO et al., 2008, Dietrich et al., 2003, Jälevik et al., 2001a, Jasulaityte et al., 2007, Lygidakis et al., 2008a, Muratbegovic et al., 2007). The distribution between the maxilla and mandible has also been investigated. Some researchers have discovered that the lesions of the FPM were more frequent in the maxilla than in the mandible (Arrow, 2008, CHO et al., 2008, Leppaniemi et al., 2001, Lygidakis et al., 2008a, Muratbegovic et al., 2007, Preusser et al., 2007). This difference in distribution was significant in two of the six previous studies mentioned (Leppaniemi et al., 2001, Preusser et al., 2007). In contrast, other studies have shown that the affected molars were more common in the mandible than the maxilla. Jälevik et al. (2001a) stated that the difference was not statistically significant and Calderara et al. (2005) failed to mention any significance.

1.12 Pain/Sensitivity related to MIH

The temperature in the oral cavity could change from ice cold to burning hot within a few seconds, depending on the food consumed. Hot or cold temperatures do not elicit nociception in the teeth under normal circumstances, unlike other tissues in the body. Nociception is the tooth's unique sensory system that senses external stimuli. The dentine and enamel are the two layers of hard tissue that cover and protect the densely vascularised innervated pulp tissue, (Byers and Narhi, 1999, Hossain et al., 2019). The hardest tissue in the body is the outermost part of the tooth, which is the enamel. 92–96% of the enamel weight is made up of minerals (Gwinnett, 1992). The high mineral content gives the enamel an excellent thermal insulating capacity (Bleicher, 2014, Chung et al., 2013, Renton, 2011, Sessle, 2011). Although the

enamel has thermal insulating capabilities, exposed dentine and pulpal inflammation could give rise to intense pain during mild temperature changes. For example, noxious heat induces dull and lasting pain while noxious cold induces sharp and transient pain (Ahn et al., 2012, Henry and Hargreaves, 2007).

Studies on the dentine microstructure by (Marshall Jr, 1993) have shown that the dentinal microstructure extends from the pulp wall to the dentino-enamel junction or the exterior cementum. Within the space of the dentinal microtubules lies the extended part of the odontoblast (odontoblastic process), as well as the non-myelinated terminal fibres, (Andrew and Matthews, 2000, Fearnhead, 1957, Hildebrand et al., 1995). While studies in the structure of the dental pulp by (Fristad and Berggreen, 2016) have shown that the pulpal nerve supply gives the dental pulp sensitivity from two main types of fibres. These fibres are responsible for pain sensation found in the pulp: unmyelinated C fibres and myelinated Aδ, the myelinated Aδ fibres tend to be more peripherally localised around the pulp chamber. While the C fibres are distributed throughout the pulpal space, they are mainly localised in the central portion of the pulp. (Fristad and Berggreen, 2016)

In the case of dentine sensitivity, the dentinal fluid outflow movement stimulates the peripherally sited A δ fibres, these myelinated fibres have a rapid action potential. Therefore a rapid onset perception of pain is related to short-acting dentinal fluid movement, but this usually resolves quickly. In an inflamed pulp, the C fibres become involved (Yu and Abbott, 2018). These fibres are slow conductors because they are smaller unmyelinated nerves that are stimulated by mediators of inflammation. The C fibres tend to have a longer duration but with low pain intensity (Yu and Abbott, 2018). When the pulp becomes irreversibly inflamed (pulpitis), the mediators of inflammation will lead to spontaneous throbbing pain; this is often

aggravated by local changes in blood pressure. As the pulp becomes progressively inflamed, the role of the Aδ becomes less prominent, while the temperature reaction in the pulp becomes more mediated by C fibres. (Närhi et al., 1994).

The pulp chamber is restricted by the surrounding dentine, therefore limiting the increase in the volume of the pulp tissue during an inflammatory response. The normal pulp pressure of the arterioles is 40-45mmHg, (Matthews et al., 2000), and a lower pressure is found in the pulpal capillaries at 30-36mmhg (Orchardson and Cadden, 2001). In general, the pulpal interstitial fluid pressure was found to be calculated as 10.4mmHg (Ciucchi et al., 1995). However, the pressure is as much as three times higher than normal during pulpal inflammation (Heyeraas and Berggreen, 1999). Nevertheless, there are special arteriovenous shunts in the dental pulp to compensate for the restricted space; this can help to reduce the intercellular fluid pressure during the existence of inflammation (Berggreen et al., 2007).
After highlighting the vital purpose of enamel in protecting the subsurface tooth structure from external stimulus, also the role of dentine and pulp in pain sensation, it is essential to highlight the three main theories that might explain the cause of dental pain sensation.

There are three main hypotheses that explain the mechanism underlying the cause of dental pain. The first hypothesis is the Neural theory (Anderson et al., 1970, Byers, 1984, Dubner, 1978, Johnsen, 1985, Närhi, 1985, Olgart, 1985, Sessle, 1979, Sessle, 1987); this theory assumes that the temperature changes on the tooth surface are conducted through enamel and dentine to the dentinoenamel junction (DEJ), this is where the sensory receptors are located, thus causing neuron excitation. The second hypothesis is the Odontoblastic transduction theory (Chung et al., 2013, Magloire et al., 2004, Thomas, 1979); this theory suggests that the odontoblast transfers the external stimulus to the nerves through the synaptic junctions that are

located between the odontoblasts and nerves. The third hypothesis is the Hydrodynamic theory (Brannstrom, 1963, Brännström, 1986, Brännström and Åström, 1964); which explains that the stimulation of mechano-sensitive nociceptors causes dentinal fluid movement within dentinal microtubules, hence causing dental pain sensation. Among these theories, the most widely accepted theory explaining dental pain sensation is the hydrodynamic theory (Andrew and Matthews, 2000, Charoenlarp et al., 2007, Vongsavan and Matthews, 2007).

Brännström (1986), Brännström and Åström (1964) originally proposed the hydrodynamic theory; they discovered that when using a paper pellet or air puff to dry the dentinal tubules, would cause dentine sensitivity. This theory focuses on the sensory component of the dentine and pulp when the dentinal fluid is activated by various stimuli on to the exposed dentine surface (Charoenlarp et al., 2007, Lin et al., 2011b, Pashley et al., 1996). Several studies (Brännström, 1966, Lin et al., 2011b) based on the hydrodynamic theory proposed that dentine external stimulation such as air puff, brushing or probing results in dentinal fluid movement in the dentine-pulp complex or could cause tubular content deformation. This has also been suggested by (Brännström and Johnson, 1970, Lin et al., 2011a) but they have added that the mechanical stimuli could create enough shear stress on the intratubular nerve terminals to initiate mechanosensitive ion channels to cause dental pain sensation.

Following the previously mentioned theories, and the essential roles of the enamel, dentine and pulp, it is important to mention the pain sensation in MIH teeth and to correlate the information on the occurrence of pain in MIH teeth provided in the literature.

Children with MIH defects report frequent sharp pain from various normal stimuli, such as osmochemichal, mechanical and thermal and this may be due to dentine hypersensitivity, (Addy, 2000). This may arise from the repeated excitability of intradental nerves and the

effectiveness of nerves transmitting the stimulation. Due to the presence of porous hard tissue and exposed dentine (pre/post-eruptive breakdown), MIH teeth are left with exposed dentine vulnerable to oral stimuli. Therefore the experienced sharp short pain is most likely caused by the dentinal fluid movement that is explained by the hydrodynamic theory, (Addy, 2000). In this situation, the high threshold mechanoreceptors (A-fibres) are activated and resulting in pain. In children, the symptoms are likely to be increased; this may be due to immature dentine (patent and wide dentinal tubules that ease the dentinal fluid movement, resulting in A fibre stimulation. It has been shown by Jälevik et al. (2001c), that the levels of calcium and phosphate in the enamel were lower in MIH teeth than in normal teeth. This could explain the accelerated posteruptive breakdown of enamel and explain that enamel defects could increase the permeability of the tooth leading to increased sensitivity (Seow, 2014).

A study by Rodd et al. (2007) on the pulpal status of Hypomineralised First Permanent Molars explored the pulps of non-carious hypomineralised FPM, comparing them to sound FPM in MIH affected individuals, and found that there was an increase in neural density at the pulp horn area and the subodontoblastic occlusal region regardless if the tooth was associated with enamel breakdown or not. Jälevik and Klingberg (2002) mentioned that despite the use of local anaesthesia for hypomineralised teeth, some teeth may remain sensitive to instrumentation. This may be due to the subclinical pulpal inflammation that might lead to hypersensitivity of these tissues (Rodd et al., 2007). The tissue inflammation in the pulpal region may lead to changes in the cytochemical and morphological sensory neurons resulting in hypersensitivity of these nerves fibres (Byers et al., 2003, Olgart and Kerezoudis, 1994)

Another finding revealed that in the subodontoblastic region, there was a significant increase in the innervation density in the pulp of hypomineralised molars associated with enamel breakdown compared to that in sound teeth with normal enamel. Furthermore, they found that

the presence of enlarged vessels may indicate that there is an increase in tissue fluid pressure, causing an outward flow of dentinal fluid, resulting in dentinal stimulation and increased pain. (Matthews and Vongsavan, 1994). In addition, they have found that some of the non-carious hypomineralised molars presented with underlying inflammation of the pulp, this was demonstrated with an increase in the pulpal innervation density. These histological findings are corresponding with what has been mentioned in the literature by (Rodd and Boissonade, 2001), which was regarding the carious first permanent molar in children of the same age group. Although (Rodd et al., 2007) recognised that their samples were small but concluded in their study that there is biological evidence showing inflammatory changes may be present within the pulpal tissue of hypomineralised teeth.

In conclusion

1- The enamel is the hardest part of the tooth and acts as a thermal insulator for the underlying tooth structure. (Bleicher, 2014, Chung et al., 2013, Renton, 2011, Sessle, 2011). However, some teeth have associated enamel defects such as MIH teeth, and these defects could be accompanied by enamel breakdown, therefore increasing the permeability of the tooth and leading to increased sensitivity (Seow, 2014).

2- The hydrodynamic theory is the most common theory that explains the process of dental pain.

3- The pulpal status plays a major role in the sensitivity of the teeth. For example, an inflamed pulp might lead to hypersensitivity. (Närhi et al., 1994, Orchardson and Peacock, 1994).

1.13 Management of MIH

1.13.1 Prevention and decreasing sensitivity

Caries Preventive advice is very important and should include appropriate dietary advice. If a child is still using low-fluoride toothpaste then the parents should be encouraged to change to one with a higher fluoride level of at least 1,350-1500 ppm F. Other topical fluoride products may also be helpful. Amongst these are topical fluoride varnishes, e.g. Duraphat 22,600ppm F and stannous fluoride gels for example Gel-kam 1,000ppm F. These products may help with reducing sensitivity and help in further mineralisation of the hypomineralised areas, although at present there is actually no evidence to indicate their efficacy in patients with MIH. In 2001, Shen et al, found that further mineralisation of sub-surface artificially demineralised enamel area was seen when using Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP). CPP-ACP creates and stabilises a supersaturated solution of calcium and phosphate followed by deposition at the enamel surface. This has been suggested to be useful for patients with hypomineralised teeth. However, there is no strong evidence to support this at the present, only anecdotal reports. Fissure sealants may be useful for FPMs that are mildly affected, while the enamel is still intact and the teeth are not sensitive (Fayle, 2003). Marthu-Muju and Wright (2006) mentioned that there was no evidence to support the effect of fissure sealants on affected molars. They suggested that if the fissures appeared opaque or yellow-brown then a 60-second pre-treatment with 5% sodium hypochlorite may be useful in that it may remove some intrinsic enamel proteins. As with sealants on normal enamel, it is important to monitor fissure sealants regularly as they may fail, and they will then require additional material or replacement (Fayle, 2003). It was found in a recent study (Crombie et al., 2014) that the use of resin infiltrate on MIH lesions increased the microhardness values when compared to the adjacent hypomineralised enamel.

1.13.2 Restorative treatment of MIH

Restoration of the MIH affected FPM can be complicated by difficulties in defining the cavity borders. Two approaches have been suggested:

1- Removal of all defective enamel to sound enamel (William et al., 2006).

This means that a lot of tooth material is lost but is better if an adhesive material is to rely upon bonding to enamel.

2- Removal of the very porous enamel of the hypomineralised tooth, until resistance to the bur is felt (Fayle, 2003). This is more conservative, but it can mean that the defective enamel may continue to breakdown.

There are various restorative materials/options available to the dental surgeon treating these patients:

- Glass Ionomer Cements
- Resin Modified Glass Ionomer Cements
- Polyacid-modified composite resins (Compomers)
- Composite resins
- Preformed Metal Crowns (PMC)
- Indirect adhesive or cast restorations

Amalgam is non-adhesive and its use should be avoided in these atypically shaped cavities. It also requires over removal of tooth tissue to achieve retention. At the present time, there is insufficient evidence to support one restorative material over another (Fayle, 2003, Marthu-Muju and Wright, 2006). Lygidakis et al. (2009) reported that after four years of placing composite resin in hypomineralised FPM they were acceptable at the time of review for the study.

1.13.3 Extraction of FPMs

If the mandibular FPMs have a poor long-term prognosis, and there is a normal occlusion developing, then an ideal dental age for their extraction is recommended to be between 8.5-9 years coinciding with the calcification of the bifurcation of the mandibular permanent second molars. This gives the best chance for the second permanent molars to come forward into a good alignment. If the mandibular FPM is extracted then consideration should be given to compensating extractions of the maxillary FPM and always the patient needs to be treated with an eye to future dental development. However, it must be emphasised that the decision to extract any of the FPM in many instances should be discussed with an orthodontic colleague as early as possible to plan for the best result possible for the occlusion (Williams and Gowans, 2003). Mejàre et al. (2005) found that at 18-years-of-age, the space closure that had occurred was acceptable in most of the cases where FPMs had been extracted earlier, in spite of the fact that in their study, the average age at which extractions were performed was later than the ideal age at 11-years-of-age. Jälevik and Möller (2007) stated that extraction of severely affected FPM in patients diagnosed with MIH was a good treatment alternative to restorative care under the right conditions.

1.14 Recent clinical studies on the treatment of MIH molars

It has been reported by Schwendicke et al. (2018) that 23.5 to 31.7% of children with MIH had or will require dental treatment due to sensitivity, pain and post eruptive breakdown. Treatment of MIH teeth verity depending on several factors, in addition molars require different treatment than central incisors. The available treatment for molars ranges from prevention to extraction of the severely affected MIH tooth. The factors that contribute in the selection of the treatment material or approach depends on the stage of dental development, defect severity, presence of other dental anomalies and patient cooperation. Other factors might be considered as well such as patient and parental preference as well as the psychosocial impact on the child.

Hypomineralised Molar treatment options:

Fissure sealants:

Fissure sealants are considered the first line of treatment approach for hypomineraliesd molars given the possibility for future post eruptive breakdown or initiation of carious lesions. Moderate success has been shown in clinical studies for the retention of fissure sealant (Fragelli et al., 2017, Kotsanos et al., 2005, Lygidakis et al., 2009).

Hypersensitivity:

Various materials have been used for the management of sensitivity in MIH teeth, of which that are mostly commonly used in clinical trials are CPP-ACP tooth mousse, Fluoride varnish, 8% arginine and toothpastes containing calcium carbonate. All of the previously mentioned dental materials showed a significant reduction of sensitivity in children with MIH affected teeth (Bekes et al., 2017, Muniz et al., 2020, Pasini et al., 2017). However, the follow up for these studies varied from only 4 weeks to 6 months follow up.

Direct restorations

A restorative approach could be undertaken if the MIH tooth was cavitated. MIH cavitation could be caused either by post eruptive break-down or caries. Glass ionomer cement (GIC) is one of the restorative choices for MIH teeth due to its chemical retention and the release of fluoride. Durmus et al. (2021) have found that the survival rate of GIC to be 87.5% while another study by (Grossi et al., 2018) reported a higher survival rate of 98%. The technique and type of GIC greatly influence the longevity of GIC restorations (Durmus et al., 2021). GIC could also be used to assess the presence of post eruptive break down as well as a provisional restoration to later be replaced with a permanent restoration such as composite.

Composite resin is well known for its reliability in dentistry, although in cases such as MIH, composite is used as an interim restoration until a final more definitive restoration could be placed, which will also greatly improve the cooperation (Taylor et al., 2019, Wall and Leith, 2020). It has been found by Linner et al. (2020) that the success rate for composite resin restoration of MIH teeth was 76.2%. In addition, composite restoration success rate using an invasive approach (complete removal of MIH defect) showed greater success 76% when compared with minimally invasive approach (without removal of MIH defect) 29.9%. The main issues of MIH teeth are that the enamel is greatly porous with reduced elasticity and hardness, as well as having an increase in organic components when compared to normal enamel (Elhennawy et al., 2017). Therefore, the use of a minimally invasive approach while leaving affected MIH enamel might result in poorer adhesion and decreased bond strength (Lagarde et al., 2020). An increase in bond strength between composite and MIH teeth was observed by (Lagarde et al., 2020, Sönmez and Saat, 2017) when rinsing the MIH enamel with sodium hypochlorite before placing the composite restoration. However, an invitro study results showed a significant decrease in bond strength (Krämer et al., 2018).

Indirect restoration

Recent studies have shown a higher success rate using preformed metal crowns on MIH teeth when compared with direct composite resin restorations. Oh et al. (2020) reported an 86% success rate regarding the use of preformed metal crowns. Meanwhile, Koleventi et al. (2018) reported a higher success rate of 100%. Taylor et al. (2019) has suggested that the preformed metal crowns might be used as a temporary measure in certain situations such while waiting for scheduled extraction. The use of preformed metal crowns could also aid in the management of hypersensitivity of MIH teeth and further post eruptive breakdown (Taylor et al., 2019).

In recent studies (Dhareula et al., 2019, Dhareula et al., 2018, Linner et al., 2020) indirect restorations have been shown to have highest success rate of 98% to 100%. The materials used

for indirect restorations were resin composite with a success rate of 100% over 36 months (Dhareula et al., 2019, Dhareula et al., 2018). Ceramic restorations had a similar success rate of 100% compared to indirect composite with the same follow up period (Linner et al., 2020), while gold cast had a slightly lower success rate of 98.2% over the period of 38 months of follow up (Gaardmand et al., 2013). In older children, indirect restoration can be considered a long-term treatment. However, removing more from the tooth structure is a requirement for indirect restorations. The higher success rate for indirect restorations may be due to removing most of the affected area with MIH which results in better adhesion (Somani et al., 2021).

Pulp therapy

A recent systematic review by Taylor et al. (2019) found a high success rate in the short and long term when performing partial and coronal pulpotomies on compromised first permanent molars. Partial and coronal pulpotomies could be considered as a possible treatment option for vital teeth affected by MIH. Meanwhile, for non-vital teeth a new approach of regenerative endodontics for molars may be undertaken, although, the limited evidence on regenerative techniques requires further investigation (Tzanetakis et al., 2021).

Extraction

Extraction may be the only option for molars that are severely affected and with a poor long-term prognosis. Children with severely affected MIH molars are often associated with a high burden of future dental treatment. Therefore, extraction may be the more suitable treatment option (Lygidakis et al., 2010). Scheduled extraction should be planned following orthodontic assessment, evaluation of malocclusion, hypodontia, absence or presence of crowding also presence of the third molar. Furthermore, extraction should be at an optimal development stage, usually it has been advised at an age between 8 and 10 years (Ashley and Noar, 2019). However, the timing should always take occlusion into account to help in supporting possible future orthodontic treatment.

1.15 Dental anomalies associated with MIH

A study by Walshaw et al. (2020) confirmed a link between MIH and hypodontia. In their study, they recorded dental anomalies associated with children that have MIH. These dental anomalies were (Hypodontia, Ectopic eruption, Infraocclusion, Diminutive maxillary lateral incisors, Crown morphology anomalies, Macrodont tooth and Root anomaly). It was found that 29 % (n= 29) of participants had a dental anomaly in addition to the dental anomaly of MIH. The most common anomaly found was hypodontia with 12% (n=12) of the total number of participants (n=101). The most common missing tooth was the lower second premolar, this finding was similar to what (Folayan, 2019) has reported. Walshaw et al. (2020) stated that the prevalence of hypodontia was higher than expected in a normal population when compared with a meta-analysis study by (Polder et al., 2004). On the contrary, a study by Ţenţ et al. (2018) has reported similar results to what was found by (Walshaw et al., 2020), but no specific data were provided in their study. Clinically, it's important for the paediatric dentists to be aware of the higher dental anomaly prevalence associated with children diagnosed with MIH. In addition, the paediatric dentists must consider the implications, especially of hypodontia, during the assessment of the initial dental radiograph, also the importance to include it in the treatment planning for children with MIH.

1.16 Laboratory Methods of assessing of MIH teeth

1.16.1 DIAGNOdent

Clinical carious lesions detection is primarily based on visual inspection, which is based on qualitative evaluation and subjectivity. Another method that was useful in caries detection was commercially introduced in the market in 1998; this device is called the DIAGNOdent (KaVo, Biberach, Germany)(Hibst, 1998). It was able to quantify the carious lesion. This method is a non-invasive method in caries detection. One of the main disadvantages of the DIAGNOdent was that it couldn't analyse the proximal carious lesions. A few years later DIAGNOdent pen appeared on the market as a smaller battery-operated portable device which was convenient to use. This device featured cordless, flexible and different probe shapes that could be used for multipurpose one of which was to identify the proximal carious lesion. The validity and reliability have shown to be strongly correlated with the original DIAGNOdent (Aljehani et al., 2007).

The DIAGNOdent pen uses a laser fluorescence (LF), which usually refers to measuring the fluorescence of a tooth, caused by its fluorescent components (known as fluorophores) after exposing the tooth to specific laser light. The fluorescence of carious dental tissues is different from that of sound tissues. This difference can be utilised to differentiate between sound and carious tooth structure. The DIAGNOdent pen uses a diode laser that emits light at 655 nm wavelength to illuminate the tooth through a fibre optic bundle. The light is absorbed by the dental tissue and then re-emitted as near-infrared fluorescent light. The re-emitted fluorescent light is received by another fibre optic bundle, and the device records the amount of reflected light as a numeric value (0–99) that increases by the increase in dental caries on the tooth structure (Mendes et al., 2004). It is believed that the actual bacteria is not what causes the enhanced fluorescence, but its metabolites this has been shown by (Hibst et al., 2001). These organic molecules that are most likely causing the enhanced fluorescence in dental caries are

bacterial metabolites called porphyrins (Hibst et al., 2001, HILBST, 1999). Therefore, the DIAGNOdent is likely to detect the fluorophores that are organic in nature. (Mendes et al., 2003, Shi et al., 2001b, Shi et al., 2001a, Tam and McComb, 2001).

Reading of the DIAGNOdent device may be influenced adversely by a number of variables in the oral environment. Several studies by Lussi et al. (1999), Lussi and Reich (2005), Shi et al. (2000) have reported false-positive readings in the presence of food deposits, plaque, calculus, toothpaste, prophylaxis paste, and stains. Lussi et al. (2006), Lussi and Hellwig (2006) compared the quantification of carious lesions on occlusal and proximal surfaces using both the DIAGNOdent pen and the original DIAGNOdent device, both studies have concluded that the DIAGNOdent pen might be a useful additional tool for caries quantification on both occlusal and proximal surfaces.

The DIAGNOdent is traditionally used as an additional method in dental caries diagnosis. However, a recent study done by Farah et al. (2008) Showed a good correlation between the DIAGNOdent readings and the severity of MIH while assessing the reduction in mechanical properties of enamel. This may strongly suggest the use of DIAGNOdent in evaluating the severity of MIH. Since LF correlates strongly with the mechanical properties (Farah et al., 2008) and the mineral density of MIH enamel, LF measured by DIAGNOdent may be used in the dental clinic as an extra indication of the severity of the MIH defects.

Mechanical properties of the hard tissues such as bone (Alho et al., 1988, Dalén et al., 1976, Hodgskinson et al., 1989, Leichter et al., 1982, Smith and Smith, 1976, Smith and Smith, 1978) and teeth.(Ge et al., 2005, Kinney et al., 1996, Kodaka et al., 1992, Mahoney et al., 2004b) are positively correlated with mineral density. Xie et al. (2007) research was based on electron microscopic imaging and nanoindentation testing and reached a conclusion similar to that of

(Spears, 1997). They concluded that the reduction in mechanical properties was related to increased organic content and reduced mineral content of the enamel prisms and enamel sheaths. This also agrees with the findings of Farah et al. (2008) where the reduced mechanical properties of MIH enamel suggest lower mineral content and the increased DIAGNOdent readings suggest increased organic content. This may also be supported by the finding of an in vivo study (Takamori et al., 2001) where DIAGNOdent readings dropped significantly after rinsing the dental fissures with a protein denaturant sodium hypochlorite (NaOCl), meanwhile, acid etching had no effect on DIAGNOdent readings. Since DIAGNOdent readings were affected by a protein denaturing agent and not by a demineralising agent, this led to the conclusion that the reflected fluorescence was mostly related to an organic fluorophore rather than the mineral content of enamel. The analysis supported by (Spears, 1997, Xie et al., 2007) shows that the organic component seems to be more abundantly in the MIH enamel and plays a stronger role in reflecting the mechanical properties of MIH enamel.

In summary, the bacterial metabolites may not be the only fluorophore resulting in an increase in DIAGNOdent readings, especially in hypomineralised enamel. Regardless of the nature of the fluorophores in MIH enamel, an increased DIAGNOdent reading indicates defective enamel. In addition, the LF assessment for the degree of staining of MIH enamel reflects the severity of the disease. However, due to the wide range of readings that DIAGNOdent presents for each colour, it shouldn't be relied on as an indicator of MIH severity. There is a strong correlation between the mineral density of hypomineralised enamel and laser fluorescence. When the DIAGNOdent device demonstrates a high fluoresce number, a lower mineral density area is revealed.

1.16.2 SEM

Many articles have been published using the use of Scanning Electron Microscopy SEM since 1962 (Stewart and Boyde, 1962). SEM was and remains a useful tool in research for some time. Nowadays, most laboratory research in universities have its own scanning electron microscope, and the technique has significantly evolved.

Use:

SEM aids in the visualisation of images at high magnification (50x – 10.000x and above). In the SEM technique, an electron beam scans the surface of the sample producing a variety of signals; the characteristics depend on many factors, this includes the nature of the sample, and the energy of an electron beam. Saghiri et al. (2012) described that a beam of electrons hits the sample and the response is collected by the detector; therefore, there is no light used, and the colour of the sample does not have an influence on the image, this is very important in dentistry, where the dental tissues and dental materials tend to be white or have light colours, which makes the use of optical microscopes hard.

