Comparative evaluation of ICON resin infiltration and Micro-abrasion followed by CPP-ACP application on colour, mineral density and surface roughness of MIH-affected teeth in vitro

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

Dedicated to my beloved family

(My mother, husband and my boys)

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Abstract

Aim

To evaluate the surface roughness, mineral density and colour change of MIH affected teeth following surface treatment using two protocols; ICON Resin Infiltration (IRF) and Micro-abrasion followed by CPP-ACP (MCP).

Materials and methods

50 extracted human teeth were used in the study, 10 sound enamel [Group 1(SE)] as a control group and 40 MIH enamel; 20 white-creamy [Group 2(IRF) and Group 3(MCP)] and 20 yellow-brown [4(IRF) and 5(MCP)] which were divided evenly based on their colour and DIAGNOdent (DD) readings followed by randomisation prior to receiving two treatment (IRF and MCP). Measurements were done at baseline and post-treatment (after 7 days, 1 and 3 months) using Image J (colour), DIAGNOdent pen (DD), profilometry (Ra) and micro-CT (MD). Data was analysed using SPSS at a significant level ($p<0.05$).

Results

IRF showed significant improvement compared to MCP in restoring lightness of MIH lesion after 1 month(white-creamy) and 3 months(yellow-brown) with the L* values close to those of SE ($p<0.05$). The largest increase in ΔE values was in MCP groups which was significance at up to 1 month for yellow-brown MIH lesions and up until 3 months for white-creamy MIH lesion ($p<0.05$).

The lowest Ra was observed in SE followed by MCP. Ra significantly increased in MCP and IRF for white-creamy MIH after 1 month ($p<0.05$) while a significant reduction in Ra was observed only in MCP for yellow-brown MIH ($p<0.05$). DD was lowest in SE group followed by white-creamy MIH. DD decreased significantly only for SE group after 7 days ($p<0.05$) and no significant difference was observed in intervention groups ($p>0.05$). At baseline, SE had the highest MD followed by white-creamy MIH and the lowest in yellow-brown MIH (IRF and MCP).

Conclusion

IRF had potential in improving lightness of the MIH lesion while MCP was more effective in colour change especially masking white-creamy MIH lesions as well as the potential for Ra reduction. SE exhibited better colour improvement, lowest Ra, DD and highest MD compared to intervention groups.

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ANOVA	Analysis of Variance	H_2O_2	Hydrogen peroxide
AF	Auto-focus	HEMA	Hydroxyethylmethacrylate
a *	Red/green axis values	HCL	Hydrochloric acid
BMP	Morphogenic protein	ISO	International organization for standardization
b*	Yellow/blue axis values	IRF	ICON resin infiltration
CPQ	Child's perception questionnaire	IQR	Interquartile range
CEJ	Cemental-enamel junction	Kda	Kilodaltons
CIELAB	Commission Internationale de l'E' clairage	KLK4	Kallikrein related peptidase-4
CPP-ACPF	Casein phosphopeptide-amorphous calcium phosphate fluoride	KV	Kilovoltage
CPP-ACP	Casein phosphopeptide-amorphous calcium phosphate	LED	Light emitting diode
DEJ	Dentino-enamel Junction	L*	Lightness values
DpSpSEEK	Six-peptide sequence	MIH	Molar Incisor Hypomineralisation
DDE	Developmental defects of enamel	MMP	Matrixmetalloproteinase
DC	Direct current	MSBS	Microshear bond strength
DD	DIAGNOdent reading	MCP	Micro-abrasion followed by CPP-ACP applicat application
EAPD	European Acedemy of Pediatric dentistry	Micro-CT	X-ray mictotomography
ExciTE F	Light-curing , fluoride releasing, single component total-etch adhesive	MD	Mineral density
FPMs	First Permanent Molars	mm	milimeters
FGF	Fibroblast growth factor	NaOCL	Sodium Hypochlorite
F	Focal length	nm	Nanometer

PRPs	Platelet rich plasma	TEGMA	Triethylene glycol dimethacrylate
pH	Potential of Hydrogen	3D	3 Dimensional
PEB	Post-eruptive breakdown	µm	Micrometer
RI	Refractive index	Δ*a*	Change in *a* * values
Ra	Roughness average	Δb	Change in b* values
RCT	Randomised controlled trial	ΔE	Colour change
SE	Sound Enamel	ΔL	Change in L* values
SPSS	Statistical package for social science		
SAP	Salivary acquired pellicle		
SD	Standard deviation		
TGF-B1	Transforming growth factor B1		

1 Introduction

Molar incisor hypomineralisation (MIH) is defined as a qualitative, demarcated, enamel defect of hypomineralisation affecting at least one first permanent molar (FPM), while often permanent incisors are also affected (Weerheijm et al 2001). According to the European Academy of Paediatric Dentistry (EAPD) diagnostic criteria, at least one or more FPMs should have one of the following characteristics: demarcated enamel opacity, post-eruptive enamel breakdown, atypical restoration, or atypical extraction due to MIH (Weerheijm et al. 2001, Lygidakis et al.2010). A global MIH prevalence has been reported as 13.1% (11.8%-14.5%) with significant differences between regions and countries (Schwendicke et al. 2018). The aetiology of MIH is unclear and believed to be multifactorial (Alaluusua et al. 2010, Fatturi et al.2019)

MIH-affected teeth are characterized by reduced mineral contents of enamel (Farah et al. 2008, Farah et al. 2010, Fearne et al. 2004) and mechanical properties leading to lower strength and hardness of the enamel. This could lead to increased tooth sensitivity to food, drinks and thermal changes which depend on the enamel porosity or reduction in mineral content. Furthermore, clinical presentation of MIH has been reported to determine the severity with dark-brown enamel considered worse than yellow enamel, which is, in turn, worse than white-creamy enamel (Farah et al.2010). MIH-affected children face several difficulties in their life like aesthetic concerns and sensitivity and pain, from these teeth which may further affect the quality of life of these children (Joshi et al. 2022).