SEM detectors:

Secondary electron detectors are the most common detectors used in SEM (ETD - Everhart-Thornley Detector or SE1/SE2 in high-vacuum or LFD - Large-Field Detector, in low-vacuum) and backscattered electron detector (BSED). According to (Paradella and Bottino, 2012), the difference between the two techniques is that secondary electrons are ejected from the outer electron shell of an atom as a result of the impact from a high energy electron, having low energies (up to about 50 eV, compared to the 1 - 30 keV of the beam electrons) and display the surface features (topography) of the sample. Alternatively, backscattered electrons are beam electrons that have undertaken sufficient elastic 'collisions' with atomic nuclei and consequently change the direction to exit the surface of the sample. In Endodontics, SEM is mainly used to evaluate bacterial biofilm formation and to evaluate the bacterial leakage within the root canal

system. SEM is also used to evaluate the fracture pattern regarding root posts and filling cement (Wang et al., 2012). The SEM is also capable of analysing and measuring the gap between the dentine wall and the root canal filling material (Souza et al., 2012).

Mahoney et al. (2004b) study aimed to determine the morphological structure of hypomineralised defects using scanning electron microscopy (SEM). Mahoney et al. (2004b) have found that the SEM analysis of the normal enamel showed an amorphous, orderly rod appearance. Meanwhile, the hypomineralised enamel was disorganised, with variable rod widths and a loss of distinct boundaries between the enamel rods. Also, there were obvious voids in the hypomineralised enamel.

The present study by Mahoney et al. (2004b) revealed significant differences between SEM photomicrographs in the sound enamel and the hypomineralised enamel. These differences included an increased porosity and consistently disorganised rod structure in the hypomineralised enamel. The increased porosity and disorganisation may contribute to the reduction in mineral content. However, it's unlikely that this can entirely account for the decrease in the mechanical properties of affected teeth found in this study.

Jälevik et al. (2005) aimed to study hypomineralised enamel's ultrastructure. They found that the enamel of the control teeth showed well-defined enamel rods with distinct borders and narrow interred zones. The crystals building up the enamel rods were densely packed and their orientation was uniform and well organised. The enamel in normal areas of the MIH teeth showed the same morphological appearance as the control teeth. Meanwhile, in the porous areas; the enamel rods had a normal course and a normal basic morphology. However, different degrees of decomposition of the structural pattern could be seen Depending on the degree of hypomineralisation. In moderately porous areas, the enamel rod borders were more or less blurred, and the interrod zones were not always distinguishable. The crystals seemed to be more loosely and irregularly packed. In severely porous areas close to the surface and adjacent to disintegrated enamel, thinner irregular enamel rods with wide interrod zones were seen. The crystals were

irregularly orientated and challenging to distinguish and the enamel surface was highly porous. In general, the hypomineralised enamel showed fewer distinct features compared with the normal enamel. The border between the normal and hypomineralised areas following the rod direction was evident.

1.16.3 Light profilometry

Surface profilometry is one of the most common laboratory methods used for determining the surface roughness of a tooth sample. There are two methods for surface profilometry measurement, contact and non-contact (Whitehead et al., 1995). The contact profilometry method uses a stylus tip to record the surface profile. While this traditional method is relatively simple, it has the potential of affecting the reading and has the risk of damaging the surface with force applied by the stylus while analysing the surface (Heurich et al., 2010, Stout, 1981). In addition, the contact profilometer generates a line profile of the surface analysed meanwhile, the non-contact profilometer creates a surface plane. This allows for volumetric loss analysis in cases of tooth surface loss analysis such as dental erosion (Rodriguez and Bartlett, 2010).

The two main non-contact profilometer uses either a light beam or a laser beam to scan and analyse the surface profile. When comparing between them, the Laser profilometry surface scanning techniques are much faster than the light profilometer, but they either require a coating of the surface or plaster replicas fabrication (Heintze et al., 2006). In addition, a careful selection of samples is required for laser profilometry because the translucent (Enamel) and reflective surfaces could cause inconsistent values of the surface profile. On the contrary to laser techniques, light profilometry allows the digitisation of the specimen's surface, potentially resulting in fewer errors in the measurements acquired (Theocharopoulos et al., 2010).

Light profilometry uses a measuring sensor to record the distance of the surface accurately. The measuring sensor contains special lenses that are able to separate the light beam of a white (polychromatic) light source into its constituent wavelengths (Litwin et al., 2006). Every wavelength could only be focused on one point that lies at a particular distance from the sensor, hence forming a continuity of monochromatic imaging points. The sensor’s ability to

sense the distance is enabled by the reflected beam's wavelengths being matched to the height of the focused point through a spectrometer. After that, a microtopographic image is created by scanning the specimen surface (Litwin et al., 2006, Cohen-Sabban et al., 2001). The white light sensor applications include reverse engineering, industrial process control and as a research tool because of its high precision. The non-contact sensor can provide analysis of texture and shape, microform and microtopography, as well as surface roughness measurements (Theocharopoulos et al., 2010).

In conclusion, there are two types of surface profilometers (contact and non-contact). The contact method uses a stylus to analyse the surface but could potentially damage the surface. On the other hand, there are two non-contact methods (laser and light); laser profilometry is faster than light in recording the surface but is very sensitive, especially with translucent and reflective surfaces. Meanwhile, light profilometry has high precision and overcomes the limitations of laser profilometry.

1.16.4 Micro CT

Micro CT is a system that uses a microfocal spot X-ray source with a high resolution detectors, this will allow projections to rotate through multiple viewing directions to produce a 3D Image. Micro CT is a three-dimensional spatial distribution map of material density within attenuating materials or tissues such as teeth (Hounsfield, 1973). Micro-CT may examine various specimens, including mineralised tissues such as teeth, bone, and materials. This imaging process is non-destructive. Therefore, the tooth sample may be scanned multiple times while the sample remains available for further mechanical testing. A study by (Neboda et al., 2017) used Micro CT to assess the mineral density of hypomineralised enamel. They found that there was statistical significance between the brown and yellow lesions compared with white lesions.

1.16.5 Microhardness

The hardness can be defined as measuring the resistance for permanent deformation when a constant compressive force is applied. This deformation can be generated by different mechanisms such as bending, mechanical wear, cutting, scratching or indentation. Hardness has a relation to other mechanical properties like ductility, fatigue resistance and strength. Therefore, hardness testing could be used in the industry as a fast and simple quality control method. The first methodical test to measure hardness was introduced by the Austrian mineralogist Friedrich Mohs in 1812. Since then, a variety of methods have been used in determining hardness. The first machine to report the indentation hardness was performed by William Wade in 1856. This has been accomplished by applying a load to a hardened tool (pyramid-shaped), and from the size of the deformed cavity on the surface, the hardness value was evaluated. In the mid 19 century, the scientific bases for the indentation hardness tests started to have great use and influence on the future work of indentation (TABOR, 1951). The physical insight for understanding the indentation phenomena represented a model based on theoretical developments and careful experiments.

There are three main methods of indentation hardness: Macroindentation, Microindentation, and Nanoindentation (Broitman, 2017):

1.16.5.1 Macroindentation Tests

What characterises the macroindentation test is the indentation loads; it ranges from 2 N to 30 kN. This method of indentation is used in the industry and research communities; the main macroindentation tests are the International Rubber Hardness Degree, Vickers, Meyer, Shore, Brinell and the Durometer. The macroindentation indenters are made of a non-deformable material that is available in various shapes such as a pyramid, ball or cone. These tests

determine the material's resistance to penetration. The hardness is measured by the correlation of the surface deformation or the penetration depth of the indenter under a given load within a certain period of time.

1.16.5.2 Microindentation Tests

What characterises the microindentation test is that the indentation load is below 2N. The two main tests for the indentation are Knoop and Vickers. These microindentation hardness tests determine the material’s penetration resistance to a diamond indenter with a pyramid shape. The method of recording the hardness is similar to that of the Macroindentation process, which explains that the hardness is correlated with the depth of the indenter under a given load within a certain period of time.

1.16.5.3 Nanoindentation Tests

The main difference between the Nanoindentation and the previously mentioned (Micro, Macro), is that the displacement and the load are continuously monitored with high precision in the nanoindentation machine. The two main nanoindenter shapes are the cube corner and Berkovich.

Microhardness tests are widely used to measure the hardness of teeth (Attin et al., 2003, Chunmuang et al., 2007, Faraoni-Romano et al., 2007) but are more commonly used to study the physical properties of the material. This method of testing the surface hardness is quick, easy and requires only a small surface area of the sample or specimen to be tested. Two of the most used indenters are the (Vickers and Knoop) they use a diamond indenter to impress the surface of the specimen/sample at a certain load for a certain period of time. After the load is

removed, the diagonals of the indentation are measured with an optical microscope. The ratio between the area of the residual impression and the indentation load defines the hardness number. The residual impression area depends on the indenter shape.

Various studies (Mahoney et al., 2004a, Mahoney et al., 2004b, Fagrell et al., 2010) have identified a significant reduction in enamel hardness of hypomineralised teeth. (Crombie et al., 2013b) have studied the microhardness of hypomineralised enamel and found that there was a significant reduction in microhardness compared with a control group. Another finding was a sharp decrease in mineral content and hardness was revealed immediately beneath the surface layer, if the surface layer wasn't available due to enamel break down the hardness values were gradually increased towards the dentinoenamel junction DEJ. This paper has also noted that mild hypomineralised lesions showed a significant reduction in hardness and mineral content and in some cases, an extensive enamel involvement. The cervical area of the enamel shows an unaffected area also the unaffected area was consistent with normal enamel in regards to the hardness values, mineral content profiles and porosities this coincides with the finding by (Jälevik and Norén, 2000, Jalevik, 2003). To summarise this paper has examined and discussed the MIH affected enamel in regards to the reduced hardness and mineral contents as well as the defective ultrastructure, especially in the surface defect of the presumably intact lesion.

Fagrell et al. (2010) studied the mechanical properties of hypomineralised enamel of permanent first molars and found that the hardness measurement values were significantly lower in hypomineralised enamel compared with normal enamel. Hypomineralised enamel showed larger variations values during the microhardness measurement, while the normal enamel values were lower. The lower hardness findings in this study of hypomineralised

enamel compared with normal enamel coincide with what has been shown in other studies (Suckling et al., 1989, Mahoney et al., 2004a, Mahoney et al., 2004b). Fagrell et al. (2010) have concluded that the hardness values are lower in teeth that have been diagnosed with MIH compared with normal enamel.

1.16.6 Photography

For many years, photography has been used in the medical and dental fields to record data, research, teaching purposes, and show different treatment aspects. Various information could be provided to the dentist just by a photographic image; this includes the tooth colour, morphology and other properties. There is tremendous benefit in the photography of the oral tissue to the clinical practitioner. Conventional radiographs were initially made using slide film (35mm). During that time, researchers used colourimeters as a secondary method to aid in observable colour change clinically (Zekonis et al., 2003, Maggio et al., 2003). Lately, digital photography has replaced conventional photography. Digital cameras have started to be used more recently by researchers to assess tooth bleaching and colour changes through assessing digital image with editing-image software on a computer. The editing-image software provides quantitative values of image brightness and colour (Carsten, 2003). The image colour and brightness depend on the camera sensor. Therefore the digital camera sensor plays a vital role in capturing the photograph of an object because it contains millions of a single photo element; after an image of an object is projected onto the sensor's surface, the image is split into millions of picture elements (pixels). Before the pixel transforms into an electric signal, the brightness is recorded for each pixel. The colour is automatically generated by the data being processed internally because the photo-diodes cannot differentiate between colours. Therefore most digital cameras consist of a colour mosaic filter.

One of the main difficulties to examine the surface of enamel is its highly reflective surface. Various minor defects may be ignored during the clinical examination due to the wet condition of the teeth. Nevertheless, Ellwood et al. (1994) have found that teeth examined under wet conditions could raise the diagnostic threshold compared to teeth that were allowed a minute to dry. In addition, the details of the enamel surface could be lost because of the reflection of the flash from the tooth surface. Although while using a flash, the enamel tooth surface and texture could be identified. However, the backscatter and direction of the reflection from the enamel surface could combine and hide the range of an enamel defect that may be present. Adams (1968) recently found that using a double polarised filter has aided in suppressing the reflection from the enamel surface of teeth. However, this method didn't eliminate the reflection completely from teeth but enhanced the surface. Dyce and Small (1950) have also suggested using a double polarised filter in their published paper 'Dental Photography with a Specially Designed Unit'. Edgar et al. (1978) Stated that changes in the appearance of the enamel are valuable when aided by photographic recording, supporting the assessment of the teeth during a single session even by multiple observers.

In the medical field, many authors (Anderson, 1991, Pekarek, 1993, McFall, 1996) have pointed out that cross polarised photography has completely eliminated the reflectance of the images of human skin. One of the authors (Anderson, 1991) has stated that the typical glare was absent in the nail plate photographs; this has allowed a clear view of the longitudinal melanonychia and nail bed abnormalities. A study by (J. ROBERTSON, 1999) managed to determine the practicality and the efficacy of using cross-polarised photography for enamel defect assessment compared with the conventional photography method. Their results showed very clear evidence to support the use of the cross polarisation method, meanwhile showing agreement between the assessors, which was greatly significant than that of conventional photography. It has also improved the visual details of enamel defects enabling assessment of

the appearance change. (J. ROBERTSON, 1999) stated that although dental defects were identifiable by both photography methods (conventional and cross-polarised), the dental defects were clearly seen in the cross-polarised method without distraction. Orr (2013) stated that the use of cross-polarised assists in the complete reflection elimination of light on the tooth surface, which helps visualise the details of the sub-surface of the tooth.

1.17 Summary

1.17.1 Study 1

In summary, none of the previously published questionnaires has documented pain severity from the various stimuli (cold, hot, brushing, and sweets) in MIH teeth nor have they correlated the reported pain to the appearance or the type of treatment planned. Therefore a designed questionnaire was developed to investigate these aspects in children with MIH.

1.17.2 Study 2

Various *in vitro* designs (Cochrane et al., 2008, Bahrololoomi et al., 2013) have been introduced in the past years to investigate the remineralisation of the enamel of teeth. In some studies, it would involve the application of a highly concentrated mineralising solution for a week or two in the experiment slurry. In addition, none of the studies has extended more than two weeks. Published studies that investigated the further mineralisation of MIH enamel are few (Crombie et al., 2013a, Batra, 2011). Neither of these studies involved the role of saliva or the application of toothpaste twice daily. Therefore, this study was designed to overcome these limitations.

1.18 Aims

1.18.1 Aim Study 1

- To establish the prevalence of self-reported sensitivity and pain in children who have Molar incisor hypomineralisation (MIH or Molar hypomineralisation)
- To establish the relationship between the severity of pain/sensitivity, appearance of hypomineralised molars clinically and the choice of treatment of hypomineralised molars

1.18.2 Aims study 2

The research aims of the *invitro* study were centred around comparing possible MIH enamel improvements with different treatments:

- To compare the effect of Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP on changes in MIH lesions.
- To assess the surface hardness of MIH lesions using Surface Microhardness Testing.
- To assess the mineralisation of MIH lesions using DIAGNOdent.
- To examine the surface roughness of MIH lesions using surface Profilometry.
- To assess the change in colour of MIH lesions using digital photographs

1.19 Hypothesis

1.19.1 Cross sectional study 1 Null hypothesis

- There is no relationship between pain/sensitivity and MIH.
- There is no association between the severity of pain or sensitivity of hypomneralised teeth and the treatment outcome of hypomineralised teeth
- There isn’t an association between the severity of pain or sensitivity of hypomneralised teeth and the appearance of hypomineralised teeth clinically
- There isn’t an association between the appearance of hypomineralised teeth clinically and the treatment decided of hypomineralised teeth

1.19.2 *In Vitro* study 2 Null Hypotheses

- There are no changes on MIH enamel lesion when using different treatments: Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP.
- There are no changes on MIH enamel surface hardness, surface roughness, mineralisation and lesion colour when using different treatments: Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP.

Chapter 2 **Study 1 Cross sectional**

2.1 Material and methods

The prevalence of pain and sensitivity in hypomineralised teeth was studied in those patients who presented with Molar incisor hypomineralisation or Molar hypomineralisation to Leeds Dental Hospital and Hull community dental centre.

2.2 Hypothesis

2.2.1 Null hypothesis

- There is no relationship between pain/sensitivity and MIH.
- There is no association between the severity of pain or sensitivity of hypomneralised teeth and the treatment outcome of hypomineralised teeth
- There isn't an association between the severity of pain or sensitivity of hypomneralised teeth and the appearance of hypomineralised teeth clinically
- There isn't an association between the appearance of hypomineralised teeth clinically and the treatment decided of hypomineralised teeth

2.3 Inclusion and exclusion criteria

2.3.1 Inclusion criteria

- Children between 6 years of age and 10 years of age
- Fit and healthy children with no medical conditions that could affect the perception of pain
- Children with the established diagnosis of Molar Hypomineralisation or Molar Incisor Hypomineralisation
- Children presenting to Leeds Dental Hospital and Hull Community Dental Centre with the clinical features and established diagnosis of hypomineralised molars including demarcated opacities, posteruptive breakdown, atypical restorations, and/or cheesy looking appearance.

2.3.2 Exclusion criteria

- No established diagnosis of MIH or MH
- Medical conditions that could affect the perception of pain
- Children presenting to other dental centres than the previously mentioned

2.4 Sample size & Power calculation:

As there are no previous reports regarding this study, statistical advice suggested a sample size of more than 100 to 200 for a questionnaire type study. (Statistician in The University of Leeds Dr Jing Kang). A Sample Size Calculator was recommended by the statistician with a confidence level of 80% and a confidence interval of 5% was used and resulted in a power calculation of 164 participants. Therefore, as advised by the statistician it was agreed to have a sample size of no more than 200 participants.

2.5 Questionnaire Piloting:

A literature search was conducted to find published questionnaire studies that documented self-reported dental pain in children. A few studies that recorded self-reported dental pain with published questionnaires were found (Petrou et al., 2015, Haidary, 2014). However, no studies that published a questionnaire had documented the report of pain in MIH teeth to various stimuli (hot, brushing, and sweets) and correlated them to the appearance as well as the treatment planned. Few studies documented self-reported pain as a secondary finding. Therefore, a designed questionnaire was developed to investigate these aspects in children with MIH (Appendix 1). Three paediatric dental consultants were asked to look at and edit the questionnaire before it was piloted with 10 child patients attending the Leeds Dental Institute (LDI). The children were asked for their feedback on the clarity and content before the questionnaire was used in the main study. The sensitivity and pain were assessed using the Wong Baker Face Rating Scale (W-BFPS) (Baker and Wong, 1987)

Figure 1 Wong Baker Face Rating Scale (W-BFPS)

The questionnaire was piloted with 10 children at the Leeds Dental Institute (LDI), and the children were asked for clarity and content. Following completion of the pilot study and changes being made, a convenient sample of children were recruited into the main study, up to 200 children with hypomineralised teeth were recruited as agreed by the statistician. Data were then collated and statistically analysed using IBM SPSS statistics software version 26.

2.6 Ethical considerations & consent:

Ethical approval was obtained from the East of Scotland Research Ethics Service (EoSRES) Approval No 17/ES/0081 for the project ID 210269 (Appendix 3). The site specific in Hull approval for the project ID 210269 was issued by the CHCP (City Health Care Partnership) approval No: 022018 (Appendix 10). Parents or legal guardians were asked to give and sign a written consent before enrolling their child in the research. Consent was only asked from the parent/legal guardian after confirming that they fully comprehended the intentions of the research and had considered all possible consequences of their child's involvement in the research. Age-appropriate assent form (Appendix 7) was given to each child participating in the study. All information sheets (Appendix 4, Appendix 5), consent and assent forms (Appendix 6, Appendix 7) were written in such a way to ensure that the parents/legal guardians were aware of their right to withdraw their child from the study at any time without interfering with the treatment of their child. The parent/legal guardian was informed that any collected data until the time of withdrawal that was taken with the consent would be kept and used in the research. If withdrawal from the research occurred, no further data from the participant would

be acquired and any further procedures or treatments undertaken would not be associated with the research. The time taken to gain consent and carry out the study was a maximum of 20 minutes in total including the photography time.

2.7 Participant recruitment and Sensitivity/pain assessment

Patient data was not reviewed by the direct care team or by the principal investigator to recruit potential participants. The participants were approached at the end of their first visit to the paediatric dental department by the principal investigator in the Dental Hospital in Leeds and by the consultant in the Dental Community Centre in Hull. This was after the diagnosis of MIH/MH was confirmed by the consultant. After that, the principal investigator or consultant gave a verbal explanation of the study; then an information sheet (Appendix 4) was given to the parent/legal guardian to read. If they agreed to participate in the study, and the child was also happy to participate (Appendix 5), they both signed the appropriate consent forms (Appendix 6, Appendix 7). After that, the questionnaire relating to the assessment of pain and sensitivity was completed by the principal investigator or consultant. The questions were divided into two sections; the first section was related to age and gender (demographic data), and the second section was an assessment of the children's perception of sensitivity and pain related to their hypomineralised teeth. The questionnaire (Figure 2, Figure 3, and Figure 4) was used.

Figure 2 First questionnaire page

IRAS Project ID: 210269

Version 3 (18 / 05 / 2017)

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The following questions are related to the assessment of pain and sensitivity in children with Hypomineralised (defected enamel) teeth

Unique Participant Number: .....

Age:..... **Gender:**.....

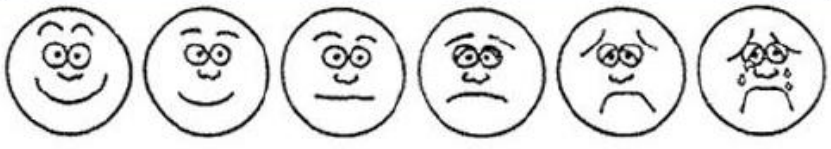
How much pain do you feel while eating cold food?

How much pain do you feel while eating hot (warm) food?

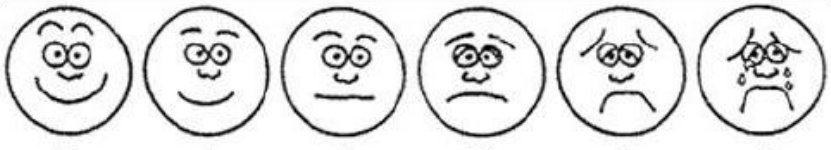
How much pain do you feel while drinking cold drinks?

Figure 3 Second questionnaire page

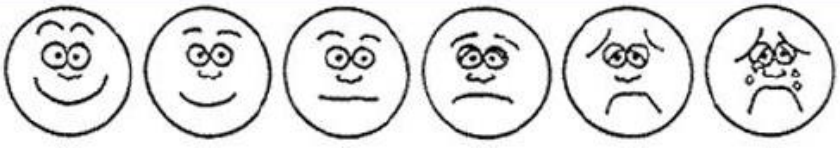
How much pain do you feel while drinking hot drinks or sipping soup?



How much pain do you feel while eating sweet food?



How much pain do you feel while brushing your teeth?



Do you know that you have teeth that are weak?

□ Yes □ No

Have you or your dentist tried to manage the pain ?

□ Yes □ No

IF yes ..... was it effective (did it decrease or lower the occurrence of pain)?

□ Yes □ No

Figure 4 Third questionnaire page (treatment planned for each tooth)

IRAS Project ID: 210269 Version 3 (18 / 05 / 2017)

(This part is to be completed by the Operator (Dentist):

Please tick one box of the treatment outcome for each tooth

Tooth number / Treatment outcome	UR6	UL6	LL6	LR6
No treatment				
Fissure sealant				
Treatment of sensitivity Desensitizing agent eg: tooth mousse				
Restoration				
Temporisation / Monitor with the view to extract later				
Extraction				

The questionnaire was piloted with 10 children at the Leeds Dental Institute (LDI), and the children were asked for clarity and content. The questionnaire was sent personally to Leeds Dental Hospital and Hull Community Dental Centre (Appendix 10). After the research was complete, the principal investigator collected the questionnaire and additional documents (consent and assent) from LDI and Hull Community Dental Centre.

2.8 Treatment outcome:

The Clinical outcome for the MH/MIH teeth was recorded by the operating dentist at each dental clinic after the questionnaire had been completed. The following data were extracted from each participant's records for each Hypomineralised First Permanent Molar (FPM):

- No treatment provided
- Treatment of sensitivity by desensitising materials (Non restorable approach)
- Fissure sealing the FPM
- Restoring the FPM
- Temporisation / Extraction at a suitable time for the FPM
- Extraction of the FPM

2.9 Clinical photos

Calibration and Kappa testing was used in this study. The kappa score was applied for the assessment of the pictures for this study analysis, it was found that the kappa

score was 0.72. Based on Cohen's kappa it describes the agreement to be a substantial agreement.

The scoring system used was the Molar Hypomineralisation Severity Index (Oliver et al., 2014) Table 3. The principal investigator and a consultant in paediatric dentistry viewed 10 participant intraoral photographs, and each intraoral photograph included four views (upper, lower right, and left views). Each tooth was scored into six categories, generating 240 entries (6 values per one permanent molar). The aim of obtaining clinical photos was to create a record that would allow the establishment of the relationship between the reported sensitivity/pain of hypomineralised molars, the clinical presentation of hypomineralised molars and the treatment plan for each hypomineralised molar. The clinical information recorded for each hypomineralised molar was determined in the following categories:

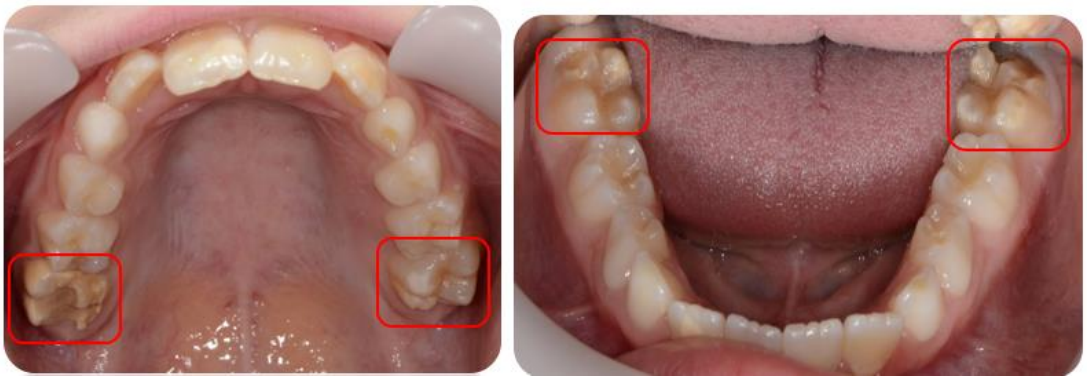
Table 3 Characteristics of molar hypomineralisation defects

Characteristics of molar hypomineralisation defects	**Severity of Characteristic**	**Weighting assigned**
Eruption status	Unerupted	0
	Erupted	1
Colour of most severe defect	None	0
	White/cream	1
	Yellow	2
	Brown	3
Location of most severe defect	None	0
	Smooth surface	1
	Occlusal surface (FPMs)	2
	Cuspal involvement (FPMs)	3
	None	0

Restorations placed/replaced	One	1
	Two or more	2
Atypical restorations	None	0
	Present	1
Post eruptive enamel breakdown (PEB)	None	0
	Present	1

Information sheet Appendix 4 Appendix 5 was handed to the parent/ legal guardian and child explaining the purpose of obtaining intraoral photos and if they agree and sign the consent and assent form, the participant was sent to the dental/ photography team to take the intraoral photos Figure 5, the photos will be stored on a password protected NHS computer in each participating dental centre. If the clinical photos had been taken as part of the routine records, the principal investigator would access them via the NHS computer in each dental clinic. Each photograph was given the participant' unique code and was sent via NHS email to be stored on a password protected computer at the University of Leeds. Access to patient data medical illustration (photography system) in the LDI by the direct care team was to reveal if the participant had previous photographs taken.

Figure 5 Intraoral photographs



The photograph on the left and right shows hypmineralised first permanent molars. the photographs also show different degree of severity ranging from white patches to yellow cream with post eruptive breakdown.

2.10 Data Confidentiality and storage

Each participant was given a unique code to limit the use of any identifying data, this code will replace their identifying information on the extraction data sheet. The Lead Researcher or Consultant recorded the PAS hospital number for each participant on the code link document. The forms were stored safely in locked cabinets in a secure office in each of the participating centres. The code link document linked each participant's PAS Number with their Study code Number was stored in a safely locked cabinet in each participating centre. After the research was complete the code link document for each participating centre was scanned and sent via NHS email to be stored on a password protected computer at Leeds University. For security purposes the password protected data was emailed to the recipient at the University of Leeds and the password was given verbally over the telephone. Patient data extraction forms were also stored safely in locked cabinets in a secure office in each of the participating centres. The data extraction sheets were collected by the chief investigator and transferred to the University of Leeds University to be stored safely in a locked cabinet in a secure office.

The study data will be stored on a password protected university computer for a period of five years or until the research is published. This data can only be accessed by the chief investigator and the Lead Supervisor. Any handwritten records or charts were scanned and uploaded to the same password protected file immediately and the original handwritten document was then destroyed. None of the documents that were transferred to the main research site will include patient identifiable data. They will only include the unique participation code.

2.11 Statistical analysis

The data was analysed using IBM SPSS statistics software for windows version 26. Descriptive analysis was used to calculate the mean, standard deviations, frequencies, and percentages. Meanwhile, the multinomial logistic regression was used to analyse the (nominal) degree of pain from various stimuli and the (independent variables) treatment plan for each hypomineralised molar. In addition, the Person chi-square correlation was used to identify the association between the degree of pain from various stimuli and the photographs as well as the association between the photographs and treatment planned for each hypomineralised molar.

Chapter 3 **Study 1 Results**

3.1 Demographic data

After entering the questionnaire data into SPSS, a test of frequencies and descriptive analysis were performed to reveal the participants' number, age and gender. 20 participants were recruited from hull community centre. While 180 participants were recruited from Leeds Dental Institute. Only 2 children refused to take part in the research. The total number of participants recruited was 200 making up 100 females (50%) and 100 males (50%). The average age was: 8.29 years with an age range of 6 to 10 years, as described in Table 4.

Table 4 Age and Gender

Age (yrs)	Gender No (%)		Total No (%)
	Male	**Female**	
6	4 (2.0)	8 (4.0)	12.0 (6.0)
7	15 (7.5)	18 (9.0)	33 (16.5)
8	34 (17.0)	39 (19.5)	73 (36.5)
9	24 (12.0)	24 (12.0)	48 (24.0)
10	23 (11.5)	11 (5.5)	34 (17.0)
Total	100 (50)	100 (50)	200 (100)

3.2 Pain reporting by age

Most of the participants who reported pain were 8 years of age. While the lowest pain recorded was by participants that were 6-year olds.

Table 5 Age relationship with pain

		No pain	Pain	Total
Age	6	2	10	12
	7	3	30	33
	8	7	66	73
	9	4	44	48
	10	2	32	34
Total		18	182	200

3.3 Pain reporting by stimulus

The Questionnaire information related to pain with different stimuli is recorded in Table 6. The available choices for each question ranged from ’no hurt’ to ‘hurts worst’ based on the Wong-Baker Face Rating Scale (W-BFPS) Figure 1. The data for the experience of pain when eating cold food shows that the most frequent choice was ‘no hurt’ at 30%, followed by ‘hurt a little bit’ - 24%; while the least frequent was that the pain ‘hurts the worst’ at 5.5%. The data for the experience of pain when eating hot food shows that ‘no hurt’ was the most frequent choice at 76.5%, followed by ‘hurt a little bit’ at 16.5%; while the least frequent was ‘hurt a whole lot’ at 1.0%. The experience of pain when drinking cold drinks data shows that the most frequent choice was ‘no hurt’ at 42%, followed by ‘hurt little bit’ at 25%; while the least frequent was ‘hurts worse’ at 4%. The experience of pain when drinking hot drinks

data shows that the most frequent choice was ‘no hurt’ at 75.5%, followed by ‘hurt a little bit’ at 14%; whereas the least frequent was ‘hurts worse’ - 0.5%. The data from the experience of pain when eating sweets shows that the most frequent choice was ‘no hurt’ at 40.5%, followed by ‘hurt little bit’ at 22%. However, the least frequent was ‘hurt whole lot’ at 5%. The data from the experience of pain when tooth brushing shows that the most frequent choice was ‘no hurt’ at 53%, followed by ‘hurt little bit’ at 23%.; while the least frequent was ‘hurts worse’ at 3%.

Table 6 Pain reporting for each stimulus

	Pain with Cold food		Pain with hot food		Pain with cold drinks		Pain with hot drinks		Pain with sweets		Pain with brushing	
Degree of pain	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
no hurt	60	30.0	153	76.5	84	42.0	151	75.5	81	40.5	106	53.0
hurts little bit	49	24.5	33	16.5	50	25.0	28	14.0	44	22.0	46	23.0
hurts little more	39	19.5	7	3.5	23	11.5	14	7.0	36	18.0	21	10.5
hurts even more	27	13.5	5	2.5	23	11.5	3	1.5	17	8.5	13	6.5
hurts whole lot	14	7.0	2	1.0	12	6.0	3	1.5	10	5.0	8	4.0
hurts worse	11	5.5	0.0	0.0	8	4.0	1	0.5	12	6.0	6	3.0
Total	200	100.0	200	100.0	200	100.0	200	100.0	200	100.0	200	100.0

3.3.1 The prevalence of pain

The overall prevalence of pain is presented in Table 7. When comparing between the experience of pain vs no pain sensation for the participants, it was found that the overall prevalence of pain was 91% (n= 182), while 9% (n=18) of participants did not experience pain from any stimulus (hot, cold, brushing or eating sweets). In Addition, there was no difference in reporting pain between male and female participants Appendix 14.

Table 7 Overall participants' prevalence of pain

Overall pain		
	Frequency	Percent
No pain	18	9.0
Pain	182	91.0
Total	200	100.0

3.4 Awareness of the MIH

The Questionnaire information related to the participant's awareness of their enamel defects and pain management is presented in Table 8. The majority of participants (67.5%) answered that they were aware that they have teeth with weaker enamel, while the rest of the participants (32%) didn't know that they had teeth with defective enamel. When the participants were asked about the management of pain, it was revealed that 61.5% of the participants had had treatment to manage the pain. However, only 67.47% of those reported that there had been a success in managing to treat or lower the pain.

Table 8 Awareness of MIH and management of pain

	Frequency	Percent
Do you know that you have teeth that are week?		
No	65	32.5
Yes	135	67.5
Total	200	100.0
Have you or your dentist tried to manage the pain?		
No	77	38.5
Yes	123	61.5
Total	200	100.0
If yes, was it effective?		
No	40	32.52
Yes	83	67.47
Total	123	100.0

3.5 Treatment planned for each individual molar

The Questionnaire information related to the treatment plan for each 1st permanent molar is recorded in Table 9. The majority of treatment planned for the UR6 was extraction at 47%, while the least treatment planned was treatment for sensitivity at 0.5%. The highest percentage of treatment planned for the UL6 was extraction at 44.5%, while the least treatment planned was no treatment with a percentage of 3%. The main treatment planned for the LL6 was extraction at 38%, while the least treatment planned was no treatment at 4.5%. The most treatment planned for the LR6 was extraction at 41%, whereas the least treatment planned for the LR6 was no treatment at 3.5%.

Table 9 Treatment planned for the 1st permanent molars

	UR6		UL6		LL6		LR6	
Treatment planned	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
No treatment	8	4.0	6	3.0	9	4.5	7	3.5
Fissure sealant	37	18.5	45	22.5	55	27.5	52	26.0
Treatment of sensitivity	1	0.5	0	0.0	0	0.0	0	0.0
Restoration	12	6.0	12	6.0	16	8.0	14	7.0
temp/monitor extract later	48	24.0	48	24.0	44	22.0	45	22.5
Extraction	94	47.0	89	44.5	76	38.0	82	41.0
Total	200	100.0	200	100.0	200	100.0	200	100.0

3.6 Photography

Photographs were able to be extracted for 65 out of the 200 participants. The information reported for each individual molar was eruption, colour, location, restoration, atypical restoration, and post-eruptive breakdown (PEB). The photography information related to the appearance of the 1st permanent molars is recorded in Table 10. The upper right first permanent molar data shows that four molars out of 65 participants were un-erupted. In contrast, three upper left first permanent molars were un-erupted. The lower-left first permanent molar recorded only one un-erupted tooth and the lower right first permanent molar had one un-erupted tooth. The highest frequency of lesion colour recorded for the UR6 was yellow at 29.2%, while the UL6 was recorded as white/creamy at 40.0%. The main lesion colour reported for the LL6 was yellow at 26% but the LR6 had white/creamy at 30.8%. The main location for the lesions showed cuspal involvement for the first permanent molars: 63.1% for the UR6, and 66.2% for the UL6, 53.8% for the LL6 and 58.5% for the LR6. The majority of 1st permanent

molars had no restorations or atypical restorations as detailed in Table 10. The post eruptive breakdown (PEB) data revealed that there was no PEB on approximately two thirds of the molars.

Table 10 Photography

	UR6		UL6		LL6		LR6	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
Eruption								
Un-erupted	4	6.2	3	4.6	1	1.5	1	1.5
erupted	61	93.8	62	95.4	64	98.5	64	98.5
Total	65	100.0	65	100	65	100	65	100
Colour								
none	14	21.5	13	20.0	16	24.6	16	24.6
white/creamy	17	26.2	26	40.0	16	24.6	20	30.8
yellow	19	29.2	14	21.5	17	26.2	12	18.5
brown	15	23.1	12	18.5	16	24.6	17	26.2
Total	65	100.0	65	100	65	100	65	100
Location								
none	12	18.5	12	18.5	15	23.1	16	24.6
smooth surface	4	6.2	3	4.6	9	13.8	6	9.2
occlusal	8	12.3	7	10.8	6	9.2	5	7.7
cusp involvement	41	63.1	43	66.2	35	53.8	38	58.5
Total	65	100.0	65	100	65	100	65	100
Restoration								
none	49	75.4	50	76.9	48	73.8	47	72.3
one restoration	16	24.6	15	23.1	17	26.2	18	27.7
Total	65	100.0	65	100	65	100	65	100
Atypical Restoration								
none	61	93.8	60	92.3	61	93.8	60	92.3
Present	4	6.2	5	7.7	4	6.2	5	7.7
Total	65	100.0	65	100	65	100	65	100

PEB								
none	43	66.2	42	64.6	37	56.9	39	60.0
Present	22	33.8	23	35.4	28	43.1	26	40.0
Total	65	100.0	65	100	65	100	65	100

3.7 Statistical analysis for the association between the degree of pain and the treatment planned

The multinomial logistic regression test was used to identify the association between the degree of pain and the treatment planned. The test reveals statistical significance between the pain from the different stimuli and the treatment plan for each molar as shown in Table 11. For example, the statistical tests show a statistical significance P-value of 0.049 between the sensitivity from hot drinks and planned fissure sealant treatment of the UR6 (Table 11). A chi-square and Cramer's V association between the treatment planned and the degree of pain of all the molars are shown in **Error! Reference source not found.**.

Table 11 Association between pain stimulus and the treatment plan for each molar

Molar location	Cause of Pain	Treatment planned	Sig
UR6	Pain with hot drinks	Fissure sealant	0.049
UL6	Pain with sweets	Fissure sealant	0.031
		Restoration	0.013
LL6	Pain with sweets	Fissure sealant	0.016
LR6	Pain with sweets	Fissure sealant	0.020

3.8 Association between the appearance of MIH teeth and the treatment planned for MIH

A chi-square and Cramer's V association strength test was used to determine the association between the two variables. The statistical analysis for the association between the treatment planned and the appearance of molars is presented in Table 12. The photography analysis shown in Table 12 includes colour, location and PEB. A significant association between the presence or absence of PEB and treatment planned for the UR6 is presented in Table 12. Detail of the association between the treatment planned and the appearance of all the molars is shown in Appendix 11. The UR6, UL6, LL6 with peb were more likely to be extracted when compared to intact lesions in the same quadrant. However, intact lesions were more likely to be (fissure sealant, restoration and temprorised/monitor extract later). The UL6, LL6, LR6 with cuspal involvement were more likely to be extracted followed by temp/monitor with the view to extract later. The LR6 with brown coloured lesion where more likely to be extracted when compared with other lesion colours. The LR6 with white/creamy lesions were more likely to be temprorised and monitored with the view of extraction later.

Table 12 Association between the appearance of MIH teeth and treatment planned for MIH

Photography Treatment	Photography data (colour,location,peb)	Sig[1]	Cramers V[2]
UR6	PEB of UR6	0.001	0.521
UL6	lesion location UL6	0.009	0.370
	PEB UL6	0.010	0.450
LL6	lesion location LL6	0.016	0.356
	PEB LL6	0.001	0.553
LR6	lesion colour LR6	0.045	0.331
	lesion location LR6	0.045	0.331

[1] Person chi-square, P-value

[2] Cramers V association strength test (0= no relationship, 0.2 or less= a weak relationship, 0.2 to 0.3= moderate relationship, 0.3 and up= a strong relationship)

3.9 Association between the pain stimulus and the appearance of MIH teeth

Chi-square and Cramer's V test was used to determine the association between the pain stimulus and the appearance of the teeth as shown in the photography analysis (location and PEB). The significant association between the pain stimulus for molars and the photography report for the location and PEB is presented in Table 13. The association description is shown in the crosstabs between the pain stimulus and the appearance of molars Appendix 12. The UR6 and UL6 with cuspal involvement were more likely to be recorded as (no hurt) from pain of hot food when compared with other lesion location. UL6 without PEB were more likely to be recorded as 'no hurt' when reporting pain from hot drinks. While UL6 with peb were more likely to be reported as 'hurts little bit'.

Table 13 Association between the pain stimulus and the appearance of MIH teeth

Photography / Pain stimulus	Photography report (location,PEB)	Sig[1]	Cramers V[2]
Hot food	Location of lesion UR6	0.015	0.324
	Location of lesion UL6	0.001	0.381
Hot drinks	PEB UL6	0.001	0.534

[1] Person chi-square, P-value

[2] Cramers V association strength test (0= no relationship, 0.2 or less= a weak relationship, 0.2 to 0.3= moderate relationship, 0.3 and up= a strong relationship)

Chapter 4 Study 1 Discussion

4.1 Research Aims

The research aims for the first study were:

- To establish the prevalence of self-reported sensitivity and pain in children who have Molar incisor hypomineralisation (MIH or Molar hypomineralisation
- To establish the relationship between the severity of pain/sensitivity, appearance of hypomineralised molars clinically and the choice of treatment of hypomineralised molars

4.2 Research design

4.2.1 Questionnaire

Pain is a subjective experience and the gold standard for assessing children's perceptual pain experience is to use self-reported measurements (Versloot et al., 2006). Stinson et al. (2006) defined pain as "a subjective phenomenon that must be measured by self-report. None of the previously published questionnaires have documented pain severity from the various stimuli (cold, hot, brushing, and sweets) in MIH teeth nor have they correlated the reported pain to the appearance or the type of treatment planned. Therefore, we designed a questionnaire to investigate these aspects in children with MIH. A validated face rating scale (Wong and Baker, 1988) was added to the questionnaire to facilitate the pain rating by children Figure 1. Garra et al. (2010) mentioned in their study that there is a debate between researchers regarding the optimum facial designs such as (facial features or shape), also the pain spectrum (eyebrows elevation, mouth opening and furrowing of the forehead). Additionally, some facial pain scales show a neutral face for no pain while others show a happy face. It might be confusing between the two faces of (hurts a little bit) and (hurts even more). Although, this comment was not

mentioned by participants, it was addressed by expert. Based on (Garra et al., 2010) the face scale might benefit from modifying the fine details in particular.

Three paediatric dental consultants were asked to look at and edit the questionnaire before it was piloted with 10 child patients attending the Leeds Dental Institute (LDI). The children were asked for their feedback on the clarity and content before the questionnaire was used in the main study. The changes made to the questionnaire was that the Wong-Baker faces pain rating scale was added for each question separately and that all questions were consistent having the same wording “how much pain do you feel while”.

4.3 Demographic data

4.3.1 Sample size

A Sample Size Calculator with a confidence level of 80% and a confidence interval of 5% was used. After discussion with the statistician, it was agreed to have a sample size of 200 participants to allow for complete questionnaires and ensure valid statistical analysis could be carried out. 20 participants were recruited from the Hull Community Dental Center. While 180 participants were recruited from Leeds Dental Institute. Only 2 children refused to take part in the research. the children stated “I don’t want to” therefore they were not involved in the research. The rest of the participants showed motivation and willingness to participate.

4.3.2 Age and Gender of Participants

The total number of participants recruited was 200 with an age range of 6 to 10 years and a mean age of 8.29 years, as shown in Table 4. This was an appropriate age group because the first permanent molars erupt around the age of 6 years (Proffit et al., 1993) and the study wished to capture the children’s evaluation during the first few years after the MIH teeth erupt to see if the symptoms remain the same or change with time. It has been reported that the recorded percentage of MIH reduces after the age of 10 (Zhao et al., 2018), possibly due to the

teeth having been extracted or filled and the real diagnosis not being made. It may also be that many MIH teeth suffer post-eruptive breakdown as the age increases (Mishra and Pandey, 2016), therefore, increasing the chances that the dental treatment provided might mask teeth from being diagnosed as MIH teeth. That would result in an apparent reduction in the prevalence percentage of MIH for children over the age of 10 years-of-age.

In the current study, there was no difference between the number of male and female participants. Additionally, there was no difference in reporting pain between males and female participants Appendix 14. This finding agrees with what has been published in the literature regarding the gender of participants (Martinez Gomez et al., 2012, Jälevik et al., 2001a, Preusser et al., 2007).

4.4 Pain reporting by stimulus

Temperature changes should not elicit nociception in teeth under normal circumstances. The dentine and enamel are the two layers of hard tissue that cover and protect the densely vascularised innervated pulp tissue (Byers and Narhi, 1999, Hossain et al., 2019). The hardest tissue in the body is the outermost part of the tooth, which is the enamel. Some 92–96% of the enamel weight is made up of minerals (Gwinnett, 1992). Although the enamel has thermal insulating capabilities, exposed dentine and pulpal inflammation can give rise to intense pain during mild temperature change (Ahn et al., 2012, Henry and Hargreaves, 2007). Various theories explain the mechanism underlying the cause of dental pain. Among these theories is the hydrodynamic theory, which is considered the most widely accepted theory in explaining dental pain sensation (Andrew and Matthews, 2000, Charoenlarp et al., 2007, Vongsavan and Matthews, 2007). Children with MIH defects report frequent sharp pain from various stimuli, such as osmochemichal, mechanical and thermal stimuli, and this may be due to dentine hypersensitivity (Addy, 2000).

The sensitivity may arise from repeated excitability of intradental nerves and the effectiveness of nerves transmitting the stimulation. MIH teeth may have more porous enamel and there may be exposed dentine due to pre/post-eruptive breakdown. When this occurs, MIH teeth are left with dentine that is vulnerable to oral stimuli. In addition, it has been shown by Jälevik et al. (2001c) that the levels of calcium and phosphate in the enamel are lower in MIH teeth than in normal teeth. This will contribute to the accelerated posteruptive breakdown of enamel and the increased permeability of hypomineralised enamel leading to increased sensitivity (Seow, 2014). It has been reported in the literature that children with MIH complained of shooting pain when they were brushing their affected molars (Parikh et al., 2012). Sensitivity of MIH teeth has been reported in various studies. None of these studies had reported the degree of pain in MIH teeth or examined the correlation between the degree of pain, treatment outcome and the appearance of the MIH teeth. In the current study, children with MIH reported the degree of pain from various stimuli. The stimuli involved thermal (cold and hot); mechanical (tooth brushing) and sweet (pain when eating sweets). Haidary (2014), reported in their study that the percentage of sensitivity recorded in MIH teeth was 98.3% in UAE/Dubai. Some 81% were mild cases where the children reported pain from air and water, while pain in severe cases (spontaneous hypersensitivity to external stimulus and pain with brushing) was reported by 18%. Petrou et al. (2015) reported the prevalence of sensitivity in MIH teeth based on the pain from daily activities such as tooth brushing and eating. While (Raposo et al., 2019) reported the prevalence of pain from compressed air and tactile stimuli. A study by (Shepherd et al., 1999) stated that much dental pain experience in children was caused by dental caries. They also reported that sweets caused 38% of the pain in children who reported toothache. A recent study by (de Aguiar Grossi et al., 2017) found that MIH teeth had significantly higher dentine caries (23.08%), compared with teeth without MIH. They also stated that the MIH teeth were more likely to develop carious lesions. A systematic review (Americano et al., 2017), found that

MIH teeth were more likely to develop caries; the more severe the MIH defect, the more likely that carious lesions will be found.

4.4.1 The prevalence of pain reported

In the current study, the overall prevalence of pain is presented in Table 7. When comparing the experience of pain vs no pain sensation for the participants, it was found that the overall prevalence of pain was 91% (n= 182). In comparison, 9% (n=18) of participants did not report experience pain from any stimulus (hot, cold, brushing or eating sweets). A study by Petrou et al. (2015), reported a 31% prevalence of self-reported pain in children with MIH while performing daily activities (brushing and eating). Raposo et al. (2019) reported a 34.7% prevalence of pain in children with MIH. For the pain assessment, Raposo et al. (2019) applied a blast of air for one second. Five minutes later, a tactile test (scraping the affected enamel with a dental probe) was administered. In their study, a visual analogue scale from 0 to 10 was used for both assessments. However, another study by Haidary (2014) reported that the prevalence of sensitivity in MIH teeth was 98.3%.

4.5 Awareness of having 'weak' teeth

The awareness of children with MIH about their teeth has not been reported in the literature. Therefore, this part of our research involved the awareness of the children having MIH and the pain management and effectiveness of the treatment provided by their general dental practitioner (GDP). The majority of participants (67.5%) answered that they knew they had teeth with weaker enamel. In comparison, the rest of the participants (32%) did not appear to realise they had teeth with defective enamel Table 8. When participants were asked about the previous management of pain, it was revealed that 61.5% of participants had had treatment to manage the pain. However, only 67.47% of those reported that there had been any success in managing to treat or lower the pain. Table 8. The parents' and children's awareness of MIH is expected to be partly related to the knowledge of the GDP that the child visits regularly.

Gamboa et al. (2018) reported that 72.8% (n=166) of GDPs were aware of the differences in developmental dental defects (MIH, fluorosis or hypoplasia). A study by Weerheijm and Mejàre (2003) reported GDPs' knowledge was 97% (n= 54) of MIH in 30 different countries in Europe and Asia. Another study by Crombie et al. (2008) found that 98.3% (n= 58) of GDPs were aware of MIH teeth. In this study, the awareness of children that they had MIH was 67.5% (n= 135). The awareness of children in this study was lower than other studies (Weerheijm and Mejàre, 2003, Crombie et al., 2008). The percentage for children's awareness might reflect the knowledge of the GDP. It is possible that they and their parents had gained some knowledge from online resources and discussions with friends. The finding regarding the awareness of MIH is similar to (Gamboa et al., 2018) study. It would be interesting to know what sensitivity treatment products were being recommended by GDPs as previous studies have reported that the majority of the GDPs would provide prevention/treatment for the sensitivity of MIH in children (Crombie et al., 2008, Gamboa et al., 2018).

4.6 Treatment planned for each first permanent molar

This part of the discussion involves the treatment planned by the Consultants for the first permanent molars. The treatment plan for each tooth depends on many factors such as the severity of MIH defect, the area of the defect and the presence or absence of post-eruptive breakdown. Potential treatment and management for hypomineralised first permanent molars was explained by (William et al., 2006, Lygidakis, 2010). Possible treatments for the molars was recorded and was discussed with the Consultants in Leeds Dental Institute before including the possible options in the study. The treatments ranged from conservative "no treatment" to severe "extraction". In this study, it was found that a significant treatment planned for hypomineralised first permanent molars was extraction with varying percentages of 38% - 47% depending on which molar was planned for extraction. This finding was similar to findings in previous studies (Jälevik and Klingberg, 2002, Oliver et al., 2014). Additionally, it was found

that molars in the upper arch had a higher percentage of extraction treatment planned when compared to molars in the lower arch. This may be due to the higher percentage of MIH occurring in the maxillary arch compared to the mandibular arch (Haidary, 2014, Petrou et al., 2015, Cabral et al., 2020).