Management of MIH-affected teeth is usually a complex process and different factors need to be considered before treating these teeth such as patient cooperation, stage of dental development and defect severity as well as patient, parental preferences, other anomalies and the psychosocial impact on the child must be taken into consideration. Management approaches for MIH-affected incisors with micro-abrasion (Bhandari et al.2019), IRF and a combination of approaches have been reported (Hasmun et al.2020). Other studies reported strategies to mineralize and reduce hypersensitivity using 5% fluoride varnish, 10% Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 10% CPP-ACP crème with 900 ppm fluoride and ozone (Özgül et a. 2013).

However, to date, there has not been any study using the range of MIH-affected teeth to investigate the effect of ICON resin infiltration (IRF) and micro-abrasion followed by CPP-ACP application (MCP) on the colour, surface roughness and mineral density of these teeth prior and post-treatment and compare with sound enamel (SE).

In this research project, the effects of IRF and MCP have been investigated on white-creamy and yellow-brown MIH enamel lesions with response to their colour, surface roughness and mineral density prior and post-treatment and compared with SE in vitro.

2 Literature Review

2.1 Dental enamel formation

Dental enamel is the visible outermost covering of the tooth. It is the hardest tissue in the human body that contains a high amount of minerals(Hu Jan C.C, et al., 2007). Embryonically, amelogenesis starts by developing dental lamina which involves the inward growth of the thickened oral epithelium. The dental lamina then folds and penetrates the underlying mesenchyme derived from cranial neural crest cells(Chai et al., 2000) to form dental placode, bud, cap and bell stages. These stages are crucial for the formation of the crown of the tooth(Lacruz, Rodrigo S. et al., 2017), later signals from mesenchyme regulate the formation of the enamel knot which results in the transition from bud to cap stage(Vaahtokari et al., 1996). Therefore the interaction between dental epithelium and mesenchyme regulates tooth morphogenesis hence the expression of the signalling molecules in the enamel knot is responsible for regulating the more advance stages of tooth morphogenesis which indicates that the enamel knot is a signalling centre that guides the development of tooth crown. Followed by basic cap stage formation, the cells of dental hard tissue continue to differentiate; the contact and interaction between mesenchymal cells and dental epithelium differentiate into odontoblasts and the adjacent inner enamel epithelium give rise to ameloblasts. Meanwhile, the bell stage of tooth development results in epithelial expansion to differentiate into four types of epithelial cells which are the inner enamel epithelium, the stratum intermedium, the stellate reticulum and the outer enamel epithelium. Matalova & Sharpe reported that this process of ameloblast differentiation is initiated by odonto blasts and the cells of stratum intermedium via molecular signals such as bone morphogenic protein(BMP) and fibroblast growth factor (FGF) (Matalova E, Lungova V and Sharpe P, 2015) and the secretion of the first layer of predentine initiate the terminal differentiation of the preameloblast (Miletich and Sharpe, 2003). On the other hand, the Inner enamel epithelium which is a single layer of cells differentiates into enamel matrix–secreting ameloblasts (Lacruz, Rodrigo S. et al., 2017) and is primarily responsible for enamel formation and mineralization. The inner and outer enamel epithelium meet at the cervical loop, a place for dental epithelial stem cells that provide a constant supply of enamel-forming cells until the full formation of the enamel crown.

The process of enamel formation which is referred to as amelogenesis involves several stages. Some studies have classified the stages into the presecretory stage, the secretory stage and the maturation stage(Warshawsky and Tissues, 1985). Robinson et al described the major stages being the secretory,

transition and maturation stages and others described the stages to be presecretory, secretory, transition and maturation (Bartlett J.D, 2013), and in each stage, ameloblasts have been reported to fulfil a more or less exclusive function.

2.1.1 Presecretory Stage

This stage starts with the deposition of predentine by odontoblasts at the future dentino-enamel junction (DEJ) before mineral formation. This begins from the cusp tip towards the cervical region of the tooth. Predentine which is composed of collagenous and non-collagenous proteins is the first to mineralize beneath the future DEJ which is followed by the differentiating pre-ameloblasts extending cytoplasmic projections through the basement membrane. It is at this stage that the differentiating ameloblasts acquire their phenotype, change polarity, develop an extensive protein synthesis apparatus and prepare to secrete the organic matrix of enamel. Not only does deposition of predentine take place at this stage but also inner enamel epithelium starts differentiating into ameloblasts that are ready for secretion (Bartlett J.D, 2013).

2.1.2 Secretory stage

Pre-ameloblasts are transformed into secreting ameloblasts; they get elongated into columnar cells to form Tome's Processes at their apical ends close to the forming enamel (Bartlett J.D, 2013). The Tomes process makes a junction that forms between enamel and the ameloblasts with a picket-fence or saw-toothed appearance.

During amelogenesis, ameloblasts secrete enamel matrix proteins such as amelogenins, enamelins, ameloblastins and Tuftelins into the enamel space and they later break down proteolytically. Amelogenin comprises approximately 80-90% of the inorganic matter while ameloblastin and enamelins comprise 5% and 3%-5% respectively (Bartlett J.D, 2013).

Ameloblastins play a crucial role in maintaining ameloblast differentiation, essential for enamel formation as well as stopping proliferation of ameloblasts. On the other hand amelogenins and enamelins are essential for the deposition of hydroxyapatite crystals. During maturation when ameloblasts produce proteinases like matrix metalloproteinase MMP, amelogenins and ameloblastins are removed (Matalova E, Lungova V and Sharpe P, 2015). It is in this way that the ameloblasts regulate the hydroxyapatite crystals within the enamel space to form enamel which is arranged in a prismatic appearance and composed of rods extending from dentino-enamel junction to the enamel space and

the interrod enamel located around the enamel rods (Miletich I, and Sharpe PT 2003). The whole process of amelogenesis terminates at the time of tooth eruption and so no production of secondary enamel or regenerative enamel appears once the tooth has erupted.

A newly erupted tooth is susceptible to acid dissolution in the oral cavity. This is because the enamel once erupted in the oral cavity is very porous and permeable and allows penetration of up to 200 µm into subsurface enamel. However, it is protected by the acquired enamel pellicle that covers the surface of the tooth as soon as the tooth erupts. Salivary-acquired pellicle(SAP) is 0.1-1.0micrometer thick (Garcia Godoy, F and Hicks MJ, 2008) composed of proteins and glycoproteins(Yang et al., 2017). Furthermore, Some of the salivary proteins (statherin, PRPs) in the pellicle also bind to hydroxyapatite crystals in the surface and subsurface enamel to protect against demineralization upon acid challenge(Garcia Godoy, F and Hicks MJ, 2008).

Statherin, a vital constituent of the SAP consists of 43 amino acids and has a high affinity of calcium phosphate that could adsorb on hydroxyapatite surface. The first six peptides; DpSpSEEK of the N-terminus of statherin has been reported to have the potential to restore demineralized enamel. Yang Y et al found that the acid-etched tooth enamel slices treated with DpSpSEEK peptide grew nano-rod crystals uniformly compared to the slices which were soaked in saliva for one day which also regenerated crystals but were distributed randomly and were in different sizes, shapes and directions(Yang et al., 2017)

2.1.3 Transition and Maturation stage

In these stages the pre-existing enamel crystals widen and thicken, this is a very fundamental process. Before the layer of enamel reaches its full thickness, the ameloblasts stop moving relative to each other, Tomes Processes are retracted and the enamel surface smoothens and the cells become flatter and shorter during the transition stage. In the maturation stage, ameloblasts carry out the activity of enamel matrix protein degradation and replace them with inorganic material. This is achieved with the help of kallikrein-related peptidase-4 (KLK4) secreted by ameloblasts (Bartlett JD, 2013) which is induced by transforming growth factor β1 (TGF-β1)

According to Simmer, the expression of KLK4 by ameloblasts starts at the beginning of the transition stage and lack of secretion and activity of KLK4 resulted in enamel defects in mice (Simmer J.P, Hu Y,

Lertlam R, Yamakoshi Y and Hu Jan.C.C, 2009). KLK4 also plays a role in proteolytic degradation of the residual matrix to simplify its removal and provide space for the expansion of pre-existing crystals; therefore any disturbance in TGF-β1 will affect the secretion of KLK4. A study conducted in rats and mice found that enamel exposure to fluoride significantly lowered TGF-β1 levels in rat enamel organ and contributed to reduced KLK4 protein levels in fluorosed enamel leading to higher protein content in fluorosed enamel than in normal enamel which in turn reduced enamel hardness (Suzuki, M.et al., 2014). Similarly, 8--21-fold higher protein content was reported in MIH teeth compared to normal enamel (Farah, RA, Monk, BC, Swain, MV and Drummond, BK, 2010).

In early enamel formation, it is partially mineralized to approximately 30% and this then increases as the organic matrix is broken down and removed. The crystals grow wider and thicker, organic matrix and water are lost and minerals are added. This happens after the full thickness of enamel has been formed and the mineral content increases to 96% which makes up the inorganic portion of the enamel whereas the remaining 4% is the organic portion. Suga S reported that mineralization starts as the enamel matrix is laid down and proceeds during the maturation stage (Suga, 1989). Initially, mineralization begins at the surface and proceeds towards the inner layer and later heavy mineralization starts from the inner layer towards the surface. It has been reported during the early stages of mineralization, enamel usually is dull, white and soft and it becomes hard translucent enamel in the late stages of development (Suckling, 1989).

2.2 Disturbance during enamel formation

Ameloblasts are very sensitive to external stimuli and any disturbance can result in an enamel defect. Remodeling does not occur after enamel formation therefore any disturbance during formation will lead to the formation of permanent marks or lesions. Several factors affect the appearance of the defect on the enamel and when these factors act simultaneously, the severity of the defect becomes even worse. Factors reported to cause these defects include the stage at which the insult occurs, the severity of the insult causing temporary or permanent cessation of the cell's activity, the duration of the insult, the specific agent involved and the phase of ameloblastic activity (Suckling, 1989). These changes during enamel matrix formation (secretion phase) lead to hypoplasia while disturbances during the maturation phase result in hypomineralization regardless of the presence of the hypoplastic defect (Suga, 1989). As a result damage during the secretory phase results in very thin enamel whereas if the maturation phase is disturbed, the resulting enamel leads to the appearance of bands of chalky opaque porous enamel (Moss-Salentijn and Hendricks-Klyvert, 1990, Suckling, 1989). This explains the fact that hypomineralized

enamel usually contains higher amounts of organic substances than normal mineralized enamel. Due to the dysfunction of the resorptive activity of ameloblasts and Inhibition of proteolytic enzyme activity, enamel proteins are left behind blocking the growth of crystals (Crenshaw, 1984) suggesting a qualitative defect rather than quantitative in hypomineralized teeth. Another study was conducted on sheep where an instrument was inserted in a developing permanent incisor crypt and reported a disturbance in ameloblasts leading to the cessation in the secretory function of the ameloblasts and resulted in hypoplasia due to damaged ameloblasts followed by hypomineralization (Suckling, 1980).

Suckling claimed that the causative insults do not appear to have significant importance in the final appearance of the defect because many different local and systemic causes present defects of similar appearance and physical characteristics, however the timing, duration and severity of the insults either during secretory or maturation stage has a great effect on the location and appearance of the defect (Suckling, 1989).

The aetiology of these defects can be classified as resulting in localized defects affecting few teeth or generalized defects involving most or all teeth and the factors involved can be as many as over 90 different factors (Small and Murray, 1978). Localized defects can be a result of trauma, infection or irradiation while generalized defects are a result of genetic or systemic disturbances including intoxication, perinatal and postnatal problems, malnutrition, infectious disease and other medical conditions (Small and Murray, 1978).