4.7 Appearance of Teeth on Clinical Photographs:

The clinical presentation of MIH has been described (Weerheijm, 2003b). She explains the presence of demarcated opacities with varying colour from chalky white to brown affecting the first permanent molars and or incisors. In addition, the presence of PEB may be clinically evident in some MIH cases. The judgement criteria for MIH (Weerheijm, 2003b), was based on the clinical presentation. The criteria involved "absence or presence of demarcated opacities; posteruptive enamel breakdown; atypical restorations; extraction of molars due to MIH; failure of eruption of a molar or an incisor". It was stated by Weerheijm (2003b) that if there was one molar affected with MIH, it was more likely that the contralateral tooth was affected with MIH. The finding in this study is consistent with what (Weerheijm, 2003b) found Appendix 15. A later study (Oliver et al., 2014) recorded further details of the clinical presentation of MIH teeth to produce a severity index. The points for recording the clinical appearance of MIH teeth were adapted and modified from (Oliver et al., 2014) study. The clinical recording of MIH characteristics included eruption status, lesion colour, lesion location, presence or absence of restoration, atypical restoration and post-eruptive breakdown. Petrou et al. (2015) found PEB and/or atypical restoration in 17.8 % of MIH teeth in their study. The current study found from the photographs that PEB ranged between 33.8% – 43.1%, depending on which molar was assessed. This was higher compared to other studies. An explanation may be that the present study population was a referred population and it is possible that only the more severe cases are being referred to the LDI. Therefore, this would not reflect the true population of children with MIH in the city of Leeds.

4.8 Association between the degree of pain from different stimuli and treatment planned for MIH teeth

The possible causes of pain and pain stimulus were mentioned in Section4.4. It was reported by Oliver et al. (2014) that sensitivity was one of the positive predictors for planning extraction of first permanent molars. In contrast, there was no association found between the degree of pain reported by the children (at least in the study) and the planned extraction of MIH teeth in this study. It is possible that other reports of pain from parents and the referring GDP could have influenced the planning by the Consultants. In the present study, the degree of pain reported from eating sweets was more likely to be associated with planned fissure sealing for the following teeth: LL6, LR6 and UL6. The degree of pain reported from sweets was also more likely to be associated with planned restoration for the UL6. In the literature, the pain from sweets has previously mostly been associated with caries (Shepherd et al., 1999). A study by (Gamboa et al., 2018) found that 42% (n=107) of dental practitioners applied fissure sealant as a prevention treatment for MIH teeth regardless of reported pain. At the same time, 67.1% (n=171) of practitioners would restore the MIH teeth with composite if required. Similarly, a study by (Crombie et al., 2008) reported 73% (n=84) of practitioners would restore MIH teeth with composite. In our study restoration was planned in 6.75 % of the teeth, Perhaps this reflects the severity of the cases being referred to the LDI. (Haidary, 2014) reported that 30.5% of children with MIH had at least one tooth with a filling. On the other hand, in (Petrou et al., 2015) study, it was found that 15.5% of children had at least one tooth with a restoration. In the current study, the treatment plan for fissure sealant application ranged from 18.5 – 27.5% for each molar. While treatment planned for restoring MIH teeth ranged from 6.0 – 8.0% for each molar. It would be interesting to record the restoration of choice in this study. However, the restorative materials/methods were not recorded in this study.

4.9 Association between the pain stimulus and the appearance of MIH teeth on the photographs

The possible causes of pain and pain stimulus have been discussed in Section4.4. This present showed that the UR6 and UL6 teeth with MIH cuspal involvement were less likely to have pain from hot food recorded by the children. In addition, the UL6 with MIH but intact enamel was less likely to have pain from hot drinks recorded but if the UL6 had PEB, there was more likelihood of pain from hot beverages being reported. It is not clear why there are differences between the upper and lower arches. Perhaps the foods or drinks contact certain teeth for longer or there is a difference in the innervation and perception of pain. It would be interesting to investigate this further. The finding in our study agrees with the literature regarding the presence of PEB and the report of pain (Oliver et al., 2014). Additionally, the location "cuspal involvement" and the report of pain was consistent to what has been reported (Oliver et al., 2014). The majority of previous studies have reported pain from cold stimuli, as well as brushing or eating, and have not included hot stimuli.

4.10 Association between the appearance of MIH teeth on photographs and the treatment planned for MIH

This part of the study involved the association between the appearance of MIH teeth (lesion colour, lesion location and presence of PEB) and the treatment planned by the Consultants. Previous studies have reported that the darker the lesion colour, the greater the chances for post eruptive breakdown to occur over time (Da COSTA-SILVA et al., 2011, Neves et al., 2019), which has been suggested might result in practitioners being more likely to plan extraction for these teeth. The finding in the present study was that the presence of PEB;

cuspal involvement of the MIH lesion and/or brown lesion colour were more likely to lead to planned extraction of the teeth. This is consistent with previous reports (Jälevik and Klingberg, 2002, Oliver et al., 2014). It suggests that practitioners are aware that the presence of these factors are more likely to the MIH lesions breaking down in the future and/or being much more difficult to restore. Given that MIH teeth are more difficult to anaesthetise, some practitioners may believe that extracting the teeth is a better solution. They may also consider that restoration failure is higher in teeth with more severe lesions and they may wish to avoid children having to have repeated restorations. Another reason may be to reduce the dental fear or anxiety that could arise from multiple dental visits. Also, the dental occlusal, crowding of teeth and suitable dental age might favour the extraction of MIH especially if they had poor prognosis.

4.11 Limitation of the first study

- The questions would be consistent with the answers if changed to (how much does it hurt) rather than (how much pain).
- The Wong baker face scale might benefit from modifying in future studies to facilitate the selection of the faces. For example, details to modify (facial features or shape), also (eyebrows elevation, mouth opening and furrowing of the forehead).
- Record of any other factors that could misinterpret the pain such as pain from caries or eruption.
- The study may be overpowered while the photos are underpowered
- Intra examiner reliability test was not performed in this study

4.12 Conclusion

This study showed that the majority of participants reported pain and this may reflect that they were being referred for tertiary care and are likely to have more severe forms of MIH. It appeared

in this group that Consultants were most likely to make decisions based on the severity of the lesions and probably the pain history they elicited at the assessment visit.

4.13 Rational of the invitro study

Fearon (2007) and Gillam (2015) reported that teeth with lower mechanical properties showed increased tooth sensitivity. Additionally, teeth with reduction in microhardness have also been shown to have increased sensitivity in tooth bleaching studies (Elfallah and Swain, 2013). Other studies (Angker et al., 2004, Farah et al., 2010) mentioned the reduction of mineral content of teeth, which may result in the increase of tooth sensitivity. From the first study results it was shown that the prevalence of pain reported was around 91%. Therefore, it was important to undertake an invitro research project, aiming to assess the mineral density and hardness of MIH lesions and to investigate the effect of available oral hygiene products on improving microhardness which in turn may decrease sensitivity *in vivo*. The products selected were Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP.

Chapter 5 **Study 2 *In vitro***

5.1 Method and Material

5.2 Ethics

Ethical approval was granted from the University of Leeds, Dental Research Ethical Committee (DREC). Application for the School of Dentistry Skeletal Tissue Research Bank was to obtain MIH non-carious first permanent molars with intact surfaces. Approval no: (070619/AS/275) Appendix 9.

5.3 Inclusion and exclusion criteria

5.3.1 Inclusion criteria:

- Established diagnosis of Molar Hypomineralisation or Molar Incisor Hypomineralisation
- Extracted MIH first permanent molars with intact enamel surfaces containing enamel opacities (white and yellow)

5.3.2 Exclusion criteria:

- No established diagnosis of Molar Hypomineralisation or Molar Incisor Hypomineralisation
- Extracted MIH first permanent molar without intact surfaces

5.4 Power Calculation

The sample number of 20 samples was confirmed with the statistician. The groups were equally distributed (5 samples in each group) to facilitate the statistical analysis method of multilevel modelling.

5.5 Experimental plan (add appendix)

Group 1: Control group

- Non-Fluoride toothpaste 0 ppm F (Negative Control).
- Fluoride toothpaste (1450 ppm F) (positive control).
- Fluoride toothpaste (1450 ppm F) (Novamin)

Group 2: experimental group

- Fluoride toothpaste (1450 ppm F) + Tooth Mousse (10% w/v CPP-ACP)

5.6 Enamel Slab Preparation

Before sectioning the teeth, they were cleaned using a toothbrush to remove any soft tissue remnants. All teeth were screened by trans-illumination and transmitted light using a large magnifying glass to detect hypomineralised lesions. The root part of each tooth was removed and disposed of in accordance with the Human Tissue Research Bank's Code of Practice. The coronal part of each tooth was mounted using 'green stick' impression compound (Kerr, UK) on the cutting machine (Well@Walter EBNER, CH-2400 Le Loche) plates Figure 6. Then the crowns were sectioned using a water-cooled diamond wire saw, cutting machine. Each crown's buccal and palatal/lingual surface was separated, and approximately 4mm x 4 mm x 2mm size slabs containing hypomineralised lesion was prepared from these sectioned surfaces (**Figure 7**). The enamel slabs were then mounted on the spoon of micro-centrifuge tubes with blue tack.

Figure 6 cutting machine (Well@Walter EBNER, CH-2400 Le Loche)

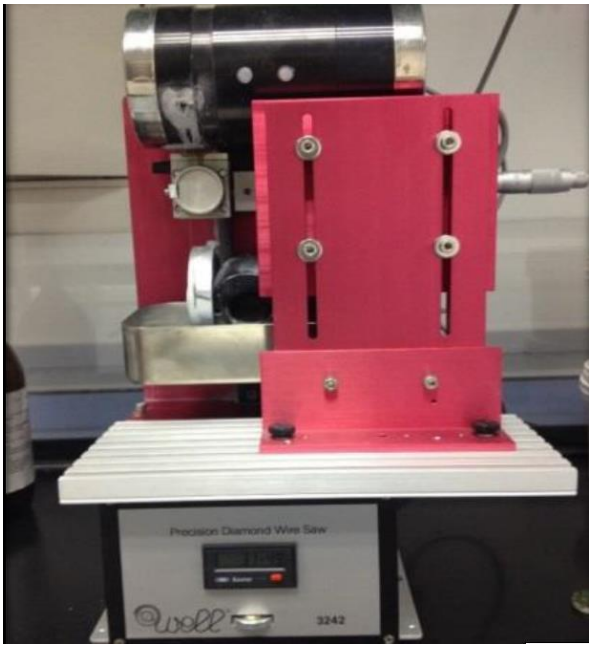
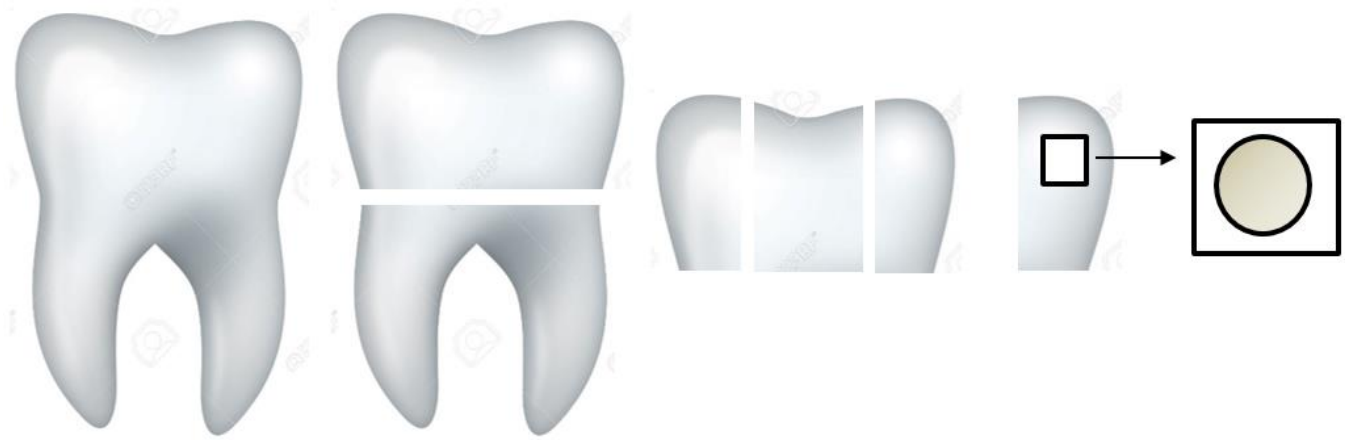


Figure 7 Tooth Cutting Procedure

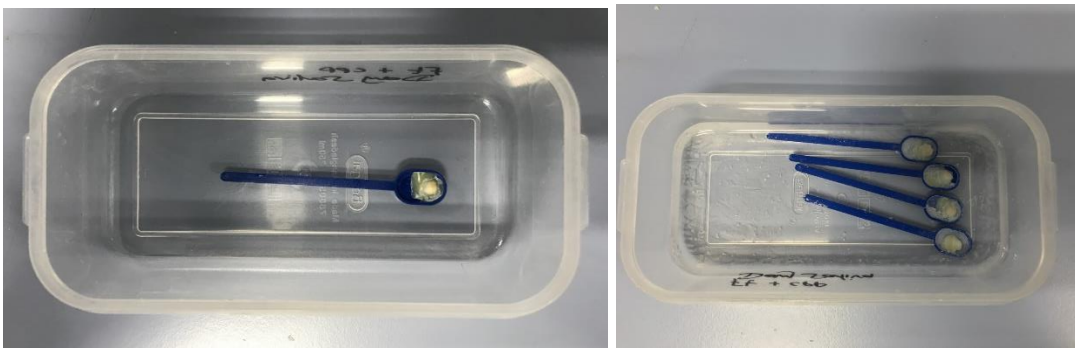


From left to right, is the procedure of cutting the samples. Second from left is a coronal cut separating the crown from the root. The third figure displays a sagittal cut that separates the cusps from each other. The fourth figure shows the tooth slab with the MIH lesion.

5.7 Storage of Enamel Slabs

After the enamel slabs were completely prepared, they were stored in micro-centrifuge tubes containing deionised water at room temperature to keep them moist and prevent dehydration. After recording the baseline readings each enamel slab was mounted with wax on the micro-centrifuge spoon Figure 8.

Figure 8 Enamel slab



5.8 The Cycling Regime

The enamel slabs with hypomineralised lesions were immersed in toothpaste slurries (Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP) for 5 minutes twice each day to simulate the use of toothpaste twice daily during tooth brushing. This was done once in the morning (time) before suspending the enamel slabs in artificial saliva and again at night (time) before suspending the enamel slabs in artificial saliva and left overnight. The toothpaste slurries were prepared by mixing 1g of the toothpaste with 4 ml of deionised water.

Each day at 8.00 am, the enamel slabs with hypomineralised lesions were taken out of the artificial saliva and were immersed in the toothpaste slurry with the selected mineralising agents for 5 minutes; then they were immersed in 50 ml of artificial saliva for 8 hours. At 4.00 pm, the enamel slabs were re-immersed in a freshly prepared toothpaste slurry with the selected mineralising agents for a further 5 minutes then suspended in 50 ml of artificial saliva until the

following day. At all times, the enamel slabs were kept in an incubator at 37˚C, except when immersed in the remineralisation solution. This cycling was carried out for 28 days Figure 9.

Figure 9 Cycling Regime

5.9 Group 1:

5.9.1 - Non-Fluoride toothpaste (Negative Control)

A non-Fluoride toothpaste Figure 10, which served as a negative control, was used twice daily, one application in the morning and another in the evening. The enamel slabs were placed in the toothpaste slurry (without any additional mineralising agent) for 5 minutes each time.

Figure 10 Non-Fluoride toothpaste (control fluoride free toothpaste)

5.9.2 - Fluoride Toothpaste (1450 ppm F) (Positive Control).

Fluoride toothpaste (1450 ppm F) Figure 11, which served as a positive control, was applied twice daily, one application in the morning and another in the evening. The enamel slabs were placed in the toothpaste slurry (without any additional mineralising agent) for 5 minutes each time.

Figure 11 Fluoride Toothpaste (control fluoride toothpaste)

5.9.3 - Fluoride Toothpaste (1450 ppm F) + NovaMin

Fluoride toothpaste (1450 ppm F) + NovaMin Figure 12, was applied twice daily, one application in the morning and another in the evening. The enamel slabs were placed in the toothpaste slurry for 5 minutes each time.

Figure 12 Fluoride Toothpaste (1450 ppm F) + NovaMin

5.10 Group 2:

5.10.1 - Fluoride Toothpaste (1450 ppm F) + Tooth Mousse (10% w/v CPP-ACP)

Fluoride toothpaste (1450 ppm F) and Tooth Mousse (10% w/v CPP-ACP) were applied twice daily Figure 13, one application in the morning and another in the evening. The enamel slabs were placed in the toothpaste slurry for 5 minutes and this was followed by being immersed in Tooth Mousse (10% w/v CPP-ACP) for a further 5 minutes. Afterwards, the enamel slabs were immersed in artificial saliva.

Figure 13 Fluoride Toothpaste + CPP-ACP

5.11 Methods used to evaluate surface hardness and potential mineralisation changes to the lesion and the surfaces using four techniques.

5.11.1 Microhardness Testing

The microhardness technique is very sensitive to changes in mineral density and can provide indirect evidence of mineral loss or gain (Featherstone and Zero, 1992).

This method measures the resistance of the enamel surface to an indenter penetration, which represents the degree of the superficial enamel layer porosity that indicates a mineral loss or gain in subsurface lesions (Koulourides, 1971). In this study, Vickers microhardness Figure 14 was used to assess the enamel surface hardness at the baseline and following treatment. Many studies have reported the microhardness loads, which range from 50 g up to 500 g. In the present study, a load of 100 g was applied on the MIH enamel samples by the Vickers microhardness diamond. Each enamel slab was measured twice, once at the baseline, and the final measurement was after the experimental period. The measurements were recorded by a computer-aided indenter machine - Duramin (Struers A/S, DK 26-10, Ballerup, Denmark). The indentations were created with a Vickers diamond under a 100 g load for 10 seconds Figure 15. Five indentations were applied to determine the mean microhardness value for each slab. The indentations were spaced at least 100 μm apart. The length of each indentation was measured in microns by image analysis. After the experiment was complete, a final indentation was recorded. The final indentations were adjacent to each indentation that was recorded at the baseline Figure 16.

Figure 14 computer-aided Duramin Indenter Machine

Figure 15 Microscopic image of square shape indentation on the enamel surface at baseline

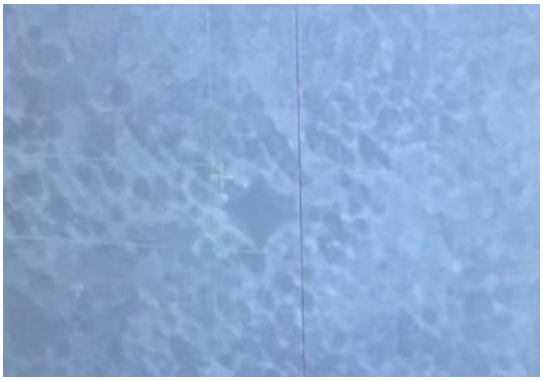
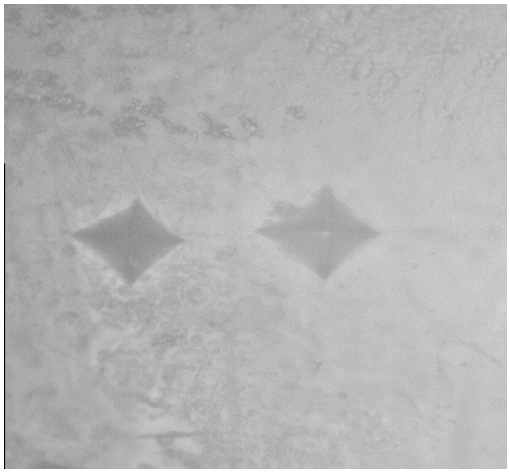


Figure 16 Microscopic image of two square shaped indentations on the enamel surface



On the right is the indentation at the baseline while the left indentation is after the experiment

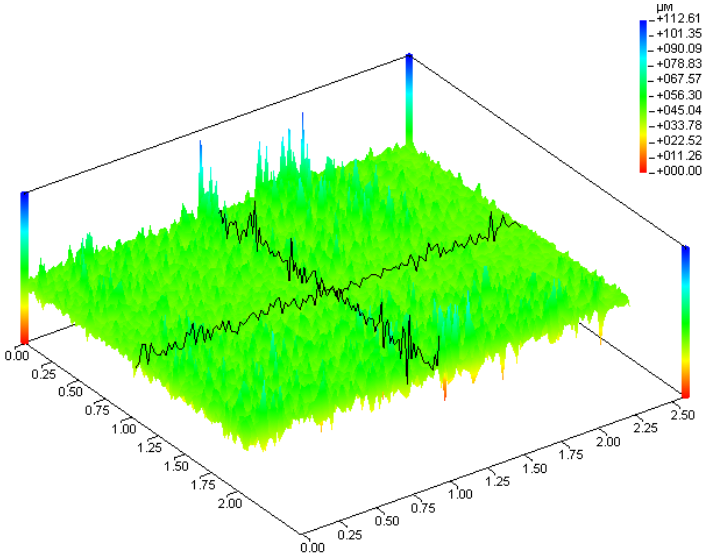
5.11.2 Light Profilometry

Surface profilometry is one of the most common laboratory methods used to determine tooth enamel surface roughness. There are two methods for surface profilometry measurement, contact and non-contact (Whitehead et al., 1995). Light profilometry is a non-contact method that allows for digitisation of the specimen surface. The light profilometer uses a measuring sensor to record the distance of the surface accurately, therefore, creating a microtopographic image (Litwin et al., 2006, Cohen-Sabban et al., 2001). The non-contact sensor can provide analysis of texture and shape, microform and microtopography, as well as surface roughness measurements (Theocharopoulos et al., 2010). The analysis for light profilometry involves two essential values, the mean of the X-axis and the mean of the Y-axis. These two values can be used to create a measurement of the roughness of the surface. The most common roughness parameter used in dentistry is the Ra value (Sunnegårdh-Grönberg and van Dijken, 2003). In this study, non-contact light profilometry was used Figure 17. A flat line reference was created on a metal base, following that the sample was placed on the metal platform and the height of the light sensor was adjusted from the top left corner for each sample until the light sensor was able to recognise the surface. After that, the initiation for scanning the surface would take

place. The scanning procedure would start by scanning a straight horizontal line, then the machine would take a step vertically to scan another line adjacent to the first one that was previously scanned, the process would continue until the whole area is completely scanned. A microtopographic image was created after scanning the specimen surface was complete Figure 18. The previous process was repeated at the end of the experiment.

Figure 17 Non-contact surface Profilometer (Scantron Proscan 2000)

Figure 18 Microtopographic image for the scanned area



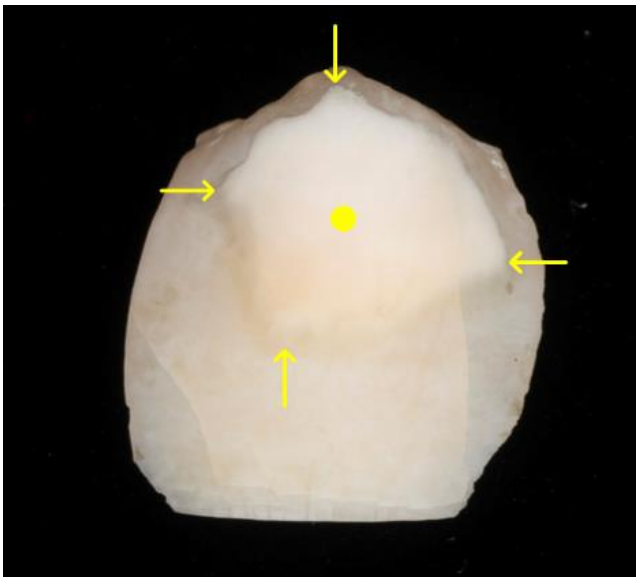
5.11.3 Laser Fluorescence - DIAGNOdent

Laser fluorescence Figure 19 was used to record differences in fluorescence and relate these to the original colours of the lesions and to changes that occurred with the mineralising products. This method has been previously shown to represent changes in the mineral density of enamel (Farah RA, et al. 2008). The DIAGNOdent device generates laser light that is absorbed by bacterial porphyrins or enamel proteins in the enamel and is reflected as fluorescence within the infrared region. The reflected laser light energy can be quantified by the device and registers a numerical value. It has been shown in the literature that increasing DIAGNOdent readings indicate decreasing mineral densities in enamel (Farah RA, et al. 2008).

Each enamel slab was measured twice, once at the baseline, and the final measurement was after the experimental was completed. The measurements were recorded by DIAGNOdent pen 2190 kavo (DIAGNOdent™) Figure 19. Five DIAGNOdent readings areas were recorded to determine the mean laser fluorescence value for each slab. Each DIAGNOdent reading was recorded separately Figure 20. The device's tip would slide on the natural tooth structure until a bleep sound is heard (usually as soon as the defective enamel area is reached), the value then was recorded for the measured area Figure 20. The measurement was only recorded when three consistent readings were achieved for each area examined. This method was used in the current study to assess changes in the mineral density of enamel before and after the treatment.