2.3 Fluorosis

Enamel fluorosis is a developmental defect characterized by hypomineralization of the enamel as a result of the excessive ingestion of fluoride during tooth development. The severity of the condition depends on the amount and duration of fluoride intake during enamel formation as well as the plasma concentration of fluoride that bathes the enamel organ (Angmar-Månsson, B., Ericsson, Y. and Ekberg, O., 1977). According to Evans and Stamm in 1991, permanent maxillary central incisors are more susceptible to fluorosis and the most critical ages of susceptibility are a four-month period after birth starting from 22 months as this relates to the development of the incisal region and exposure prior to this period carried less risk and lesion are localized while a continued exposure for a long time after this period up to 36 months result in the spread of the risk (Evans and Stamm, 1991). This has been explained further by Suga that the central incisors are presumed to have formed 16 months after birth but not matured. The presence of fluoride during maturation interferes with the maturation process by

blocking the removal of organic substances leading to cessation of crystal growth and subsequent hypomineralization (Suga, 1989). This indicated that the fluorotic changes are related to the disturbance during the maturation stage of tooth development. Ishii T and Suckling G reported that fluorosis was related with high fluoride intake and that the longest exposure to high fluoride from 35-42 months showed more severe effects while exposure between 24-33 months had very mild, mild or moderate and the exposure between 11-22 months had minor enamel abnormalities(Ishii and Suckling, 1986). These changes begin with lower incisors which complete mineralization around 2-3 years of age (Denbesten, P. and Li, W., 2011). Similarly, another study reported a high risk of fluorosis in children who were taking fluoride supplements at the age of 2.5 years compared with children who started these supplements at a later age(Larsen et al., 1985). However dental fluorosis reduced significantly when fluoride concentration in water was lowered from 1.0 to 0.7mg/L among children in Hong Kong (Evans, 1989).

2.3.1 Aetiology of dental fluorosis

Excessive intake of fluoride during tooth formation leads to dental fluorosis. It is a result of constant exposure of the developing tooth germ to elevated plasma fluoride concentrations. Dental fluorosis has been linked with the use of systemic fluorides such as the high amount of fluoride intake from water which exceeds 1ppm, infant formula reconstituted with fluoridated water, supplements (tablets, lozenges and drops) and denitrifies (Ismail, A.I et al, 1990). This was associated with the frequency and amount of fluoride supplements and the age of the child when brushing was initiated meaning that the effects of fluoride supplements together with brushing with fluoridated toothpaste before age 2 places the children at risk of fluorosis. However, Pendrys, Katz and Morse found a strong association between mild-moderate fluorosis and the use of fluoridated infant formula and frequent brushing with fluoridated toothpaste for children lived in optimal fluoridated water (Pendrys et al., 1994).

The optimal level of fluoride has been reported to be between 0.05–0.07 mg/kg (Levy 1994), whereas the Public Health Commission, 1995 suggested an optimal level between 0.7 and 1.0ppmF in drinking water and so when the level of fluoride is above 1.5ppm, dental fluorosis can occur. However, the level of fluoride in daily life is difficult to calculate because of variations in different beverages and foods a person consumes that may contain fluoride together with the use of other dental products. Therefore multiple sources of fluoride a person consumes daily are reported to be related to the increased incidence of dental fluorosis

The high fluoride intake has been suggested to affect the enamel organ during the transition stage from the presecretory to the secretory stage. Kierdorf reported that fluoride prevents ameloblasts from completing Tomes Processes formation leading to the cessation of enamel matrix formation and formation of aprismatic enamel resulting in reduced enamel thickness as seen in severe forms of fluorosis (Kierdorf et al., 1997).

Some other studies found that fluoride when ingested is associated with incomplete crystal growth at prism peripheries where degradation products of ameloblastin build up during development. Since it has been demonstrated that removal of these matrix proteins is crucial prior to crystal growth (Robinson et al., 1989), impaired removal due to fluoride results in incomplete growth of apatite crystals in this area (Robinson et al., 1998)

2.3.2 Dental Fluorosis Presentation

The degree of fluorosis may range from mild as white spot lesions (Fejerskov et al., 1988) to severe forms characterized by brown discolouration and discrete or confluent pitting depending on the timing, duration, and dose of fluoride exposure (Denbesten and Thariani, 1992). Fluorosis can present as white opacities when fluoride in the water is 1ppm. This indicates some porosities in the tissue and as levels of fluoride increase the porosities increase and enamel tissue is compromised as a result of a significant reduction of serum calcium which in turn leads to hypomineralization (Fejerskov, O., Thylstrup, A. and Larsen, M.J., 1977) (Thylstrup, A. and Fejerskov, O., 1978). Yaeger 1966 reported that hypoplastic defects in rats started developing earlier with higher amounts of fluoride in the diet, and the severity of hypoplasia was proportional to the amount of fluoride in the diet as well as the exposure time (Yaeger, 1966)

2.4 Molar Incisor Hypomineralization (MIH)

Molar incisor hypomineralization is a type of enamel defect that affects the first molars and incisors in the permanent dentition. One to four first molars can be affected and more frequently central incisors are involved (Garg, N., Jain, A.K., Saha, S. and Singh, J., 2012). The condition has been given different names including hypomineralized first permanent molars (Beentjes, V.E, Weerheijm, K.L. and Groen, H.J., 2000), Idiopathic enamel hypomineralization in First Permanent Molars (Jälevik, B., Klingberg, G., Noren, J. and Barregard, L., 2000), (Koch et al., 1987), non-fluoride hypomineralization in first permanent molars (Leppaniemi et al., 2001) and cheese molars (Weerheijm, K.L., 2003) (Weerheijm, K. L., et al., 2000.) and (Van Amerongen, W.E. and Kreulen, C.M., 1995) However to avoid confusion,

Weerheijm suggested that it would be best to use one name that has no reference to any possible aetiology and so molar Incisor hypomineralization (MIH) was proposed (Weerheijm, K.L., Jalevik, B. and Alaluusua, S., 2001).

The prevalence of MIH has been reported by several epidemiological studies worldwide to range from 2.4% to 40.2% worldwide (Almuallem and Busuttil-Naudi, 2018). MIH prevalence in Northern Europe ranged from 3.6% to 25% (Weerheijm, 2003), 9.7% in Chennai (Yannam SD, Amarlal D and Rekha CV, 2016), 0.48% in India (Subramaniam P, Gupta T and Sharma A,2016), 19.8% in Japan(Saitoh et al., 2018). However, Jalevik argued that these figures could be an underestimate due to the lack of standardization in the diagnosis of these lesions (Jälevik, B, 2010). A meta-analysis conducted recently showed an overall MIH prevalence worldwide to be 14.2%(Zhao et al., 2018). Another recent systematic review highlighted a global MIH prevalence of 12.9% (11.7%-14.3%) with significant differences between regions and countries (Schwendicke et al. 2018).

2.4.1 Aetiology of MIH

Although the aetiology is still not clear, several studies have reported that MIH is not caused by one specific factor but rather by different factors acting together (Alaluusua, 2010). Possible causes reported were the association of MIH with urinary tract infection in mothers in the last trimester of pregnancy (Freden and Gronvik, 1980), perinatal conditions associated with urgent Caesarean section, prolonged delivery, premature birth and twinning (Lygidakis et al., 2008) Similarly, hypoxia due to prolonged labour and prematurity (Seow, 1996); hypocalcaemia (Jälevik et al., 2001) and childhood illness during the first four years of life(Beentjes et al., 2002) have all been linked with development of MIH lesions.

The condition ranges from mild to severe forms, presenting as white, yellow or brown well-demarcated opacities on FPM and incisors indicating mild form to severe forms with post-eruptive breakdown. The severity can be asymmetrical, however when the FPM is severely affected the chance that the contralateral molar is also affected is high, when incisors are affected the condition is usually less severe compared to when molars are affected (Garg N, Jain A.K, Saha S. and Singh, J, 2012). In a review that evaluated the structure, mechanical and chemical composition of MIH teeth reported that MIH teeth were found to have a reduction in mineral quantity and quality (calcium and phosphate were reduced), reduction of hardness and modulus of elasticity, increase in porosity, carbon/carbonate and protein content (Elhennawy et al., 2017).

Similarly, Elfrink found a significantly lower reduction of mineral density (measured as hydroxyapatite) in yellow and brown lesions of hypomineralised second primary molar and the greatest reduction was found in yellow lesions whereas the white opacities did not show mineral density reduction (Elfrink et al., 2013).In another study involving permanent molars, MIH higher protein content of around 821-fold compared to sound enamel and the brown lesion showed the highest protein content of about 15-21-fold (Farah et al., 2010b). The colour of the lesion was found to be associated with the mineral density of the enamel meaning that the darker the colour the less the mineral content and in particular brown lesions had a marked reduction of mineral density (Farah R, Drummond B, Swain M. and Williams S, 2010).

2.5 Impact of developmental defects on enamel

Developmental defects of enamel (DDE) can cause several oral problems like aesthetics, occlusion, tooth wear, erosion and sensitivity leading to an increased risk of dental caries which can affect the physical, social and psychological well-being of the children (Salanitri and Seow, 2013). A recent article published in Brazil on the impact of these defects found that these defects have a negative impact on the oral health-related quality of life of children. Children with DDE had a 7% reduced positive impact in oral health-related quality of life; this was further reduced to 13% and 29% on their physical impact of social life and overall score on general health-related quality of life respectively. Boys were 1.15 to 1.13 times more likely to report on positive impact on physical and emotional aspects compared to girls (Andrade et al., 2019).This has been observed to be more associated with hypoplastic lesions as these type of defects are more severe (Ferreira VF and Ardenghi TM, 2011) and results in tooth sensitivity and more susceptible to dental caries (Schluter PJ., Kanagaratnam S, Durward, CS. and Mahood, R., 2008). Enamel hypoplasia has been reported to be associated with a compromise in the physical function of these teeth and a compromise of oral health while diffuse opacity affected the social life of the children (Andrade et al., 2019). Furthermore, enamel hypoplasia with caries had a more negative impact on children than those with enamel hypoplasia but not decayed (Folayan et al., 2018). Similarly, it has been reported that discoloured MIH teeth can suffer severe enamel breakdown after eruption which can lead to food impaction and eventually the development of caries. Enamel loss is also associated with dentine hypersensitivity due to exposure of dentinal tubules and the presence of bacteria in the dentinal tubules indicating that bacteria can penetrate through the hypomineralized enamel into the dentine thus contributing to hypersensitivity (Fagrell et al., 2008). Marks caused by these defects appeared to have a negative impact, especially on young people who desire a good appearance and this can compromise a

sense of self in these young groups of people as they believe that these defects act as a threat to their sense of self resulting in low self-esteem and low self-confidence. Furthermore, social interactions with peers which are essential to young people and are part of their development are affe cted by these defects especially when they first meet. Teasing by being called or given strange names due to the appearance of their teeth was reported more in young people with enamel defects which led to conflict between young people (Marshman Z, Gibson B. and Robinson PG, 2009).