Figure 19 DIAGNOdent pen 2190 kavo (DIAGNOdent™)

Figure 20 DIAGNOdent scanned areas



Five scanned areas are showing in this figure, each yellow arrow or circle represents a scanned area

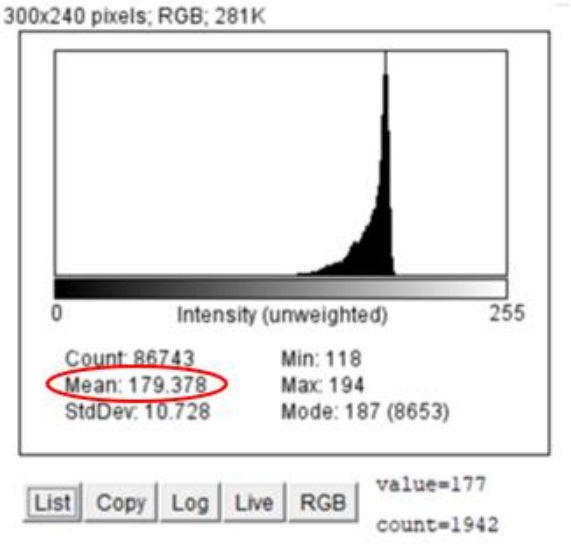
5.11.4 Photography

Photographs were taken of each lesion before and after the selected mineralisation treatment to assess changes in brightness and colour for each tooth sample. The imaging system used a digital camera mounted in a fixed position from the object Figure 21. The measuring distance between the tooth slab and the camera lens was set at (0.32m). The light source was provided by a ring flash that was mounted onto the camera lens. The light was standardised using a flashlight meter (Sekonic L-308S Flashmate Digital Incident, Reflected & Flash Light Meter). A cross polarised filter was used in this research to reduce the specular reflection from the

enamel tooth surface. The cross polarised filter was attached to the ring flash and the camera lens via small magnets. The colour of the photographs was calibrated using a grey Pantone graph at the beginning and the end of each photography session. The white light meter was also standardised and checked at the beginning and end of each photography session. The camera aperture (opening of the lens) was set at f/25 with a shutter speed of 160^{th} of a second; meanwhile, the ISO (sensitivity to light) was set at 100. The speed of the flash was set at 1/2 of a second. Brightness and colours for the area of interest in each photograph were analysed using ImageJ software (Abràmoff et al., 2004). ImageJ software could be operated by the Apple Macintosh, Microsoft Windows and Unix (Schneider et al., 2012). The photographs were opened with ImageJ on an Apple Macintosh computer. Following that, the pre and post photograph were opened together while using the tracing tool to select the area of interest for the pre experiment photograph. A reference point was recorded for each sample to facilitate the tracing area to articulate on the post experiment photograph Figure 22. The pre- and post-experiment photographs were analysed simultaneously. The selected area analysed the pre and post experiment photographs for each sample while maintaining the area traced. Numeric values were obtained from each photograph which analyses the three primary colours (red, green, blue). The mean for the three colours forms the brightness for each selected area Figure 22. The range for each brightness is between 0 and 255. The closer the values are to 0, the darker the image becomes, while the closer they are to 255, the brighter the image becomes.

Figure 21 Digital camera mounted in a fixed position from the object

Figure 22 Photograph showing traced MIH lesion



5.12 Preparation of solutions used in the study

5.12.1 Artificial Saliva

In this study, artificial saliva was used and prepared according to that recommended by Dr P Shellis (Department of Oral and Dental Science, University of Bristol, UK) Table 14.

Table 14 formulation of the artificial saliva

Salt	Concentration g/L
Calcium carbonate	0.07
Magnesium carbonate (hydrated basic)	0.019
Potassium di-hydrogen phosphate	0.554
HEPES buffer (acid form)	4.77
Potassium chloride	2.24

900 ml deionised water, 1.8 ml 1 mol/L HCl, and the prepared formulations were stirred using a shaker until they all dissolved. The pH was adjusted to 6.8 by adding a KOH solution made up to 1L with deionised water.

5.13 Analysis of Data

The data was analysed using IBM SPSS statistics Software for Windows Version 26. Descriptive analysis was used to calculate the mean and to test for normality. Paired Sample t-tests and the Wilcoxon tests were used to determine the mean difference between the baseline and final readings. Also, General Linear Modelling was used to test the relationships between the groups and within the groups.

Chapter 6 **Study 2 Results**

6.1 Descriptive analysis

6.1.1 Description of the Slabs and Treatments

Eleven teeth were collected and sectioned, resulting in 20 enamel slabs. The enamel slabs with hypomineralised lesions were then allocated randomly to the experimental groups (Control F Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Test F toothpaste + CPP-ACP) **Table 15**.

Table 15 Sample and Group Distribution

Sample number	Tooth number	Section number	Experiment groups
1	1	A	Test F toothpaste + CPP-ACP
2	1	B	Control F toothpaste
3	2	C	Control F Free toothpaste
4	3	A	Control F toothpaste
5	3	B	Control F toothpaste
6	4	A	Test F toothpaste + CPP-ACP
7	4	B	Control F toothpaste + Novamin
8	4	C	Control F Free toothpaste
9	5	A	Control F Free toothpaste
10	6	A	Control F toothpaste + Novamin
11	6	B	Test F toothpaste + CPP-ACP
12	6	C	Control F toothpaste + Novamin
13	6	D	Control F toothpaste
14	6	E	Control F Free toothpaste
15	7	A	Test F toothpaste + CPP-ACP

16	7	B	Control F toothpaste + Novamin
17	8	A	Control F Free toothpaste
18	9	A	Control F toothpaste + Novamin
19	10	A	Control F toothpaste
20	11	A	Test F toothpaste + CPP-ACP

6.1.2 The mean change in surface microhardness of hypomineralised enamel

The descriptive microhardness analysis demonstrates the mean values of the baseline and final readings for the control and test groups and the mean difference for each group in **Table 16 Figure 23**. The Control F Free toothpaste group microhardness baseline mean was 219 ± 79.5 SD. The final readings reveal an increase in the mean microhardness compared to the baseline at 274.3 ± 78.5 SD. The biggest increase in microhardness mean was shown in the Control F Free toothpaste group – 2C, at 174.1 to 302.7 **Table 16**. Furthermore, the microhardness mean baseline value for the Control F toothpaste was 193.8 ± 52.8 SD, while the final reading was 205.6 ± 87.6 SD. A decrease in microhardness was observed in one Control F toothpaste group, from 184.4 to 85.1.

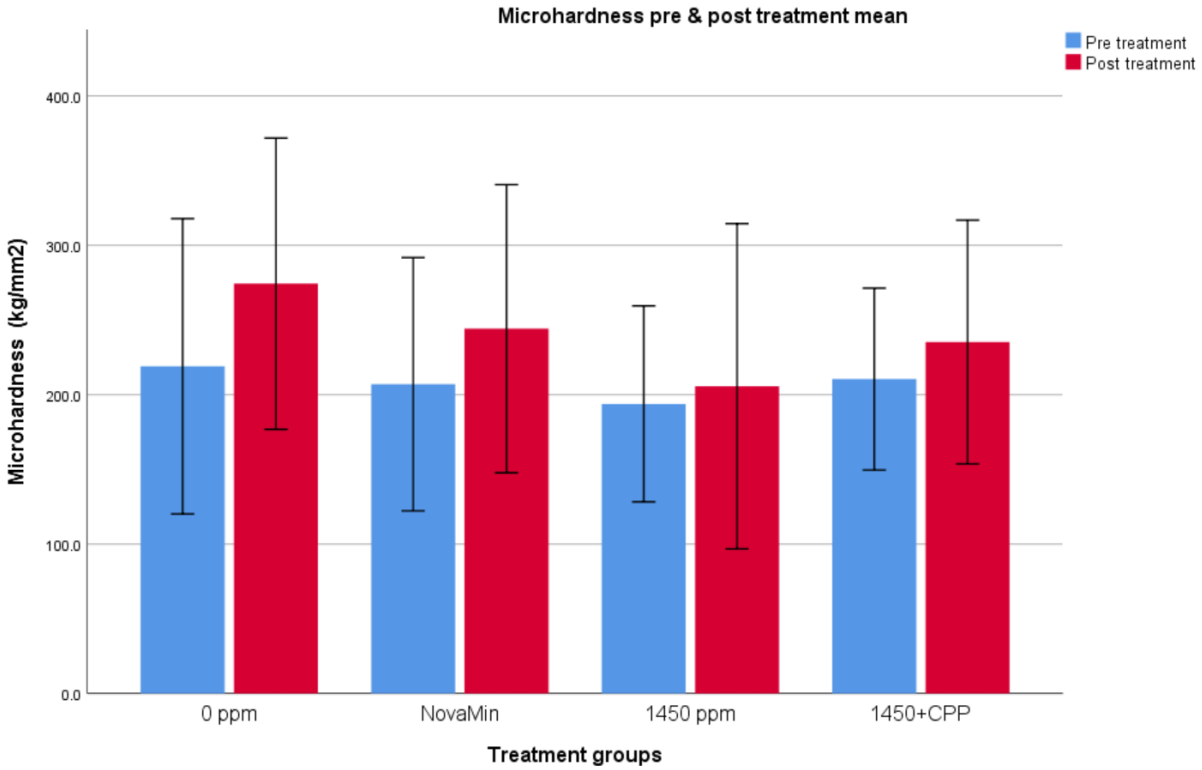
The Control F toothpaste + Novamin baseline mean value was 207 ± 68.2 SD, while the final readings revealed an increase in the mean compared to the baseline at 244.18 ± 77.6 SD. The Test F toothpaste + CPP-ACP group microhardness baseline mean was 210.4 ± 48.9SD and the final readings revealed an increase in the mean compared to the baseline at 235.2± 65.7 SD.

Table 16 Enamel Surface Microhardness Pre and Post Treatment

Microhardness	Baseline (kg/mm^2)	Final (kg/mm^2)	Difference (kg/mm^2)
Control F Free toothpaste			
2C	174.1	302.7	128.6
4C	234.0	257.5	23.5
5A	116.8	149.4	32.6
6E	240.9	303.4	62.5
8A	329.3	358.7	29.4
Mean	219.0	274.3	55.3
SD	79.5	78.5	
Control F toothpaste			
1B	252.0	280.8	28.8
3A	133.4	168.6	35.2
3B	155.1	193.0	37.9
6D	244.3	300.7	56.4
10A	184.4	85.1	-99.3
Mean	193.8	205.6	11.8
SD	52.8	87.6	
Control F toothpaste + Novamin			
4B	182.2	201.6	19.4
6A	120.5	150.9	30.4
6C	297.2	347.2	50.0
7B	184.5	225.5	41.0

9A	250.6	295.7	45.1
Mean	207.0	244.1	37.1
SD	68.2	77.6	
Test F toothpaste + CPP-ACP			
1A	263.0	271.4	8.4
4A	247.2	329.3	82.1
6B	143.6	196.4	52.8
7A	218.6	162.6	-56.0
11A	179.7	216.6	36.9
Mean	210.4	235.2	24.8
SD	48.9	65.7	

Figure 23 Microhardness Pre and Post treatment group means



6.1.3 The mean change in laser fluorescence (Diagnodent) of hypomineralised enamel

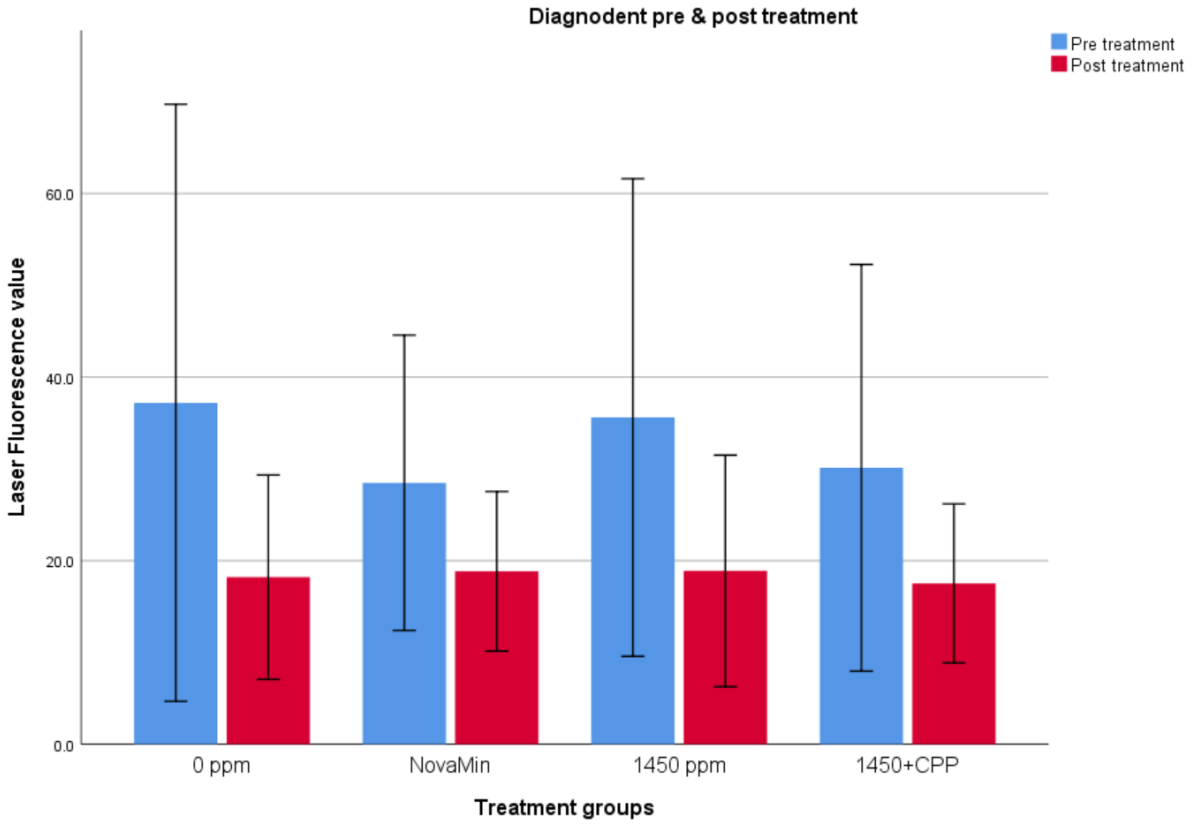
The Diagnodent descriptive analysis compared the mean values of the baseline and final readings for the control and test groups and the difference between the means in **Table 17** **Figure 24**. The Control F Free toothpaste group Diagnodent baseline mean was 37.2 ± 26.1 SD; meanwhile, the final readings revealed a decrease in the mean compared to the baseline at 18.2 ± 8.9 SD. The greatest decrease in Diagnodent readings was shown In the Control F Free toothpaste group - 6E at 83.2 to 33.2 in **Table 17**. The Diagnodent mean baseline value for the Control F toothpaste was 35.6 ± 20.9 SD, while the final mean reading was 18.8 ± 10.1 SD. The only laser fluorescence readings that increased was found in the Control F toothpaste group 10A, from 15 to 15.8. As for the Control F toothpaste + Novamin, the baseline mean was 28.4 ± 12.96 SD, while the final mean readings revealed a decrease in the mean compared to the baseline at 18.8 ± 10.1 SD. The Test F toothpaste + CPP-ACP group baseline mean was 30.1 ± 17.8 SD. and the final readings revealed a decrease in the mean compared to the baseline to 17.5 ± 6.9 SD.

Table 17 Diagnodent (LF) Measurements Pre and Post Treatment

Diagnodent	Baseline (LF)	Final (LF)	Difference (LF)
Control F Free toothpaste			
2C	32.6	12.6	-20.0
4C	26.6	15.2	-11.4
5A	25.0	19.2	-5.8
6E	83.2	33.2	-50
8A	18.6	10.8	-7.8
Mean	37.2	18.2	-19.0
SD	26.1	8.9	
Control F toothpaste			
1B	54.4	17.6	-36.8
3A	18.0	8.8	-9.2
3B	29.8	16.2	-13.6
6D	60.8	36	-24.8
10A	15.0	15.8	0.8
Mean	35.6	18.8	-16.7
SD	20.9	10.1	
Control F toothpaste + Novamin			
4B	33.6	21.6	-12.0
6A	35.8	19.8	-16.0
6C	43.0	28.6	-14.4
7B	18.0	13.0	-5.0

9A	12.0	11.2	-0.8
Mean	28.4	18.8	-9.6
SD	12.96	7.0	
Test F toothpaste + CPP-ACP			
1A	30.8	21.8	-9.0
4A	27.8	16.8	-11.0
6B	59.6	26.8	-32.8
7A	18.6	13.0	-5.6
11A	13.8	9.2	-4.6
Mean	30.1	17.5	-12.6
SD	17.8	6.9	

Figure 24 Diagnodent (LF) pre and Post treatment group means



6.1.4 The mean change in surface roughness of hypomineralised enamel

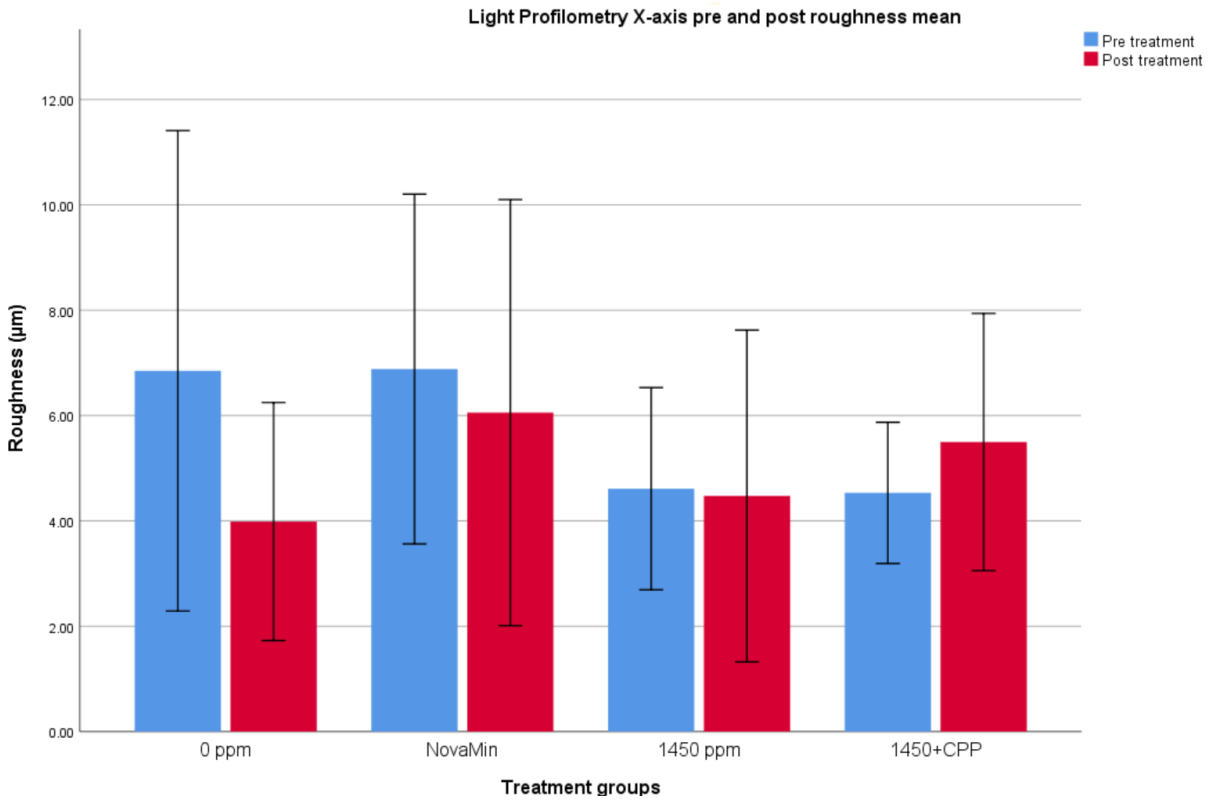
There are two parameters (X-axis and Y-axis) used to represent the roughness (Ra) value in light profilometry. The descriptive analysis for the light profilometry X-axis compares the mean roughness (Ra) values of the baseline and final readings for the control and test groups and the difference between the means in **Table 18 Figure 25**. The Control F Free toothpaste group baseline mean was 6.85 ± 3.6 SD. Meanwhile, the final mean readings revealed a decrease in the mean compared to the baseline to 3.98 ± 1.8 SD. The greatest decrease in the light profilometry X-axis means was shown In the Control F Free toothpaste group 5A - 13.02 to 5.88 shown in **Table 18**. The light profilometry X-axis mean baseline value for the Control F toothpaste was 4.61 ± 1.54 SD while the final mean reading was 4.47± 2.53 SD. The highest increase in the light profilometry X-axis value was found in the Control F toothpaste group 6D, from 5.18 to 8.96. As for the Control F toothpaste + Novamin, the baseline mean was 6.88 ± 2.67 SD, while the final mean value revealed a decrease in the mean compared to the baseline to 6.05 ± 3.25 SD. The Test F toothpaste + CPP-ACP group baseline mean was 4.53 ± 1.07 SD. Meanwhile, the final readings showed an increase in the mean compared to the baseline at 5.49 ± 1.96 SD.

Table 18 Light Profilometry X-axis Values Pre and Post Treatment

Light Profilometry X axis	Baseline (µm)	Final (µm)	Difference (µm)
Control F Free toothpaste			
2C	5.1	2.7	-2.4
4C	3.4	2.2	-1.2
5A	13.02	5.88	-7.14
6E	5.7	3.1	-2.6
8A	6.9	6.0	-0.9
Mean	6.85	3.98	-2.86
SD	3.6	1.8	
Control F toothpaste			
1B	5.64	2.95	-2.69
3A	6.13	3.89	-2.24
3B	3.77	3.03	-0.74
6D	5.18	8.96	3.78
10A	2.34	3.54	1.2
Mean	4.61	4.47	-0.13
SD	1.54	2.53	
Control F toothpaste + Novamin			
4B	5.02	3.79	-1.23
6A	3.2	3.88	0.68
6C	8.56	11.59	3.03
7B	9.61	4.69	-4.92

9A	8.03	6.33	-1.7
Mean	6.88	6.05	-0.82
SD	2.67	3.25	
Test F toothpaste + CPP-ACP			
1A	3.75	5.99	2.24
4A	4.65	4.88	0.23
6B	4.04	3.35	-0.69
7A	3.86	4.68	0.82
11A	6.36	8.59	2.23
Mean	4.53	5.49	0.96
SD	1.07	1.96	

Figure 25 Light Profilometry X-axis Pre and Post Treatment group means



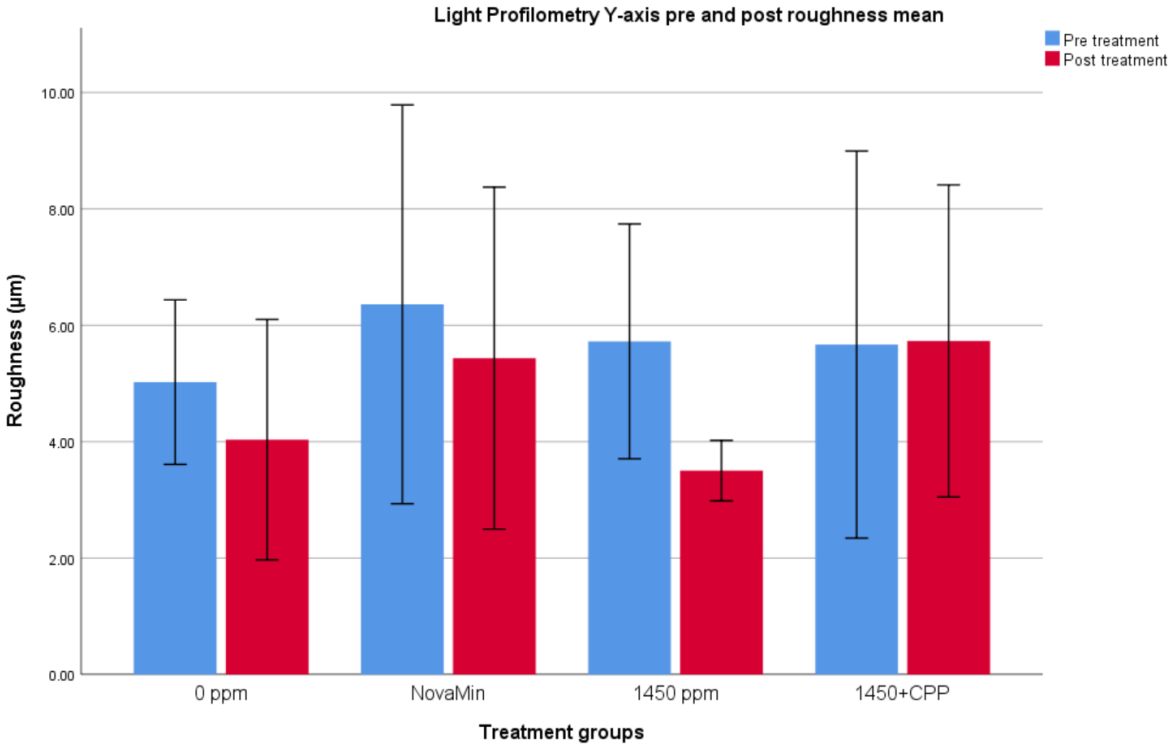
The second parameter that represents the surface roughness value is the light profilometry Y-axis. The descriptive analysis for the Y-axis compares the values of the baseline and final readings for the control and test groups and the difference between the means in Table 19 Figure 26. The Control F Free toothpaste group baseline mean was 5.02 ± 1.13 SD and the final mean readings revealed a decrease in the mean compared to the baseline to 4.03 ± 1.66 SD. The light profilometry Y-axis baseline mean for the Control F toothpaste was 5.72 ± 1.62 SD, whereas the final mean reading decreased to 3.50 ± 0.41 SD. The greatest decrease in Y-axis roughness value was observed in the Control F Free toothpaste group from 6.83 to 2.24 Table 19. As for the Control F toothpaste + Novamin, the baseline mean was 6.36 ± 2.75 SD, while the final mean reading revealed a decrease in the mean to 5.43 ± 2.36 SD. The Test F toothpaste + CPP-ACP group baseline mean was 5.66 ± 2.67 SD and the final readings show an increase in the mean compared to the baseline at 5.73 ± 2.15 SD.