2.6 Oral Health-Related Quality of Life (OHRQoL) and MIH

MIH has been reported to have a negative impact on the quality of life of children. In a study conducted in Mexico for children aged 8-10 years to evaluate the OHRQoL, they used the Spanish version of the child's perceptions Questionnaire (CPQ) which comprises of 25 questions. They found that 78.9% of children with MIH experienced more oral health problems which in turn affected the general well-being of these children. They also found that school children with MIH experienced a higher rate of negative impact compared to those without MIH, and this was more significant in children with moderate/severe forms of MIH compared to those with mild forms meaning that MIH in these forms was associated with oral symptoms, functional limitations, emotional and social wellbeing (Güngör, D. et al., 2019). Similarly, this was found in another study conducted in Brazil, where se vere MIH had a negative impact on the quality of life of children and this was associated more with oral symptoms and functional limitations (Dalledone M, 2018). However, the Influence of family on children's oral health conditions, family monthly income and mother's education were among the factors reported to contribute to the improvement of OHRQoL. These resulted in increased children's perception of their oral health, easy access to health care and preventive interventions (Paula et al., 2012). Another study found that children who received minimally invasive treatment for these lesions to reduce the visibility of enamel opacities resulted in a positive impact on children's well-being.

2.7 Treatment of Aesthetic Defects

Management of enamel defects is affected by several factors such as the type of the affected teeth, extent of defects, tooth surface loss, prognosis and associated symptoms. Different management options have been proposed to improve the aesthetic appearance of these defects from non-invasive to invasive approaches depending on the severity. Non-invasive approaches include bleaching (Gugnani et al., 2017), bleaching with fluoridation (Choi et al., 2018), bleaching with ICON resin infiltration (Gugnani et al., 2017, Schoppmeier et al., 2018, Pan et al., 2019), bleaching, micro abrasion and ICON resin

infiltration (Oliveira et al., 2020) and ICON resin infiltration alone (Kim et al., 2011, Giannetti et al., 2018, Auschill et al., 2015) whereas invasive methods such as veneers (Zorba et al., 2010), indirect resin crowns (Quinonez et al., 2000), have been used to manage these defects.

Mild to moderate cases of fluorosis have been effectively managed by bleaching with 25% hydrogen peroxide (H_2O_2) prior to the application of ICON resin infiltration (Schoppmeier, CM., et al., 2018). H_2O_2 was used to stabilize colour as well as remove the oxygen ions to enhance adhesion of ICON resin infiltration. On the other hand, Gugnani reported significant results when treated non pitted fluorosed teeth with double application of ICON resin infiltration compared to those treated with a combination technique of bleaching and ICON resin infiltration (Gugnani et al., 2017). Another study used mega-abrasion in severe cases of dental fluorosis to remove the most unaestheic superficial layer of enamel and deeper stains (200–400µm) using a high-speed hand-piece with a 105-µm fine diamond bur. This was followed by micro-abrasion and bleaching and lastly, ICON resin infiltration was applied to improve the aesthetic appearance of the teeth which produced great results (Wang Y, Sa Y, Liang S. and Jiang T, 2013). The effect of bleaching prior to ICON resin infiltration was further explained in a case report which used different approaches in three cases of stained enamel opacities (a case of traumatic dental injury and two cases of MIH). No bleaching was performed prior to ICON resin infiltration in one case while the other two cases underwent bleaching sessions prior to ICON resin infiltration. The study reported that failure to perform bleaching in the first case produced unsatisfactory results as bleaching helped in the removal of stains and ignoring this step when managing stained enamel will result in an anaesthetic outcome arguing that the colour masking of resin infiltration is related to the refractive index of the low viscosity of the material and this will not cover the brownish staining (Marouane O, Douki, N. and Chtioui, F, 2018). In another recent case of MIH, a 16 years old female patient presented with diffuse white-yellowish chalky, opaque over the maxillary and mandibular teeth were managed invasively by lithium di-silicate (E-max) crowns and veneers which produced optimal results (Alkaabi AM, Alhumaidan AA, Alqarawi FK. and Alshahrani FA, 2019). This treatment not only improved the aesthetic condition of the teeth but also their function and strengthened the structural weakness. Although ceramic materials are known to be among the excellent materials in cosmetic dentistry, the procedure requires the removal of a certain amount of tooth structure and can cause dentine sensitivity. Another drawback is the difficulty in repairing in case of failure which is also contraindicated to patients with para-functional habits such as bruxism. Since the passive eruption of maxillary central, lateral incisors, canines and mandibular canines continues until at least age 18-19 years, performing these restorations to patients below this age can compromise aesthetics in the maxillary teeth (Morrow et al., 2000).

Another case study of a 9-year-old patient with hypoplastic white spots in the anterior teeth which were a consequence of the trauma to the primary teeth and in turn affected these permanent teeth during their development was treated with a combination technique. As the patient's teeth were naturally yellow and the opacities involved both enamel and dentine, bleaching with 10% carbamide peroxide was first done to remove the yellowness and then the hypoplastic defects were removed using diamond bur under water cooling followed by restoration with composite resins (Carvalho LD. et al., 2013). This technique produced significant aesthetic results and restored both appearance and function.

Giovanni compared different treatment modalities for the management of dental fluorosis and reported resin infiltration to be more effective for the treatment of mild to moderate fluorosis followed by bleaching and micro-abrasion (Di Giovanni et al., 2018). In fact when non-pitted fluorosis was treated with ICON resin infiltration with additional application of the infiltrant, significant aesthetic results were achieved compared to normal infiltration, bleaching and a combination of bleaching and resin infiltration (Gugnani et al., 2017). However when post orthodontic white spot lesions were treated with ICON resin infiltration compared with those treated with Clinpro™ XT varnish, both ICON resin infiltration and Clinpro™ XT varnish showed significantly better improvement in mild lesions immediately after the intervention while at 6 months Clinpro™ XT varnish treated teeth showed clear improvement clinically compared to ICON resin infiltration suggesting that this further improvement was due to continued release of fluoride from the fluoroaluminosilicate glass particles (Kannan, A. and Padmanabhan, S., 2019).

ICON Resin infiltration has been reported to offer advantages over other treatment options. It masks the defect and reinforces the enamel surface hardness of the porous enamel lesion thereby improving the mechanical strength of enamel. However significant colour change was observed when bleaching was used prior to resin infiltration than when treated with either bleaching or ICON resin infiltration alone (Horuztepe SA. and Baseren M, 2017).

2.8 ICON Resin infiltration

ICON resin infiltration was developed by a company (ICON; DMG, Hamburg, Germany) as a micro-invasive technique for treating incipient caries in patients. It is a low-viscosity unfilled resin and has the advantage of masking white spot lesions through optical manipulation and without tissue removal. The resin flows into the demineralized enamel which has a similar refractive index (optical properties) to the enamel thus it reflects light to match the shade of the tooth's natural shade (Manoharan, V., et al.,

2019), (Mazur et al., 2018). The low-viscosity resin penetrates the incipient carious lesion, blocking further bacteria penetration and lesion development(Paris and Meyer-Lueckel, 2010) thereby strengthening and stabilizing the demineralized enamel. It is a micro-invasive technique which means no drilling or anaesthesia is required(Kugel et al., 2009)

The method has been widely used as a micro-invasive technique for treating white spots and demineralized early carious lesions developed during or after a course of orthodontic treatment (Ogodescu et al., 2011). It works by penetrating the porous surface of the enamel lesion thereby preventing further acid penetrating and dissolving minerals (Phark et al., 2009, Paris and Meyer-Lueckel, 2010), this results in the increase in the microhardness of the demineralized enamel (Torres et al., 2012). However, microhardness was reduced in teeth treated with bleaching prior to ICON resin infiltration compared to the teeth treated with ICON resin infiltration alone. Microhardness reduction in the bleaching-resin infiltrated teeth was further explained to be caused by the fluoride in the bleaching agent leading to mineral precipitation on the surface of the enamel blocking the penetration of the resin infiltrant (Horuztepe SA. and Baseren M, 2017). On the other hand, the highest colour change was observed in the group of teeth which received bleaching prior to ICON resin infiltration (Horuztepe SA. and Baseren M, 2017). Similarly, another recent study which reassessed the effect of ICON resin infiltraion in masking the post-orthodontic white-spot lesions for 24 months using a spectrophotometer found Significant colour change after ICON resin infiltration and this was stable for more than 24 months without changing in vivo (Knösel et al., 2019).

Reduction in shear bond strength was another problem associated with ICON resin infiltration. Significant reduction in shear bond strength of bands cemented to teeth which received ICON resin infiltration prior to cementation with glass ionomer (Elhiny et al., 2016) and this was presumed to be due to the interposing layer of resin infiltrant which disturbed the chemical bond of glass ionomer to the tooth (Skinner and Phillips, 1982). Likewise, Chay, Manton and Palamara found a decrease in mean microshear bond strength(MSBS) of resin composite to enamel when ICON resin infiltration was used as a pre-treatment however when hypomineralised (MIH)teeth were pre-treated with 5.25% sodium hypochlorite(NaOCl) followed by ICON resin infiltration, the mean MSBS reported being the highest (Chay et al., 2014) implying that the bond of composite resin to hypomineralised enamel is increased by pre-treatment with NaOCl, this was probably due to the higher protein content in MIH teeth acting as a barrier preventing infiltration of ICON resin infiltration deeper into the lesion (Chay et al., 2014).

Similarly, the method has been used in treating mild to moderate fluorosis and has proven effective. A systematic review published in 2018, evaluated and compared different interventions for the treatment of dental fluorosis. The review found that aesthetic improvement was observed for the fluorosed teeth treated with either ICON resin infiltration alone or a combination technique(bleaching with ICON resin infiltration) compared to bleaching and micro-abrasion (Di Giovanni et al., 2018). Home bleaching with ICON resin infiltration was also reported to be more effective in a recent prospective study and resulted in not only aesthetic improvement but also psychological well-being of the patients (Pan et al., 2019). Another different approach in the management of non-pitted fluorosis using ICON resin infiltration was reported by Gugnani who treated these enamel defects by increasing infiltration time (either alone or with bleaching) and found that the aesthetic results were even better compared to those treated with bleaching alone (Gugnani et al., 2017). However, when hypoplastic teeth were treated with ICON resin infiltration the colour of the lesion was not masked completely compared to fluorosed teeth. This was suggested that the stains in the hypoplastic enamel fail to allow total blending of the lesion by ICON resin infiltration due to their depth and thicker surface layer. The infiltration behaviour of these lesions is said to be similar to the inactive lesions which are difficult to infiltrate compared to active lesions for the reason that active lesions are thin and more porous (Muñoz et al., 2013).

Likewise, a study by Bhandari found successful results when Grade I MIH lesions were treated with ICON resin infiltration which showed colour alteration immediately after ICON resin infiltration. He also observed that the whiteness of MIH lesions was reduced even more after 6 months following treatment with ICON resin infiltration (Bhandari et al., 2018). Several factors have been suggested by researchers to influence Icon penetration into MIH lesions including higher protein content in these defects. MIH lesion was reported to have 8-21-fold higher protein content than sound enamel, brown enamel lesion showed 15-21-fold higher protein content while yellow and chalky enamel showed 8-fold higher protein (Farah et al., 2010b). This contributes to the sub-surface porosity and mechanical weakness exhibited in MIH lesions and can be very useful in characterizing these lesions clinically. Different types of proteins were found in these lesions, 13 among them identified in MIH lesions are found in saliva and crevicular fluid and the other 3 (haemoglobin, albumin and complement C3) are the major components of blood. This was then reported that the integrity of the enamel surface in MIH teeth was significantly influenced by the protein profiles found in these lesions. Magnum and other researchers found that intact lesions contained a 66-kDa band of albumin only whereas a 12-kDa band with the major protein content being haemoglobin was found in broken lesions; however albumin was also found but in a very low content (Mangum et al., 2010). Infiltration of ICON into these lesions has been challenging and the results

showed inconsistent and superficial infiltration as compared to carious lesions (Meyer-Lueckel and Paris, 2008). This was related to the fact that these lesions have different characteristics and mineral content which determine the penetration of ICON resin infiltrant into the lesion (Crombie et al., 2014). There are several factors have been reported to contribute to the inconsistent penetration of ICON resin infiltrant in these lesions and protein content is not the only factor to prevent complete infiltration of the ICON and that there many factors which render ICON resin infiltration difficult to MIH lesions. The authors argued and stated that if protein content was the only factor then mild MIH lesions would have infiltrated to a greater extent than severe MIH lesions which was not observed in their study. Therefore they suggested other factors such as incomplete removal of the surface layer and depth of the lesion may have played a role in impeding ICON resin infiltration (Kumar et al., 2017).