Table 19 Light Profilometry Y-axis Values Pre and Post Treatment

Light Profilometry Y axis	Baseline (µm)	Final (µm)	Difference (µm)
Control F Free toothpaste			
2C	3.84	3.19	-0.65
4C	6.83	2.24	-4.59
5A	5.04	5.77	0.73
6E	4.31	3.12	-1.19
8A	5.1	5.85	0.75
Mean	5.02	4.03	-0.99
SD	1.13	1.66	
Control F toothpaste			
1B	7.45	3.02	-4.43
3A	6.72	3.92	-2.8
3B	4.29	3.11	-1.18
6D	6.44	3.86	-2.58
10A	3.72	3.6	-0.12
mean	5.72	3.50	-2.22
SD	1.62	0.41	
Control F toothpaste + Novamin			
4B	4.55	3.69	-0.86
6A	3.38	3.91	0.53
6C	10.61	9.44	-1.17
7B	6.85	4.47	-2.38

9A	6.41	5.67	-0.74
mean	6.36	5.43	-0.92
SD	2.75	2.36	
Test F toothpaste + CPP-ACP			
1A	5.32	6.02	0.7
4A	5.41	5.55	0.14
6B	3.45	3.3	-0.15
7A	3.95	4.66	0.71
11A	10.21	9.12	-1.09
mean	5.66	5.73	0.06
SD	2.67	2.15	

Figure 26 Light Profilometry Y-axis Pre and Post Treatment group means



6.1.5 The mean change in brightness of hypomineralised enamel

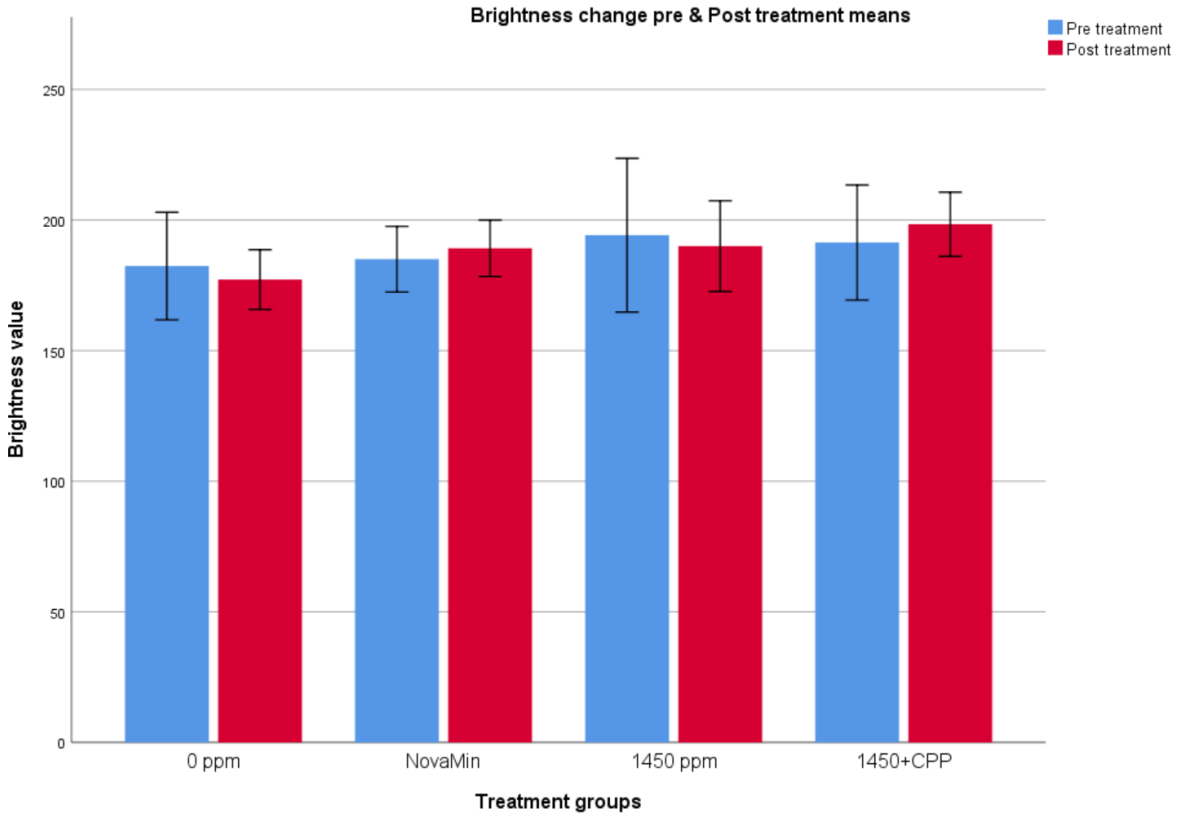
The descriptive analysis for digital photography compared the primary colours (red, blue, green) mean values of the baseline and final readings for the control and test groups and the difference between the means, shown in **Table 20 Figure 27**. The Control F Free toothpaste group digital photograph brightness baseline mean was 182.4 ± 16.5 SD, while, the final mean reading showed a decrease in brightness compared to the baseline to 177.2 ± 9.2 SD. The greatest decrease in brightness reading was seen In the Control F Free toothpaste group 1B, 222 to 185 in **Table 20**. Meanwhile, the brightness mean baseline value for the Control F toothpaste was 194.2 ± 23.7 SD and the final mean reading was 190 ± 13.9 SD. The greatest brightness decrease was observed in the Control F toothpaste group 1B, from 222 to 185. As for the Control F toothpaste + Novamin, the baseline mean value was 185 ± 10.1 SD, while the final mean reading showed an increase in the mean brightness compared to the baseline at 189.2 ± 8.7 SD. The Test F toothpaste + CPP-ACP group baseline mean was 191 ± 17.7 SD and the final readings also showed an increase in the mean compared to the baseline at 198 ± 9.8 SD. The greatest increase in brightness mean was shown in the Test F toothpaste + CPP-ACP group 6B from 172 to 195 - **Table 20**.

Table 20 Digital Photography Brightness Values Pre and Post Treatment

Photography	Baseline	Final	Difference
Control F Free toothpaste			
2C	179	178	-1
4C	179	167	-12
5A	186	192	6
6E	161	174	13
8A	207	175	-32
Mean	182.4	177.2	-5.2
SD	16.5	9.2	
Control F toothpaste			
1B	222	185	-37
3A	210	206	-4
3B	199	200	1
6D	166	170	4
10A	174	189	15
Mean	194.2	190	-4.2
SD	23.7	13.9	
Control F toothpaste + Novamin			
4B	191	202	11
6A	185	189	4
6C	168	179	11
7B	187	184	-3

9A	194	192	-2
Mean	185	189.2	4.2
SD	10.1	8.7	
Test F toothpaste + CPP-ACP			
1A	208	184	-24
4A	186	201	15
6B	172	195	23
7A	179	201	22
11A	212	211	-1
Mean	191	198	7
SD	17.7	9.8	

Figure 27 Digital Photography Brightness Values Pre and Post Treatment group means



6.2 Test for Normality

In order to check the normality of the data, the Shapiro-Wilk test was used for each testing method (Microhardness, Diagnodent, Light Profilometry and Digital Photography) as shown in **Table 21**. It was revealed that the microhardness and the digital photography data were normally distributed; therefore, the Paired Sample t-test was used. On the other hand, the Diagnodent and light profilometry data were not normally distributed. As a result, the Wilcoxon Signed Ranks Test was used.

Table 21 Test of Normality

Data from testing methods		Shapiro-wilk
Microhardness		0.530
Diagnodent		0.014*
Light profilometry	X-axis	0.035*
	Y-axis	0.018*
Digital photography		0.632

* Significant P<0.05

6.3 Difference of Within-Groups Statistical Analysis

The microhardness and the digital photography data were normally distributed. Thus the paired sample t-test was administered to assess the difference within the same group at the baseline and after treatment. Meanwhile, the diagnodent and light profilometry data were non-normally distributed. Therefore, a nonparametric test Wilcoxon test was applied to assess the difference within the same group at the baseline and after treatment. The test results showed statistical significance $p< 0.05$ in the microhardness, diagnodent, and the Y axis of the light profilometry. This means that there was a statistically significant difference between the baseline and the final reading in all treatment groups. This means shows that there was a statistically significant difference after the treatment compared with the baseline **Table 22**. However the test results for the light profilometry X axis and the photography didn't show statistical significant difference. This reveals that there wasn't a statistical significance between the baseline and final readings. **Table 22** also represents the mean difference for each testing method at the baseline and after treatment.

Table 22 Test of within the same group

Test Methods		Baseline mean	Final mean	Dif	SD	95% Confidence Interval		Sig (2 tailed)
						Lower	Upper	
Microhardness (kg/mm^2)		207.5	239.8	32.2	46	10	53	.005*
Diagnodent (LF)		32.8	18.3	-14.5	12.8	-20.4	-8.4	.000*
Light profilometry	X axis (µm)	5.7	5.0	-0.7	2.6	-1.9	0.5	.240
	Y axis (µm)	5.6	4.6	-1	1.6	-1.7	-0.2	.011*
Photography		188.2	188.7	0.45	16.3	-7.20	8.10	.903

*Significant P<0.05

6.4 Difference of between groups statistical analysis

The general linear model was used for the normally distributed data, while the non-normally distributed data was a nonparametric test Kruskal-Wallis test. The previous tests were applied to assess the difference between the mineralising products (Control F Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Test F toothpaste + CPP-ACP) for each test method. The results reveal no statistical significance $p > 0.05$ between any groups regardless of the test method **Table 23**.

Table 23 Test of between experiment groups

		Control F Free toothpaste		Control F toothpaste		Control F toothpaste + Novamin		Test F toothpaste + CPP-ACP		Sig
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Microhardness		219	274.3	193.8	205.6	207	244.1	210.4	235.2	.748
Diagnodent		37.2	18.2	35.6	18.8	28.4	18.8	30.1	17.5	.946
Light profilometry	X axis	6.85	3.98	4.61	4.47	6.88	6.05	4.53	5.49	.545
	Y axis	5.02	4.03	5.72	3.50	6.36	5.43	5.66	5.73	.541
Digital photography		182.4	177.2	194.2	190	185	189.2	191	198	.244

Chapter 7 **Study 2 Discussion**

7.1.1 Research Aims

The research aims of the *invitro* study were centred around comparing possible MIH enamel improvements with different treatments:

- To compare the effect of Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the Control F toothpaste + CPP-ACP on changes in MIH lesions.
- To assess the surface hardness of MIH lesions using Surface Microhardness Testing.
- To assess the mineralisation of MIH lesions using DIAGNOdent.
- To examine the surface roughness of MIH lesions using surface Profilometry.
- To assess the change in colour of MIH lesions using digital photographs.

7.1.2 Research design

7.1.2.1 *In vitro* model

Various *in vitro* designs (Cochrane et al., 2008, Bahrololoomi et al., 2013) have been introduced in the past years to investigate remineralisation of enamel of teeth. In some of these studies, it would involve the application of a highly concentrated mineralising solution for a week or two in the experiment slurry. In addition, none of the studies has extended more than two weeks. Published studies that investigated the further mineralisation of MIH enamel are few (Crombie et al., 2013a, Batra, 2011). Neither of these studies involved the role of saliva or the application of a toothpaste twice daily. In the present study the model includes incubating the enamel samples in artificial saliva and using the mineralising product twice a day to mimic tooth brushing twice a day as is recommended in the SDCEP Prevention and Management of Dental Caries in Children (SDCEP, 2018) . The main advantage of the *in vitro* model was the ability to carry out the experiment under controlled conditions to allow the current study to

investigate the mineralising effect of fluoride toothpastes with and without the addition of Tooth Mousse when applied twice daily for 28 days on MIH lesions.

7.1.2.2 MIH teeth

Eleven MIH teeth were collected from the Tissue Bank after obtaining the ethical approval and were immersed in deionised water for up to one month. This was because the teeth collected were a mixture of dry teeth and freshly extracted teeth. It was important to standardise the storage media for the teeth and allow the dry teeth to rehydrate. Therefore, the teeth were stored in deionised water for up to a month before the start of the research. The reason for choosing the deionised water as a storage medium was that a previous study (Aydın et al., 2015) showed that the deionised water resulted the least percentage change in microhardness values after being immersed in deionised water for up to 12 months. Aydın et al. (2015) study aimed to determine the changes in healthy dentine and enamel using Vickers microhardness when stored in different storage media (deionised water, 0.2% glutaraldehyde, Hanks' Balanced Salt Solution (HBSS), 0.1% sodium hypochlorite (NaOCl) and 0.1% thymol). The microhardness readings were recorded after two and 12 months. The results showed that the teeth stored for up to two months in the different solutions had maintained the same microhardness values as at the baseline. In their study, the pH was measured before and after the experiment to assess if the storing solution became acidic which would result in mineral loss from the tooth samples. The deionised water pH (7.2) didn't change from immersing the tooth samples for the experiment time.

7.1.2.3 Experimental materials

7.1.2.3.1 Control F-Free toothpaste

The toothpaste used was no Fluoride toothpaste Kingfisher 100 ml Mint Fluoride- Free Toothpaste (Kingfisher). This was used as a negative control to observe the mineralisation effect of the artificial saliva on MIH teeth.

7.1.2.3.2 Control F toothpaste

The fluoride toothpaste used in this study was Colgate Cool Stripe Toothpaste 75ml (Colgate Palmolive). This was used as the positive control. The concentration of 1450 ppm F is the recommended most widely used fluoride toothpaste for preventing caries in older children and adults (Walsh et al., 2019). The UK Prevention Toolkit for Delivering Better Oral Health guidance advises brushing teeth twice a day: brushing the last thing at night before going to bed and at least on one other occasion (Duckworth and Moore, 2001) (Marinho et al., 2003).

7.1.2.3.3 Control F toothpaste + Novamin

The toothpaste used was Sensodyne Repair & Protect NOVAMIN (GSK, Sensodyne). This toothpaste includes Calcium Sodium Phosphosilicate known as Novamin. (Wang et al., 2011) found that the Calcium Sodium Phosphosilicate is able to adhere to exposed dentine surfaces and that the layer formed was more resistant to mechanical and acid challenges. In addition, the properties and appearance of the layer formed were very much like hydroxyapatite in nature. Furthermore, An invitro study by KARGÜL (2019) showed that the toothpaste containing novamin showed greater hardness results compared to Pronamel and other toothpastes. The current study wished to examine if this effect occurred in MIH enamel.

7.1.2.3.4 Tooth Mousse

GC Tooth Mousse with CPP-ACP was used in this study. Many studies (Cochrane and Reynolds, 2012, Memarpour et al., 2015) have shown the remineralisation potential of CPP-ACP on human teeth with demineralisation. The current study investigated the effect on hypomineralised enamel.

7.1.2.3.5 Control F toothpaste + CPP-ACP

Fluoride toothpaste and Tooth Mousse used together has been shown to have the potential of remineralisation of enamel subsurface lesions (Cochrane et al., 2008, Memarpour et al., 2015). An *in situ* study (Reynolds et al., 2008) aimed to determine the remineralisation of white spot lesions with Tooth Mousse with CPP-ACP and different fluoride concentrations (1100 ppm, 2800 ppm). They found that the combination of CPP-ACP and fluoride (1100 ppm F) could result in substantial remineralisation of enamel lesions when compared with CPP-ACP, 1100 ppm F or even 2800 ppm F alone. In addition, they found that when fluoride is used alone, fluoride does deposit in the enamel and remineralises the enamel surface. Meanwhile, CPP-ACP in combination with fluoride was found to remineralise throughout the structure of white spot lesions rather than settle on the surface or form a layer on the outer surface of the enamel.

In the current study, the control fluoride toothpaste slurry was first applied on the MIH enamel slabs, then the Tooth Mousse slurry (CPP-ACP) was applied. There was no water rinsing between the products. The application of Tooth Mousse was in accordance with the manufacturer's instructions.

7.1.3 Microhardness Testing

Vickers is one of the most common used microhardness indenters. Vickers indenter is a reliable indenter in tooth hardness studies. Because a square shape must always be conserved and that the indentation is easily detectable, especially if the tooth surface was not flat. Vickers

microhardness was used in the current study to assess the enamel hardness at the baseline and following the experimental treatment.

In the present study, a load of 100 g was applied on the MIH enamel samples by the Vickers microhardness diamond. This load has been shown to facilitate optical perceptibility (Davidson et al., 1974). Each enamel slab was measured twice, once at the baseline, and the final measurement was after the experimental period. The measurements were recorded by a computer-aided indenter machine - Duramin (Struers A/S, DK 26-10, Ballerup, Denmark). The indentations were created with a Vickers diamond under a 100 g load for 10 seconds. Five indentations were applied to determine the mean microhardness value for each slab. The indentations were spaced at least 100 µm apart. The length of each indentation was measured in microns by image analysis.

7.1.3.1 Results of Changes in Microhardness

The mineralisation effect of each test material on MIH teeth was assessed in this study. The groups were assessed using the Vickers microhardness test: the control groups: Control F-Free toothpaste, Control F toothpaste, Control F toothpaste + Novamin and the test group: F toothpaste + CPP-ACP. Intact enamel slabs affected with MIH were assessed at baseline and 28 days after the daily mineralisation cycle (application of treatment twice daily, five minutes for each dipping).

The results revealed that all the groups had increased enamel hardness. The descriptive analysis shows that the greatest increase in enamel microhardness was achieved using the Control F-Free toothpaste followed by the Control F toothpaste + Novamin; after that, F toothpaste + CPP-ACP, while the least mineralisation was seen in the Control F toothpaste. The statistical test reveals a significant difference in enamel hardness in all the groups between the baseline and final microhardness values. However, there were no statistically significant

differences detected between the control groups or the test group. This may have been partly due to the limited sample size. However, considering the ingredients of each of the treatments, all contained materials that have the potential to increase mineralisation – the calcium carbonate in the fluoride free paste (Hannig and Hannig, 2012), the fluoride in the fluoride toothpastes (Walsh et al., 2019), the Novamin (Calcium Sodium Phosphosilicate) (Wang et al., 2011) and the CPP-ACP in the tooth mousse (Cochrane and Reynolds, 2012). This suggestes that recommending any toothpaste with mineralisaing ingredients may be useful for MIH enamel.

There was an evident increase in enamel microhardness in all groups compared to the values at baseline. The highest enamel hardness was seen in the negative Control (F Free toothpaste). This could be because of the positive effect of artificial saliva. Also, there wasn't a true negative control group to observe the effect of artificial saliva alone on MIH enamel. The finding of improvement in the F-Free toothpaste group was not expected due to the lack of fluoride in this negative control group. However, one ingredient in the Control F Free toothpaste which may have resulted in an increase in enamel hardness is calcium carbonate. A study by (Dizaj et al., 2015) found that the calcium carbonate nanostructure could act as a mineral source for calcium and phosphate to preserve the ions in a supersaturation state in enamel minerals. Furthermore, the deposition of ions on a demineralised enamel surface could support the remineralisation process of outer enamel caries lesions (Hannig and Hannig, 2012). It was also reported by (Nakashima et al., 2009) that the use of toothpaste containing 1% amorphous calcium carbonate nanoparticles on artificial caries lesions showed remineralisation and significant mineral gain. This is an aspect that would be worth further investigation on hypomineralised enamel.

Another interesting finding in the present study was in the test group using Tooth Mousse which did not follow the expected response based on earlier studies. It would have been reasonable

to have predicted that the test group F toothpaste + CPP-ACP would have had a higher mineralisation potential due to the CPP-ACP and fluoride potentially increasing mineralisation of the surface and in the depth of the hypomineralised lesion as had been shown previously with demineralised lesions (Reynolds et al., 2008). A possible explanation for this result could be the presence of higher levels of proteins in the MIH lesions, which could affect the mineralisation of the body of the lesion and possibly interfere with mineral deposition of mineral in the enamel surface. Another reason could be the application sequence of the dental products. In the current study, CPP-ACP was applied immediately after the use of fluoridated toothpaste. This application method was carried out based on the manufacturer's instructions for use in the mouth. On the contrary, a study by Al-Batayneh et al. (2017) found that the CPP-ACP exhibited better enamel remineralisation when it was applied before the fluoridated toothpaste. In the present study it may be that when fluoride is used on the surface of enamel hypomineralised lesions, it tends to cause hypermineralisation, therefore, causing blockage of the enamel pores by surface aggregation of calcium fluoride and other compounds resulting in reduced enamel surface permeability and inhibiting further penetration of phosphate and calcium ions and mineralisation in the depth of the enamel lesion as suggested by (O'reilly and Featherstone, 1987) in demineralisation studies. Further investigation is required to understand the effect of calcium carbonate on MIH teeth.

7.1.4 DIAGNOdent Laser Fluorescence

The laser fluorescence (LF) DIAGNOdent pen was used in the current study. In the literature (Farah et al., 2008), it has been shown that an increase in DIAGNOdent readings correlates well with the level of mineral density in hypomineralised enamel. Therefore, this method was used in the current study to assess changes in the mineral density of enamel before and after the treatment.

7.1.4.1 Results of Changes in DIAGNOdent Rreadings

The study results revealed that all the experimental groups showed decreased Diagnodent readings after the study period. This indicates increased mineral density in the MIH enamel lesions. The greatest drop in Diagnodent reading was in the Control F free toothpaste group which was similar to the microhardness. This was followed by the Control F toothpaste, then the F toothpaste + CPP-ACP, while the lowest drop was found with the Control F toothpaste + Novamin. The statistical test reveals a significant difference in terms of the DIAGNOdent values of enamel when compared between the baseline and the final values in all groups. However, no statistically significant differences were detected between the control groups and test group when compared with each other. As with the other analyses, this may also be due to the limited sample size and due to the apparent improvement in the enamel in all the groups. The Diagnodent values suggest that there was an increase in enamel mineral density in all groups compared to the values at baseline. The greatest drop in diagnodent mean value was shown in the Control F Free toothpaste group Table 17. The findings of improvement in all groups were not expected due to the lack of fluoride in the negative control group. The possible reason for the decrease in Diagnodent values in the Control F Free toothpaste group was the same as that mentioned previously in the microhardness part 7.1.3.1.

In summary, it was evident from the descriptive analysis that there was an improvement in the degree of mineralisation even in the Control F Free toothpaste group. The statistical test reveals a significant difference in the laser fluoresence values of enamel lesions when compared between the baseline and the final DIAGNOdent values. However, there were no statistically significant differences revealed when the groups were compared to each other suggesting that all the groups showed improvement in the enamel.

7.1.5 Light Profilometry

In the current study, light profilometry was used for the assessment of surface roughness. The most common roughness parameter used in dentistry is the Ra value (Sunnegårdh-Grönberg and van Dijken, 2003); the lower the Ra values, the smoother the surface, while the higher the Ra values, the rougher the surface.

7.1.5.1 Results of Light Profilometry

The current study results indicate that all the control groups had reduced surface roughness of the MIH enamel surfaces. Interestingly the Test group (F toothpaste + CPP-ACP) did not show improvement in the surface roughness. Indeed, an increase in surface roughness was observed in the test group. The best surface roughness improvement measured on the X-axis with the light profilometer was achieved using the Control F Free toothpaste followed by the Control F toothpaste + Novamin followed by the Control F toothpaste. The best surface roughness improvement measured on the Y-axis with the light profilometer was achieved using the Control F toothpaste followed by the Control F Free toothpaste then the Control F toothpaste + Novamin. Meanwhile, an increase in surface roughness was observed in the test group F toothpaste + CPP-ACP.

The present study findings regarding the CPP-ACP group disagrees with the previous studies (CARVALHO et al., 2014, Baroni et al., 2014) that used Tooth Mousse CPP-ACP and assessed the surface roughness with contact and non-contact profilometry. Those studies reported improvement in the surface roughness. The studies used human teeth and found improvement of the surface with the enamel surface becoming smoother when compared to the enamel in the control groups. The possible reason could be that the fluoride minerals could have blocked the MIH porous enamel while leaving the CPP-ACP to deposit on the lesion's surface, resulting in a rougher enamel surface Al-Batayneh et al. (2017). It would be useful in future studies to

analyse the enamel surface using scanning electron microscopy to gather more detail and assess the mineral deposits on the enamel surface.

In summary, the statistical test reveals a significant difference in enamel surface roughness when compared between the baseline and the final analysis with the light profilometer. However, although the treatment group did have increase in roughness there were no statistically significant differences detected between the groups.

7.1.6 Digital Photography of the MIH samples

Digital cameras are used by researchers both in the clinic and in the laboratory to assess tooth colour change through analysing digital images. The digital images can be analysed and edited using editing-image software such as Adobe Photoshop or ImageJ (ImageJ, Fiji). In the current study, ImageJ was used to analyse the digital images. Additionally, a cross polarised filter was used to enhance detection of the surface detail and facilitate the tracing for the outline of the MIH lesions. The main reason for using ImageJ was because the software could analyse brightness and colours for the area of interest for each photograph.

7.1.6.1 Results of Digital Photography Comnparison of the Lesions

There was an increase in image brightness detected in the test group (F toothpaste + CPP-ACP) and to a lesser extent in the Control F toothpaste + Novamin group. In contrast, the Control F Free toothpaste and Control F toothpaste groups showed a decrease brightness of the lesions. The greatest increase in image brightness values in all samples was observed in the test F toothpaste + CPP-ACP group. On the other hand, the greatest decrease in image brightness was observed in the Control F toothpaste group. While two groups did show increases in brightness values, statistical analysis revealed no significant differences in image brightness between the baseline and the final mean photograph brightness values in any group. There were no statistically significant differences detected between the control groups and the test group. An increase in the brightness values would suggest that the enamel had become whiter or brighter. Normal enamel has the property of being transparent or colourless (Lee, 2016), but the colour of MIH enamel defects ranges from white opaque to creamy yellow

to brown (Chawla et al., 2008, Oliver et al., 2014). In this study, the increase in lesion brightness values suggests that the lesions improved with colours changing from creamy-yellow to more white-opaque. A shortcoming for the photography analysis method used is that the image analysis used mainly the primary colours (red, green, blue) and the brightness was created from the means of the primary colours. A study by (Fuchs et al., 2020) has shown a new method to examine the translucency and the opalescence of enamel which could provide more details for enamel colour changes. Another suggestion would be to use a reference material with a known colour/shade guide, such as a dental shade guide which could be used as a reference measurement for each photograph.

7.1.7 Affect of artificial saliva

Another interpretation for the effect of artificial saliva could be that artificial saliva had a positive effect on all the test samples. Because there wasn't a true negative control group, the effect of artificial saliva alone was not recorded in this study. Shellis et al. (2011), has explained that artificial saliva could be used in remineralisation studies. Additionally, the salivary proteins in the mouth usually bind to calcium, meanwhile the artificial saliva formula doesn't contain proteins, resulting in a high level of supersaturation and precipitation of large amounts of phosphate and calcium on the surface of teeth. Therefore, remineralisation and mineralisation may be found to be greater in *in vitro* studies compared to *in vivo* studies.

7.1.8 Implication of clinical care

The mineralisation products (Calcium Carbonate, Tooth Mousse and Novamin) have been shown in this study and in the literature that they may increase the hardness and mineral density of hypomineralised enamel. Therefore, the products in this study may be used

clinically as a management for sensitivity, to attempt to increase mineral density of enamel, also to increase the resistance to acid challenges, thus, preventing further post eruptive breakdown.

7.1.9 Limitation of the study

- The study would benefit from a true negative Control group without any treatment just tested in artificial saliva
- Intra-examiner (within) reliability was not recorded for this study. However, several readings were taken for each sample, especially in one of the testing methods (Diagnodent) were the readings were repeated until a consistent number was achieved.

7.1.10 Conclusion

The use of F-Free toothpaste, F toothpaste, F toothpaste + Novamin or F toothpaste + CPP-ACP all resulted in improvement in the MIH enamel lesions, this may be due to the positive effect of artificial saliva on all experiment groups. The addition of CCP-ACP or Novamin to the toothpaste did not have any further statistically significant benefit. This may suggest that there is benefit in regular use of toothpastes containing calcium and phosphate as well as fluoride on the potential ongoing mineralisation of hypomineralised MIH lesions. Further research is needed to determine how other components in toothpaste might add benefit to this mineralisation process.

7.1.11 Future Studies

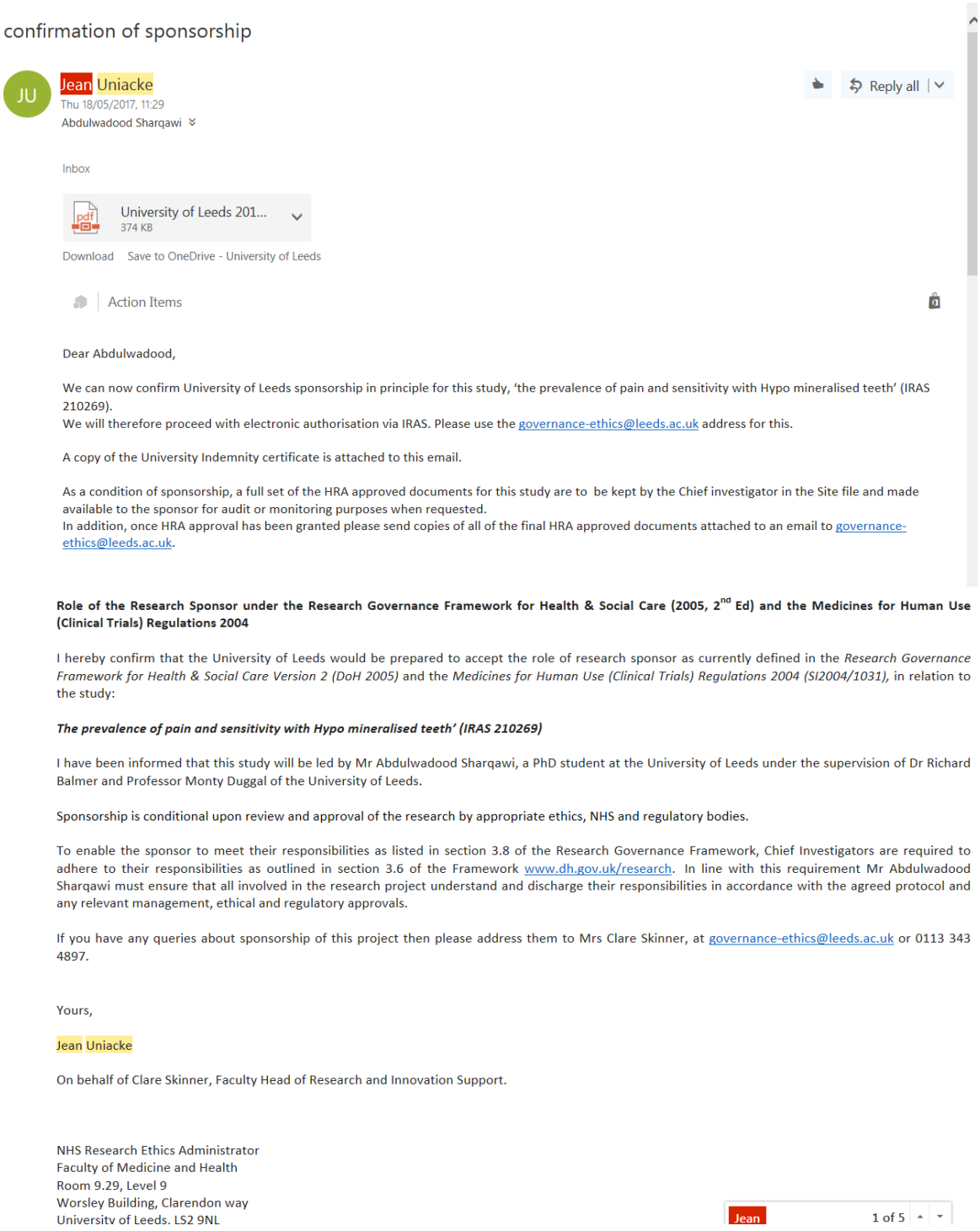
- In this study Control F Free toothpaste was used as a negative control; it was found that it included a mineralising product.
- In future *in vitro* studies, a true negative control group would be advised to investigate the effect of artificial saliva alone.
- Further investigation is required to understand the effect of calcium carbonate on MIH lesions.

- In this study, there was no statistical difference between the control and treatment groups; this may be due to the limited samples. In future *in vitro* studies, the sample size could be increased, which may show a statistical difference.
- An *In situ* model with a cross-over design: A removable appliance with sterilised MIH slabs could be worn by participants. The use of similar products in this study but potentially added a high fluoride concentration toothpaste. SEM and Micro-CT could be added as additional test methods in the study.
- One of the main advantages of the diagnodent device is its portability and light weight, which may be helpful in future *in vivo* studies.

Appendix

Appendix 1 Sponsor Confirmation (University Of Leeds)

Screenshot of the sponsor's confirmation email



confirmation of sponsorship

JU

Jean Uniacke
Thu 18/05/2017, 11:29
Abdulwadood Sharqawi

Reply all

Inbox

University of Leeds 201...
374 KB

Download Save to OneDrive - University of Leeds

Action Items

Dear Abdulwadood,

We can now confirm University of Leeds sponsorship in principle for this study, 'the prevalence of pain and sensitivity with Hypo mineralised teeth' (IRAS 210269).
We will therefore proceed with electronic authorisation via IRAS. Please use the governance-ethics@leeds.ac.uk address for this.

A copy of the University Indemnity certificate is attached to this email.

As a condition of sponsorship, a full set of the HRA approved documents for this study are to be kept by the Chief investigator in the Site file and made available to the sponsor for audit or monitoring purposes when requested.
In addition, once HRA approval has been granted please send copies of all of the final HRA approved documents attached to an email to governance-ethics@leeds.ac.uk.

Role of the Research Sponsor under the Research Governance Framework for Health & Social Care (2005, 2nd Ed) and the Medicines for Human Use (Clinical Trials) Regulations 2004

I hereby confirm that the University of Leeds would be prepared to accept the role of research sponsor as currently defined in the *Research Governance Framework for Health & Social Care Version 2 (DoH 2005)* and the *Medicines for Human Use (Clinical Trials) Regulations 2004 (SI2004/1031),* in relation to the study:

The prevalence of pain and sensitivity with Hypo mineralised teeth' (IRAS 210269)

I have been informed that this study will be led by Mr Abdulwadood Sharqawi, a PhD student at the University of Leeds under the supervision of Dr Richard Balmer and Professor Monty Duggal of the University of Leeds.

Sponsorship is conditional upon review and approval of the research by appropriate ethics, NHS and regulatory bodies.

To enable the sponsor to meet their responsibilities as listed in section 3.8 of the Research Governance Framework, Chief Investigators are required to adhere to their responsibilities as outlined in section 3.6 of the Framework www.dh.gov.uk/research. In line with this requirement Mr Abdulwadood Sharqawi must ensure that all involved in the research project understand and discharge their responsibilities in accordance with the agreed protocol and any relevant management, ethical and regulatory approvals.

If you have any queries about sponsorship of this project then please address them to Mrs Clare Skinner, at governance-ethics@leeds.ac.uk or 0113 343 4897.

Yours,

Jean Uniacke

On behalf of Clare Skinner, Faculty Head of Research and Innovation Support.

NHS Research Ethics Administrator
Faculty of Medicine and Health
Room 9.29, Level 9
Worsley Building, Clarendon way
University of Leeds. LS2 9NL

Jean 1 of 5

Appendix 2 Health Research Authority (HRA) Approval

NHS
Health Research Authority

Mr Abdulwadood Sharqawi
Leeds Dental Institute
Paediatric Dental Department
University of Leeds
Clarendon Way
Leeds. UK
LS2 9LU

Email: hra.approval@nhs.net

31 July 2017

Dear Mr Sharqawi

Letter of HRA Approval

Study title:	**A Cross sectional study of the prevalence of pain and sensitivity in children who present with Hypomineralised Molars and/or incisors**
IRAS project ID:	**210269**
REC reference:	**17/ES/0081**
Sponsor	**University of Leeds**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also

provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators",* issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained

in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA

website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **210269**. Please quote this on all correspondence.

Yours sincerely

Michael Pate

Assessor

Email: hra.approval@nhs.net

Copy to: *Dr Clare Skinner – University of Leeds – Sponsor contact*
Ms Anne Gowing - Leeds Teaching Hospitals NHS Trust – Lead NHS R&D contact.

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_06062017]		31 May 2017
Letter from sponsor [University of Leeds 2016 17 Liability Confirmation Letter]		08 September 2016
Other [REC amendments]	4	29 June 2017
Other [Statement of Activities]	1	27 June 2017
Other [Schedule of Events]	1	27 June 2017
Other [HRA amendments]	4	29 June 2017
Participant consent form [Parent Consent (highlighted changes)]	4	29 June 2017
Participant consent form [Child Assent (highlighted changes)]	4	29 June 2017
Participant information sheet (PIS) [Parent Information Sheet (highlighted changes)]	4	29 June 2017
Participant information sheet (PIS) [Child Information Sheet (highlighted changes)]	4	29 June 2017
Research protocol or project proposal [(highlighted changes)]	4	29 June 2017
Summary CV for Chief Investigator (CI) [CV]		
Summary CV for student [CV]		
Summary CV for supervisor (student research) [Prof Toumba CV]		
Summary CV for supervisor (student research) [DR Balmer CV]		
Validated questionnaire [Questionnaire]	3	18 May 2017

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations*, *capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Name: Dr Clare Skinner

Tel: 0113 343 7587

Email: governance-ethics@leeds.ac.uk

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments

2.1	Participant information/consent documents and consent process	Yes	Records will be accessed by the direct care team to determine a diagnosis which would make the child eligible for participation. The dentist/consultant will make the initial approach to the child and their parent/guardian. Parental consent and child assent will be taken. The dentist (under supervision of the consultant) at each participating centre will be taking the consent after the parent or legal guardian is handed the Information sheet explaining the purpose and rational of the study. None of the researchers outside the care team will have access to medical records. Access to patient data (only NHS photography system) by the direct

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			care team is to reveal if the participant had previous photographs taken. This will only be undertaken after obtaining informed consent. The PIS and ICF were updated following REC provisional opinion to bring them in line with HRA assessment standards.
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	A Statement of Activities will form the agreement between the sponsor and participating sites.

4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	No funding is being offered to participating sites. There is no external funding for this study.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	None of the researchers outside the care team will have access to medical records. After the research is conducted the principle investigator will access the clinical photography software on the NHS computer to transfer the clinical photos by NHS email. The dentist in each participating centre will record the PAS hospital number for each participant on the anonymisation/linkage form. The participant at this point will have a unique code in front of the PAS hospital number on the linkage form. The unique code will then be used on all

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			documents for each participant. This form will be stored safely in a locked cabinet in a secure office in each of the participating centre included in the study. As soon as the code link is transferred to the University of Leeds it will be destroyed (shredded). After the clinical photos are transferred to the University of Leeds they will be deleted from the NHS computer. There will only be the PAS hospital number on the clinical photo The code link from each centre will be transferred to the sponsor by NHS mail. The REC suggested that the password for the file be given to the recipient over the phone, to ensure confidentiality.
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.

Multi-site study where all organisations are conducting the same activities. Therefore, one site type.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.

Participating NHS organisations in England **will be expected to formally confirm their capacity and capability to host this research**.

- Following issue of this letter, participating NHS organisations in England may now confirm to the sponsor their capacity and capability to host this research, when ready to do so. How capacity and capacity will be confirmed is detailed in the *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* section of this appendix.
- The Assessing, Arranging, and Confirming document on the HRA website provides further information for the sponsor and NHS organisations on assessing, arranging and confirming capacity and capability.

IRAS project ID	210269

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A local Principal Investigator should be in place at each participating site.

No additional training is expected of each Principal Investigator in order to conduct the study.

GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken

Activities will be conducted by a dentist at each site. Dentists not already holding a contract with a participating site would require a letter of access to take consent, or complete questionnaires. Evidence of standard DBS and OH clearance would be expected through a Research Passport (for non-NHS employees), or an NHS-to-NHS proforma for NHS employees.

For staff not holding a contract with a participating site, he taking of clinical photos would require an Honorary Research Contract through a Research Passport (if not NHS employed), or a Letter of Access through an NHS-to-NHS proforma. Evidence of enhanced DBS, the appropriate barred list check and OH clearance would be expected.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.

- The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.
- No IP rights will be generated by the study.

Appendix 3 Research Ethics Committee Approval

East of Scotland Research Ethics Service (EoSRES) **Research Ethics Service**

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

TAyside medical Science Centre
Residency Block Level 3
George Pirie Way
Ninewells Hospital and Medical School
Dundee DD1 9SY

Mr Abdulwadood Sharqawi
Leeds Dental Institute
Paediatric Dental
Department University of
Leeds Clarendon Way
Leeds
LS2 9LU

Date: 31 July 2017
Your Ref:
Our Ref: LR/**17/ES/0086**
Enquiries to: Mrs Lorraine Reilly
Direct Line: 01382 383878
Email: eosres.tayside@nhs.net

Dear Mr Sharqawi

Study title:	**A Cross sectional study of the prevalence of pain and sensitivity in children who present with Hypomineralised Molars and/or incisors**
REC reference:	**17/ES/0081**
IRAS project ID:	**210269**

Thank you for your letter dated 20 July 2017, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact please contact hra.studyregistration@nhs.net outlining the reasons for your request.
Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).
Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.
There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" above).

Approved documents

The documents reviewed and approved by the Committee are:

Document	Version	Date
IRAS Application Form [IRAS_Form_06062017]		31 May 2017
IRAS Application Form XML file [IRAS_Form_06062017]		06 June 2017

IRAS Checklist XML [Checklist_20072017]		20 July 2017
Letter from sponsor [University of Leeds 2016 17 Liability Confirmation Letter]		08 September 2016
Other [HRA amendments]	4	29 June 2017
Other [REC amendments]	4	29 June 2017
Participant consent form [Parent Consent (highlighted changes)]	4	29 June 2017
Participant consent form [Child Assent (highlighted changes)]	4	29 June 2017
Participant information sheet (PIS) [Parent Information Sheet (highlighted changes)]	4	29 June 2017
Participant information sheet (PIS) [Child Information Sheet (highlighted changes)]	4	29 June 2017
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Summary CV for Chief Investigator (CI) [CV]		
Summary CV for student [CV]		
Summary CV for supervisor (student research) [Prof Toumba CV]		
Summary CV for supervisor (student research) [DR Balmer CV]		
Validated questionnaire [Questionnaire]	3	18 May 2017

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments Adding new sites and investigators Notification of serious breaches of the protocol Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

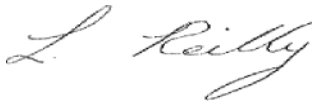
You are invited to give your view of the service that you have received from the Research Ethics Service and the application procedure. If you wish to make your views known please

use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance

We are pleased to welcome researchers and R & D staff at our RES Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of

this project. Yours sincerely



pp
Dr Anthony Davice
Vice-chair

Email: eosres.tayside@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Anne Gowing, Leeds Teaching Hospitals NHS Trust R&D Department

Appendix 4 Parent Information Sheet

Participation Information Sheet:

School of Dentistry

University of Leeds

Clarendon Way

Leeds LS2 9LU

T +44 (0) 113 343 6199

UNIVERSITY OF LEEDS

Parental Information Sheet

Study Title: A cross-sectional study of the prevalence of pain and sensitivity in children who present with hypomineralised molars and or incisors

(Occurrence of pain / sensitivity of teeth with weaker enamel surface, defective enamel)

Name of researcher: Abdulwadood J. Sharqawi

Introduction:

Your child is being invited to take part in a research study. Before you decide on whether your child should take part, it is important for you to understand why the research is being undertaken and what will be involved. Please take the time to read the following information carefully, and discuss it with others if you wish. Please do not hesitate to ask us if there is anything that is not clear, or if you would like more information. Take your time to decide whether or not you wish to take part in this study.

What is the purpose of the study?

This study aims to establish the occurrence of sensitivity and pain in children who have molar incisor hypomineralisation (defective enamel) or molar hypomineralisation.

Who is conducting this study?

This study is being conducted by a postgraduate student under the supervision of his research supervisors, as part of a PhD degree (Doctorate Degree) at the University of Leeds.

Who is being asked to participate?

We are asking the children who are diagnosed with MIH on attending the children's dentistry clinic in five different dental centres to participate in this study.

What will be involved if my child take part in the study?

If you are happy for your child to participate in this study, we will ask them to answer some questions related to the sensitivity / pain of their teeth with defective enamel. Your child will only need to answer the questions once. We may also collect further information at this appointment which includes: dental photographs. All this would usually take about 20 minutes. Dental photos will be taken for research purposes if they haven't been taken previously as a normal procedure for teeth with defective enamel. If the clinical photos were taken previously the research team will access them via on the NHS dental photography system.

What are the Advantages and Disadvantages of taking part in this study?

The only disadvantage is that you might spend 20 minutes more than usual at the visit to the dentist. While there may be no personal benefits to your child's participation in this study, the information your child provides may contribute in the establishment of the prevalence of pain and sensitivity in hypomineralised teeth. However, all the information you provide will be kept confidential at all times. All responses to our questions and information you provide will only be accessible by members of the research team.

Does my child have to take part & what will happen if my child withdraws from the study?

Your child's participation is voluntary. We would like your child to participate in this study, as we believe that your child can make an important contribution to this research. If your child does not wish to participate, your child does not have to do anything in response to this request. Please be assured that your child declining to participate will not affect the treatment provided. If your child at any time decides to withdraw from the study, the collect data will still be used in this study. Please be assured that this will not affect the treatment provided to your child.

Will my child taking part in the study be kept confidential?

All information your child provides in the questionnaire will be kept confidential. Only members of the research team will have access to it. Information from this study will only be made public in a completely anonymous format in order to ensure that no participant will be identified. Each participant in this research will be given a unique code and this code will replace there identifying information (PAS hospital number) on the data set. The data extraction form will be stored safely in a locked cabinet in a secure office in each of the dental participating centres included in the study. Any paper records that contains identifiable information (code link document, clinical photos) will be sent via NHS email to ensure security and will be stored on a password protected computer in Leeds University. Other documents (consent, assent, data extraction sheet) will be collected by the research team and transferred in a locked briefcase to Leeds University and will be stored safely in a locked cabinet in a secure office.

What will happen to the results of this research study?

All information provided by you will be stored on a password protected computer. The analysis of the information obtained will be undertaken by the research team based at the School of Dentistry, University of Leeds. The results from this analysis may be available in one or more of the following forms: 1) scientific papers in peer reviewed academic journals; 2) presentations at a conference; 3) local seminars.

Who has reviewed this study?

This Study has been reviewed by The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. This research will be monitored and regularly checked by The University of Leeds.

If you agree for your child to take part and would like more information or have any questions, concerns or complaints about the study please contact:

Principle Investigator
Abdulwadood J Sharqawi
Postgraduate student
Principal Investigator
Child Dental Health
School of dentistry
University of Leeds
Clarendon Way
Leeds
LS2 9LU
United Kingdom
01133438454

dnajs@leeds.ac.uk

Research Supervisor
Dr Richard Balmer
Clinical Lecturer / Honorary Consultant
Child Dental Health
School of dentistry
University of Leeds
Clarendon Way

Research Sponsor
Dr Clare Skinner
Faculty Head of Research and Innovation Support
Faculty Research Office
University of Leeds
Clarendon Way
Leeds

Thank you for taking the time to read this information sheet

Appendix 5 Child Information Sheet

Study Title: A cross-sectional study of the prevalence of pain and sensitivity in children who present with hypomineralised molars and or incisors

Project Title: We are counting the number of children who have toothache from their weak teeth

Information for children 6-10 years of age

Why has the dentist asked me to read this paper?

Because we think you have weak teeth, but you have strong teeth as well.
We want to know if your weak teeth hurt you.
First we will ask you questions about your weak teeth

Second we will take pictures of your te

Do I have to take part?

No. This will not change your treatment

How long will it take?

It will take twenty minutes (20 minutes)

Can I change my mind?

Of course you can change your mind anytime. You do not need to tell us why you changed your mind.

Will you still fix my teeth if I choose not to answer the questions you want?

Yes, of course.

Thank you for reading this information leaflet.

Appendix 6 Parent consent

Consent form

Patient unique participation code:

Project Title:

The prevalence of sensitivity and pain in Hypomineralised teeth (teeth with weaker enamel surface, defective enamel

1	I confirm that I have read and understand the information sheet explaining the above research project and I have had the opportunity to ask questions about the project.	☐
2	I understand that my child's participation is voluntary and that my child is free to withdraw at any time without giving any reason and without there being any negative consequences. In addition, should my child not wish to answer any particular question or questions, he/she are free to decline.	☐
3	I understand that my child's name will not be linked with the research materials, and we will not be identified or identifiable in the report or reports that result from the research.	☐
4	I agree my child's notes can be looked at by the researchers/dental team for the purpose of this research	☐
5	I agree for my child's data collected from our participation can be used anonymously in this research	☐
6	I understand and agree that the images requested here will form part of my child's confidential record and will be used for this dental research	☐
7	I agree for my child to take part in the above research project.	☐

Initials of the Parent Date Signature

(*Or legal representative*)

Dental Operator Date Signature

Appendix 7 Child assent

Project Title: We are counting the number of children who have toothache from their weak teeth

Assent form (for patients 6 - 10 years old)

Hi! I have a project.

I need help

Can you help me to answer questions about your weak teeth? Circle one

YES / NO

Can we take pictures of your teeth? Circle one

YES / NO

Appendix 8 Sensitivity Questionnaire

School of Dentistry

University of Leeds
Clarendon Way
Leeds LS2 9LU

T +44 (0) 113 343 6199
F +44 (0) 113 343 6165
E dentistry@leeds.sc.uk

IRAS Project ID: 210269
Version 2 (17 / 05 / 2017)

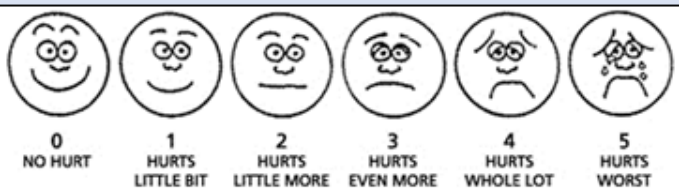
UNIVERSITY OF LEEDS

The following questions are related to the assessment of pain and sensitivity in children with Hypomineralised (defected enamel) teeth

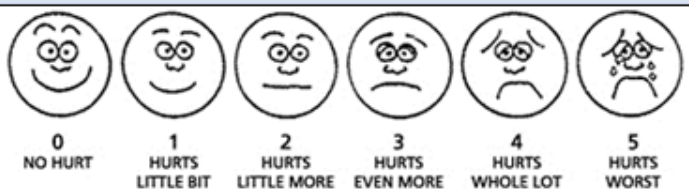
Unique Participant Number: .....

Age:..... **Gender:**.....

How much pain do you feel while eating cold food?



How much pain do you feel while eating hot (warm) food?



How much pain do you feel while drinking cold drinks?

How much pain do you feel while drinking hot drinks or sipping soup?

How much pain do you feel while eating sweet food?

How much pain do you feel while brushing your teeth?

Do you know that you have teeth that are weak?
□ Yes □ No
Have you or your dentist tried to manage the pain ?
□ Yes □ No
IF yes ..... was it effective (did it decrease or lower the occurrence of pain)?
□ Yes □ No

(This part is to be completed by the Operator (Dentist):

Please tick one box of the treatment outcome for each tooth

Tooth number / Treatment outcome	UR6	UL6	LL6	LR6
No treatment				
Fissure sealant				
Treatment of sensitivity Desensitizing agent eg: tooth mousse				
Restoration				
Temporisation / Monitor with the view to extract later				
Extraction				

Appendix 9 DREC Approval In vitro study

DREC ref: 070619/AS/275

Julie McDermott
Wed 12/06/2019 14:10
To: Abdulwadood Sharqawi
Cc: Jinous Tahmassebi; Bernadette Drummond

Dear Abdulwadood

DREC ref: 070619/AS/275
Title: In vitro study on improving the surface of hypomineralised molars using different remineralising therapies

I am pleased to inform you that the above Tissue Bank application has been accepted by the Dental Research Ethics Committee for 20 fresh, hypomineralised first permanent molars showing white yellow and brown opaque lesions.

Documents reviewed by the Committee

Document name	Version number and date
Tissue Bank application form	Signed 05.06.2019
Protocol	Version 1 05.06.2019

NB: Please be aware that teeth may not be supplied to you all at once and will be made available to you as and when they become available.

With best wishes for the success of your project.

For and on behalf of
Dr Jinous Tahmassebi
DREC Chair

Appendix 10 CHCP (City Health Care Partnership) Approval

City Health Care Partnership
5, Beacon Way
Hull
HU3 4AE
Quality & Governance Team
Tel (01482) 976948

15th February 2018

Dear Abdulwadood,

Study title:	Cross-sectional study of prevalence of pain and sensitivity in children who present with hypomineralised molars and/or incisors
REC ref:	17/ES/0081
IRAS ref:	210269
CHCP ref:	022018

Thank you for submitting the above study for consideration. The necessary organisational reviews of the submitted study documentation have taken place and I can confirm approval from City Health Care Partnership (CHCP CIC) giving you permission to commence this study with CHCP CIC patients from 15.02.2018.

You will need to arrange access to CHCP CIC patients and staff via Dr Elizabeth O'Sullivan contact details: elizabeth.o'sullivan1@nhs.net

I understand that you have already made contact with her to seek approval for the feasibility of the study and distribution of the questionnaire.

In accordance to our research governance requirements please note that all protocol amendments should be communicated promptly and any incidents or adverse events relating to this study **must** be reported to the local senior operational manager who will adopt the CHCP CIC risk management policies in managing the responsive processes.

Please note that in accepting this agreement, you also accept your professional and legal duties to comply with the requirements given by the Ethics Committee; the Department of Health's Research Governance Framework for Health and Social Care and associated legal and statutory duties. CHCP CIC note the researcher and sponsor declarations made within the IRAS R&D form (D1 items 1-11 and D2 items 1-7).

These standards are accepted by CHCP CIC as minimum standards for the conduct of research within this organisation. CHCP CIC would welcome mid-stage and end stage reports, especially any findings that could positively contribute to practice and service developments.

Yours sincerely

Sue Pender, Quality Improvement Lead Practitioner
On behalf of the Research Approval group, CHCP CIC.

Providing Quality Care

Appendix 11 Crosstabs between appearance of teeth and treatment planned

UR6 treatment planned / UR6 PEB

The UR6 without peb where more likely to be treated for (fissure sealant, restoration and temprorised/monitor extract later) when compared with UR6 with PEB. However, the UR6 with peb were more likely to be extracted when compared with UR6 without peb.

Cross tabulation for treatment planned of UR6 with absence/ Presence of PEB in UR6				
		UR6.PEB		Total
		none	present	
UR6	no treatment	3	0	3
		100.0%	0.0%	100.0%
	fissure sealant	13	1	14
		92.9%	7.1%	100.0%
	restoration	4	1	5
		80.0%	20.0%	100.0%
	temp/monitor extract later	14	4	18
		77.8%	22.2%	100.0%
	extraction	9	16	25
		36.0%	64.0%	100.0%
Total		43	22	65
		66.2%	33.8%	100.0%

UL6 treatment planned / UL6 lesion location

The UL6 with cuspal involvement were more likely to be extracted followed by temp/monitor with the view to extract later.

Cross tabulation for treatment planned for UL6 with lesion location of UL6						
		UL6.location				
		none	smooth surface	occlusal	cusp involvement	Total
UL6	no treatment	0	0	1	0	1
		0.0%	0.0%	100.0%	0.0%	100.0%
	fissure sealant	6	1	2	7	16
		37.5%	6.3%	12.5%	43.8%	100.0%
	restoration	3	0	0	1	4
		75.0%	0.0%	0.0%	25.0%	100.0%
	temp/monitor extract later	2	1	1	16	20
		10.0%	5.0%	5.0%	80.0%	100.0%
	extraction	1	1	3	19	24
		4.2%	4.2%	12.5%	79.2%	100.0%
Total		12	3	7	43	65
		18.5%	4.6%	10.8%	66.2%	100.0%

UL6 treatment planned / UL6 PEB

The UL6 without peb where more likely to be treated for (fissure sealant, restoration and temprorised/monitor extract later) when compared with UL6 with PEB. However the UL6 without peb were almost equally to be extracted when compared with UL6 with peb.

Cross tabulation between treatment planned of UL6 and absence/ Presence of PEB in UL6				
		UL6 PEB		Total
		None	Present	
UL6	no treatment	1	0	1
		100.0%	0.0%	100.0%
	fissure sealant	15	1	16
		93.8%	6.3%	100.0%
	restoration	4	0	4
		100.0%	0.0%	100.0%
	temp/monitor extract later	11	9	20
		55.0%	45.0%	100.0%
	extraction	11	13	24
		45.8%	54.2%	100.0%
Total		42	23	65
		64.6%	35.4%	100.0%

LL6 treatment planned / LL6 lesion location

The LL6 with cuspal involvement were more likely to be extracted followed by temp/monitor with the view to extract later.

Cross tabulation between treatment planned for LL6 and lesion location for LL6							
			LL6.location				Total
			none	smooth surface	occlusal	cusp involvement	
LL6	no treatment	Count	1	0	1	0	2
		Percent	50.0%	0.0%	50.0%	0.0%	100.0%
	fissure sealant	Count	9	5	0	8	22
		Percent	40.9%	22.7%	0.0%	36.4%	100.0%
	restoration	Count	1	0	1	3	5
		Percent	20.0%	0.0%	20.0%	60.0%	100.0%
	temp/monitor extract later	Count	1	4	3	9	17
		Percent	5.9%	23.5%	17.6%	52.9%	100.0%
	extraction	Count	3	0	1	15	19
		Percent	15.8%	0.0%	5.3%	78.9%	100.0%
Total		Count	15	9	6	35	65
		Percent	23.1%	13.8%	9.2%	53.8%	100.0%

LL6 treatment planned / LL6 PEB

The LL6 without peb where more likely to be treated for (fissure sealant and temprorised/monitor extract later) when compared with LL6 with PEB. However the LL6 without peb were more likely to be extracted when compared with LL6 without peb.

Cross tabulation between treatment planned for LL6 and PEB of UL6					
			LL6.PEB		Total
			none	present	
LL6	no treatment	Count	2	0	2
		Percent	100.0%	0.0%	100.0%
	fissure sealant	Count	19	3	22
		Percent	86.4%	13.6%	100.0%
	restoration	Count	2	3	5
		Percent	40.0%	60.0%	100.0%
	temp/monitor extract later	Count	10	7	17
		Percent	58.8%	41.2%	100.0%
	extraction	Count	4	15	19
		Percent	21.1%	78.9%	100.0%
Total		Count	37	28	65
		Percent	56.9%	43.1%	100.0%

LR6 treatment planned / LR6 lesion colour

The LR6 with brown coloured lesion where more likely to be extracted when compared with other lesion colours. The LR6 with white/creamy lesions were more likely to be temprorised and monitored with the view of extraction later followed by fissure sealant treatment. The majority of LR6 without lesion were more likely to have fissure sealant treatment.

Cross tabulation between treatment planned for LR6 and lesion colour for LR6							
			LR6.colour				Total
			none	white/creamy	yellow	brown	
LR6	no treatment	Count	2	0	0	0	2
		Percent	100.0%	0.0%	0.0%	0.0%	100.0%
	fissure sealant	Count	9	6	2	3	20
		Percent	45.0%	30.0%	10.0%	15.0%	100.0%
	restoration	Count	1	2	2	0	5
		Percent	20.0%	40.0%	40.0%	0.0%	100.0%
	temp/monitor extract later	Count	3	7	3	5	18
		Percent	16.7%	38.9%	16.7%	27.8%	100.0%
	extraction	Count	1	5	5	9	20
		Percent	5.0%	25.0%	25.0%	45.0%	100.0%
Total		Count	16	20	12	17	65
		Percent	24.6%	30.8%	18.5%	26.2%	100.0%

LR6 treatment planned / LR6 lesion location

The LR6 with cuspal involvement were more likely to be extracted followed by temp/monitor with the view to extract later. However, the LR6 without MIH lesion were more likely to have fissure sealant treatment.

Cross tabulation between treatment planned for LR6 and lesion location for LR6

			LR6.location				
			none	smooth surface	occlusal	cusp involvement	Total
LR6	no treatment	Count	2	0	0	0	2
		Percent	100.0%	0.0%	0.0%	0.0%	100.0%
	fissure sealant	Count	9	3	2	6	20
		Percent	45.0%	15.0%	10.0%	30.0%	100.0%
	restoration	Count	1	0	0	4	5
		Percent	20.0%	0.0%	0.0%	80.0%	100.0%
	temp/monitor extract later	Count	3	2	2	11	18
		Percent	16.7%	11.1%	11.1%	61.1%	100.0%
	extraction	Count	1	1	1	17	20
		Percent	5.0%	5.0%	5.0%	85.0%	100.0%
Total		Count	16	6	5	38	65
		Percent	24.6%	9.2%	7.7%	58.5%	100.0%

Appendix 12 Crosstabs between the appearance of teeth and pain stimulus

Pain with hot food /UR6 location

UR6 with cuspal involvement were more likely to be recorded as (no hurt) from pain from hot food when compared with the experience of pain and the lesion location.

Crosstabulation between pain from hot food and UR6 lesion location							
			UR6.location				Total
			none	smooth surface	occlusal	cusp involvement	
pain.hot.food	no hurt	Count	12	2	6	32	52
		Percent	23.1%	3.8%	11.5%	61.5%	100.0%
	hurts little bit	Count	0	1	1	7	9
		Percent	0.0%	11.1%	11.1%	77.8%	100.0%
	hurts little more	Count	0	0	1	2	3
		Percent	0.0%	0.0%	33.3%	66.7%	100.0%
	hurts whole lot	Count	0	1	0	0	1
		Percent	0.0%	100.0%	0.0%	0.0%	100.0%
Total		Count	12	4	8	41	65
		Percent	18.5%	6.2%	12.3%	63.1%	100.0%

Pain with hot food / UL6 location

UL6 with cuspal involvement were more likely to be recorded as (no hurt) from hot food when compared with the experience of pain and the lesion location.

Crosstabulation between pain from hot food and UL6 lesion location							
			UL6.location				Total
			none	smooth surface	occlusal	cusp involvement	
pain.hot.food	no hurt	Count	12	1	4	35	52
		Percent	23.1%	1.9%	7.7%	67.3%	100.0%
	hurts little bit	Count	0	1	2	6	9
		Percent	0.0%	11.1%	22.2%	66.7%	100.0%
	hurts little more	Count	0	0	1	2	3
		Percent	0.0%	0.0%	33.3%	66.7%	100.0%
	hurts whole lot	Count	0	1	0	0	1
		Percent	0.0%	100.0%	0.0%	0.0%	100.0%
Total		Count	12	3	7	43	65
		Percent	18.5%	4.6%	10.8%	66.2%	100.0%

Pain with hot drinks / UL6 PEB

UL6 without PEB were more likely to be recorded as (no hurt) when reporting pain from hot drinks. While the presence of peb were more likely to be reported as (hurts a little bit) compared to the absence of peb.

Crosstabulation between pain from hot drinks and UL6 PEB					
			UL6.PEB		Total
			none	present	
pain.hot.drink	no hurt	Count	37	12	49
		Percent	75.5%	24.5%	100.0%
	hurts little bit	Count	1	9	10
		Percent	10.0%	90.0%	100.0%
	hurts little more	Count	2	1	3
		Percent	66.7%	33.3%	100.0%
	hurts even more	Count	0	1	1
		Percent	0.0%	100.0%	100.0%
	hurts whole lot	Count	2	0	2
		Percent	100.0%	0.0%	100.0%
Total		Count	42	23	65
		Percent	64.6%	35.4%	100.0%

Appendix 13 Crosstabs between the treatment planned and the pain stimulus

Chi-square and Cramer's V test were administered to determine the statistical significance and association between the pain stimulus and the treatment planned for each hypomineralised molars. The results showed a weak relationship between the pain stimulus and the treatment planned. In addition, there were no statistical significance found in the Chi-square results.

Cramer's V significance								
	UR6		UL6		LL6		LR6	
	Sig[1]	Cramers V[2]	Sig[1]	Cramers V[2]	Sig[1]	Cramers V[2]	Sig[1]	Cramers V[2]
Cold food	0.11	0.18	0.47	0.15	0.45	0.15	0.35	0.16
Hot food	0.64	0.14	0.50	0.13	0.54	0.13	0.49	0.13
Cold drinks	0.82	0.13	0.65	0.14	0.69	0.14	0.74	0.13
Hot drinks	0.67	0.14	0.73	0.14	0.77	0.13	0.86	0.12
Sweets	0.45	0.15	0.23	0.17	0.53	0.15	0.75	0.13
Tooth brushing	0.96	0.11	0.88	0.12	0.81	0.13	0.89	0.12

[1] Person chi-square, P-value

[2] Cramers V association strength test (0= no relationship, 0.2 or less= a weak relationship, 0.2 to 0.3= moderate relationship, 0.3 and up= a strong relationship)

Pain from cold food

Pain from cold food and treatment of UR6

Crosstabulation between Pain from cold food and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	2	12	0	5	14	27	60
Hurts little bit	0	14	0	3	10	22	49
Hurts little more	3	5	1	2	11	17	39
Hurts even more	0	5	0	1	7	14	27
Hurts whole lot	0	1	0	1	4	8	14
Hurts worse	3	0	0	0	2	6	11
Total	8	37	1	12	48	94	200

Pain from cold food and treatment of UL6

Crosstabulation between Pain from cold food and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	3	13	3	14	27	60
Hurts little bit	1	15	6	7	20	49
Hurts little more	2	6	1	14	16	39
Hurts even more	0	6	0	7	14	27
Hurts whole lot	0	3	2	4	5	14
Hurts worse	0	2	0	2	7	11
Total	6	45	12	48	89	200

Pain from cold food and treatment of LL6

Crosstabulation between Pain from cold food and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	4	16	3	14	23	60
Hurts little bit	1	20	7	5	16	49
Hurts little more	3	7	2	11	16	39
Hurts even more	1	7	1	7	11	27
Hurts whole lot	0	3	2	5	4	14
Hurts worse	0	2	1	2	6	11
Total	9	55	16	44	76	200

Pain from cold food and treatment of LR6

Crosstabulation between Pain from cold food and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	2	13	6	14	25	60
Hurts little bit	1	23	3	6	16	49
Hurts little more	3	7	2	11	16	39
Hurts even more	1	5	1	7	13	27
Hurts whole lot	0	2	1	5	6	14
Hurts worse	0	2	1	2	6	11
Total	7	52	14	45	82	200

Pain from hot food

Pain from hot food and treatment of UR6

Crosstabulation between Pain from hot food and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	7	29	1	12	39	65	153
Hurts little bit	0	6	0	0	5	22	33
Hurts little more	1	1	0	0	3	2	7
Hurts even more	0	1	0	0	0	4	5
Hurts whole lot	0	0	0	0	1	1	2
Hurts worse	0	0	0	0	0	0	0
Total	8	37	1	12	48	94	200

Pain from hot food and treatment of UL6

Crosstabulation between Pain from hot food and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	4	36	12	37	64	153
Hurts little bit	1	7	0	6	19	33
Hurts little more	1	2	0	3	1	7
Hurts even more	0	0	0	1	4	5
Hurts whole lot	0	0	0	1	1	2
Hurts worse	0	0	0	0	0	0
Total	6	45	12	48	89	200

Pain from hot food and treatment of LL6

Crosstabulation between Pain from hot food and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	45	14	35	54	153
Hurts little bit	3	6	2	6	16	33
Hurts little more	1	3	0	2	1	7
Hurts even more	0	1	0	0	4	5
Hurts whole lot	0	0	0	1	1	2
Hurts worse	0	0	0	0	0	0
Total	9	55	16	44	76	200

Pain from hot food and treatment of LR6

Crosstabulation between Pain from hot food and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	41	13	37	57	153
Hurts little bit	1	7	1	5	19	33
Hurts little more	1	2	0	3	1	7
Hurts even more	0	1	0	0	4	5
Hurts whole lot	0	1	0	0	1	2
Hurts worse	0	0	0	0	0	0
Total	7	52	14	45	82	200

Pain from cold drinks

Pain from cold drinks and treatment of UR6

Crosstabulation between Pain from cold drinks and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	3	19	0	5	17	40	84
Hurts little bit	2	9	1	5	11	22	50
Hurts little more	0	3	0	1	8	11	23
Hurts even more	2	2	0	1	9	9	23
Hurts whole lot	0	3	0	0	2	7	12
Hurts worse	1	1	0	0	1	5	8
Total	8	37	1	12	48	94	200

Pain from cold drinks and treatment of UL6

Crosstabulation between Pain from cold drinks and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	3	19	7	15	40	84
Hurts little bit	2	11	4	12	21	50
Hurts little more	0	5	0	7	11	23
Hurts even more	1	4	1	11	6	23
Hurts whole lot	0	4	0	2	6	12
Hurts worse	0	2	0	1	5	8
Total	6	45	12	48	89	200

Pain from cold drinks and treatment of LL6

Crosstabulation between Pain from cold drinks and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	4	26	6	17	31	84
Hurts little bit	2	13	5	8	22	50
Hurts little more	2	3	3	5	10	23
Hurts even more	1	6	1	10	5	23
Hurts whole lot	0	5	0	3	4	12
Hurts worse	0	2	1	1	4	8
Total	9	55	16	44	76	200

Pain from cold drinks and treatment of LR6

Crosstabulation between Pain from cold drinks and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	3	22	8	16	35	84
Hurts little bit	3	16	4	8	19	50
Hurts little more	1	6	0	7	9	23
Hurts even more	0	4	2	9	8	23
Hurts whole lot	0	2	0	4	6	12
Hurts worse	0	2	0	1	5	8
Total	7	52	14	45	82	200

Pain from hot drinks

Pain from hot drinks and treatment of UR6

Crosstabulation between Pain from hot drinks and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	6	32	1	12	36	64	151
Hurts little bit	0	4	0	0	5	19	28
Hurts little more	1	1	0	0	5	7	14
Hurts even more	0	0	0	0	1	2	3
Hurts whole lot	1	0	0	0	1	1	3
Hurts worse	0	0	0	0	0	1	1
Total	8	37	1	12	48	94	200

Pain from hot drinks and treatment of UL6

Crosstabulation between Pain from hot drinks and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	39	12	35	60	151
Hurts little bit	0	4	0	6	18	28
Hurts little more	1	1	0	5	7	14
Hurts even more	0	0	0	1	2	3
Hurts whole lot	0	1	0	1	1	3
Hurts worse	0	0	0	0	1	1
Total	6	45	12	48	89	200

Pain from hot drinks and treatment of LL6

Crosstabulation between Pain from hot drinks and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	6	46	15	31	53	151
Hurts little bit	2	7	1	5	13	28
Hurts little more	1	1	0	5	7	14
Hurts even more	0	0	0	2	1	3
Hurts whole lot	0	1	0	1	1	3
Hurts worse	0	0	0	0	1	1
Total	9	55	16	44	76	200

Pain from hot drinks and treatment of LR6

Crosstabulation between Pain from hot drinks and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	40	14	35	57	151
Hurts little bit	1	8	0	5	14	28
Hurts little more	1	2	0	4	7	14
Hurts even more	0	0	0	1	2	3
Hurts whole lot	0	2	0	0	1	3
Hurts worse	0	0	0	0	1	1
Total	7	52	14	45	82	200

Pain from sweets

Pain from sweets and treatment of UR6

Crosstabulation between Pain from sweets and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	2	20	1	5	19	34	81
Hurts little bit	2	6	0	6	10	20	44
Hurts little more	1	6	0	0	10	19	36
Hurts even more	1	3	0	1	3	9	17
Hurts whole lot	0	1	0	0	5	4	10
Hurts worse	2	1	0	0	1	8	12
Total	8	37	1	12	48	94	200

Pain from sweets and treatment of UL6

Crosstabulation between Pain from sweets and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	20	9	19	28	81
Hurts little bit	1	11	3	9	20	44
Hurts little more	0	9	0	9	18	36
Hurts even more	0	3	0	4	10	17
Hurts whole lot	0	1	0	5	4	10
Hurts worse	0	1	0	2	9	12
Total	6	45	12	48	89	200

Pain from sweets and treatment of LL6

Crosstabulation between Pain from sweets and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	24	8	22	22	81
Hurts little bit	3	14	4	8	15	44
Hurts little more	1	9	2	6	18	36
Hurts even more	0	5	1	2	9	17
Hurts whole lot	0	2	0	4	4	10
Hurts worse	0	1	1	2	8	12
Total	9	55	16	44	76	200

Pain from sweets and treatment of LR6

Crosstabulation between Pain from sweets and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	3	24	7	22	25	81
Hurts little bit	2	12	3	10	17	44
Hurts little more	1	10	2	4	19	36
Hurts even more	1	3	1	3	9	17
Hurts whole lot	0	2	0	4	4	10
Hurts worse	0	1	1	2	8	12
Total	7	52	14	45	82	200

Pain when tooth brushing

Pain from toothbrushing and treatment of UR6

Crosstabulation between Pain from toothbrushing and treatment of UR6							
	No treatment	Fissure sealant	Treatment of sensitivity	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	6	22	0	7	27	44	106
Hurts little bit	1	7	1	2	11	24	46
Hurts little more	0	4	0	1	4	12	21
Hurts even more	0	2	0	2	3	6	13
Hurts whole lot	1	1	0	0	2	4	8
Hurts worse	0	1	0	0	1	4	6
Total	8	37	1	12	48	94	200

Pain from toothbrushing and treatment of UL6

Crosstabulation between Pain from toothbrushing and treatment of UL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	4	26	7	25	44	106
Hurts little bit	2	7	3	14	20	46
Hurts little more	0	4	0	5	12	21
Hurts even more	0	3	2	2	6	13
Hurts whole lot	0	3	0	2	3	8
Hurts worse	0	2	0	0	4	6
Total	6	45	12	48	89	200

Pain from toothbrushing and treatment of LL6

Crosstabulation between Pain from toothbrushing and treatment of LL6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
no hurt	5	27	12	23	39	106
hurts little bit	4	15	1	12	14	46
hurts little more	0	6	2	3	10	21
hurts even more	0	4	1	2	6	13
hurts whole lot	0	2	0	3	3	8
hurts worse	0	1	0	1	4	6
Total	9	55	16	44	76	200

Pain from toothbrushing and treatment of LR6

Crosstabulation between Pain from toothbrushing and treatment of LR6						
	No treatment	Fissure sealant	Restoration	Temp/monitor extract later	Extraction	Total
No hurt	5	28	10	23	40	106
Hurts little bit	2	12	2	12	18	46
Hurts little more	0	7	1	3	10	21
Hurts even more	0	4	1	3	5	13
Hurts whole lot	0	1	0	3	4	8
Hurts worse	0	0	0	1	5	6
Total	7	52	14	45	82	200

Appendix 14 Age and gender relationship with the presence or absence of pain

Pain has been scored either with one or zero (1-0) (pain / no pain). Most of the participants who reported pain were 8 years of age. While the lowest were 6 years old. This may explain that with time further post eruptive breakdown and sensitivity occurs because of the exposed dentine.

Age relationship with pain				
		No pain	Pain	Total
Age	6	2	10	12
	7	3	30	33
	8	7	66	73
	9	4	44	48
	10	2	32	34
Total		18	182	200

Gender relationship with pain

There was no difference in reporting pain between males and female participants.

Gender relationship with pain				
		No Pain	pain	Total
Gender	Male	9	91	100
	Female	9	91	100
Total		18	182	200

Appendix 15 Contralateral involvement of MIH teeth

The table represents the number of molars affected in each arch (maxillary and mandibular).

	Maxillary arch		Mandibular arch	
	Frequency	percentage	Frequency	MIH
One tooth affected by MIH	6	9.2%	7	10.8
Both teeth affected by MIH	59	90.8%	58	89.2%
Total	65	100%	65	100%

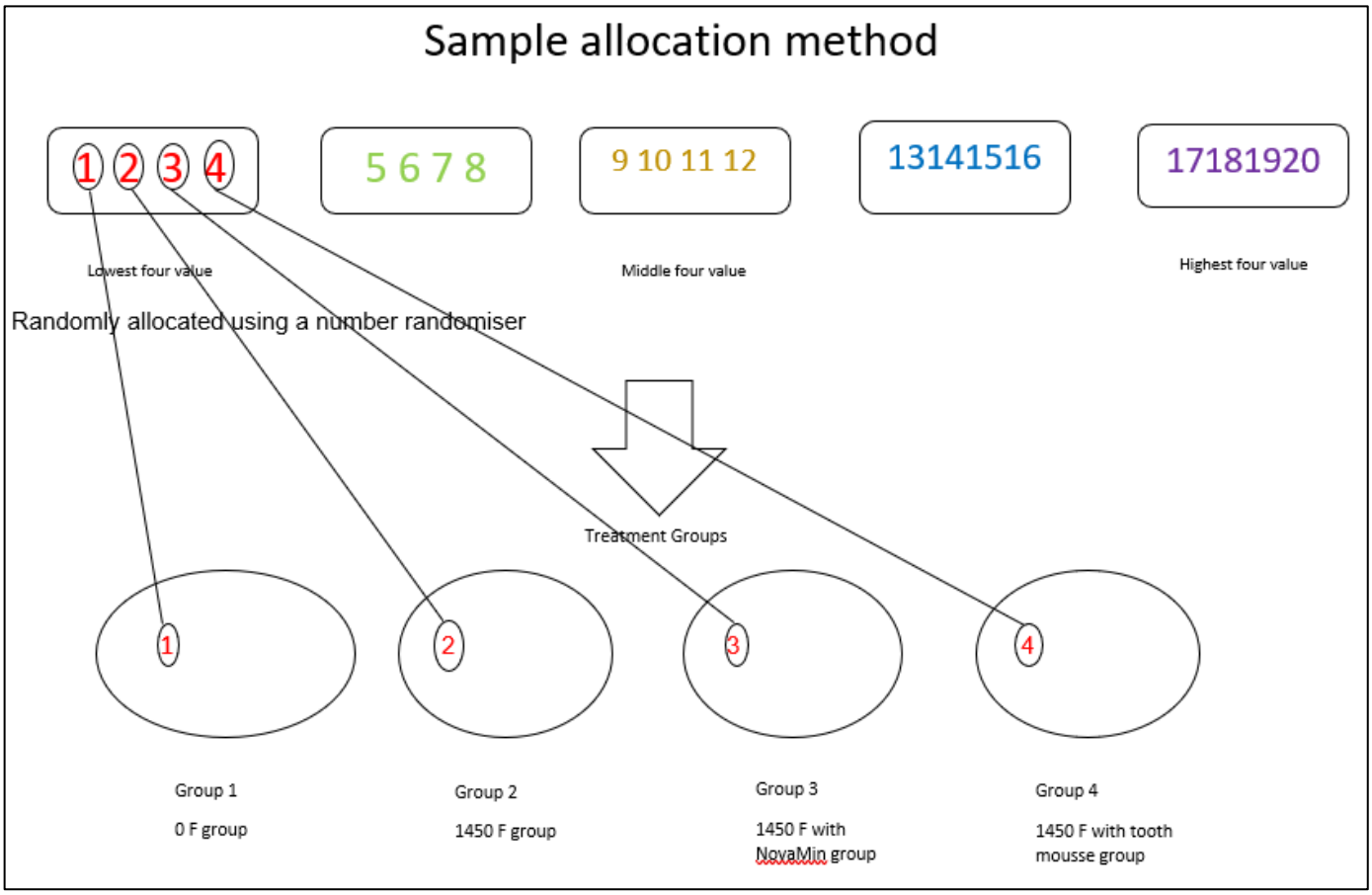
In this study, the photographic analysis of the maxillary arch reveals that 90.8% of teeth with MIH had contralateral teeth affected as well. Whereas, the mandibular arch showed similar results to the maxillary arch. The finding in this study is consistent with what (Weerheijm, 2003b) found, it was stated that if there was one molar affected with MIH, it was more likely that the contralateral tooth was affected with MIH.

Appendix 16 Randomisation strategy (Invitro study)

The baseline microhardness mean values was recorded for each sample and samples values were arranged in an ascending order from the lowest to the highest hardness value. After that, the 20 values were divided into 5 groups, each group contained four hardness values. Following that, an online sample randomiser was used to allocate the sample to each one of the four group.

Group 1: 0 F ppm, Group 2: 1450 F ppm

Group 3: 1450 F ppm (Novamin) Group 4: Tooth Mousse and 1450 F ppm



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