Another drawback of ICON resin infiltration is its susceptibility to staining which is reported by several studies. Triethylene glycol dimethacrylate(TEGMA) which is the main component of ICON resin infiltration has a higher coefficient of penetration into the lesion and ICON resin infiltration containing mainly TEGMA, has been found to be more discoloured when stored in staining solutions compared with other adhesive materials (Rey et al., 2014) due to its water sorption ability (Dietschi et al., 1994, Park et al., 2011, Vichi et al., 2004) which has been reported to allow fluid to penetrate and lead to discolouration of teeth treated by ICON resin infiltration. However, the use of bleaching has been reported to improve the colour of these stains (Araújo et al., 2015).

2.9 Tests used on sound and defective enamel

2.9.1 Profilometry

A profilometer is an instrument used to measure the roughness of the surface. It is composed of a Y-table, an X-Y control unit, a diamond-tipped stylus, a microcomputer with an IO interface, an A/D converter and a linear gauge sensor. The tip radius of the stylus is around 2µm and the force applied on it is usually 0.7mN. The purpose of profilometry is to get the morphology of the surface, step heights and surface roughness. The stylus profilometry has a diamond stylus probe that touches a surface and when moving the height variations are measured which are then converted to electrical signals and amplified to produce a DC(direct current) output signal (Li Y, Su X. and Zhang, H, 2008).

A study by Ersahan evaluated the effect of different treatment methods(acid etching, Er:YAG laser etching, and Nd:YAG laser etching) on enamel surface roughness using a non-contact optical profilometry and found significantly higher values in surface roughness compared to intact enamel surfaces. He also found that those teeth which received laser treatment demonstrated even higher surface roughness as compared to acid-etched surfaces (Ersahan, S. and Sabuncuoglu FA, 2016) which resulted in an increase in surface area available for bonding. The increased roughness after laser treatments was associated with laser removing some tissues leading to modification in the structure by melting or transforming inorganic substances, and expansion of organic matrix resulting in ion diffusion blockage. An in vitro study on the effect of ICON resin infiltration and other sealant types (dental adhesive and amorphous calcium phosphate (ACP) containing fluoride varnish) on enamel surfaces and their influence on bacteria adhesion. The study used a highly sensitive contact-type surface profilometer with a stylus to measure the enamel surface roughness and reported that enamel with ICON resin infiltration exhibited the lowest surface roughness and highest microhardness compared to Enamel Pro varnish(Amorphous calcium phosphate, 5% NaF) and ExciTE F adhesive bonding agent(Phosphoric acid acrylate, HEMA, dimethacrylate, highly dispersed silicon dioxide, initiators, stabilizers, potassium fluoride) whereas higher surface roughness was found in the group treated with Enamel Pro Varnish. The concentration of streptococcus mutans was reported to be the lowest on the teeth treated with Enamel Pro Varnish and lower in ICON resin infiltration treated teeth compared to the other two groups (control group and teeth treated with ExciTE F). However, as the fluoride concentration decreased in the teeth which received Enamel Pro varnish the bacterial count increased. The lower bacteria count observed on teeth receiving ICON resin infiltration was said to be related to the lower surface roughness obtained after treatment (Arslan S.et al, 2015).

2.9.2 DIAGNOdent

Caries have usually been diagnosed by using visual observation, a probe and radiographs. DIAGNOdent (KaVo, Biberach, Germany) which uses a laser diode to detect changes in the enamel density, is a revolutionary way to detect early changes before cavitation. It uses a light-emitting diode which shines on the enamel giving a digital readout. The dentist is able to use this to confirm or consider caries. When the reading is high it indicates the presence of caries and with additional diagnosis may indicate the need for some kind of intervention. In this way, it allows non-cavitated lesions to be detected early and be treated non-invasively. DIAGNOdent has been reported in several studies to have good performance

and reproducibility for the detection and quantification of occlusal and smooth surface carious lesions in vitro studies (Lussi et al., 1999, Shi et al., 2001a, Shi et al., 2000) However; when compared with visual inspection and radiographs similar accuracy was found and in fact visual inspection had shown to have better values for sensitivities for both enamels and dentin lesion (Rocha et al., 2003). The system has also been reported to have good qualitative properties in caries detection but is unable to determine the exact depth of the lesion in vivo studies (Ástvaldsdóttir et al., 2004). However, due to the possibility of false positive diagnosis by identifying sound sites as carious when using laser fluorescence (Bader and Shugars, 2004), it has been suggested to be used together with other traditional caries detection such as visual inspection and radiographs (Angnes et al., 2005). Similarly, it has been reported in a study by Farah et al who studied the relationship between laser fluorescence and enamel hypomineralization that in the absence of caries, Diagnodent reading was high in MIH teeth. This was correlated with the decrease in mineral density meaning that whenever there was a drop in the mineral content, the DIAGNOdent reading went up and so did the organic content. This has been reported to be associated with a decrease in enamel hardness and modulus of elasticity (Farah R.A., Drummond BK., Swain MV. and Williams S, 2008).

2.9.3 Micro-CT (X-ray Microtomography)

Micro-CT is a 3D imaging technique that uses X-ray to take slice by slice of the object which is analysed and can then be processed into 3D models (Du Plessis, A., Broeckhoven C, Guelpa, A. and Le Roux, SG, 2017). The advantage of Micro-CT is that it can be used to analyse the interior structure of samples and help scientists study the morphology of the sample, porosity, structure, defect analysis, density, particle size and voids.

X-ray micro-tomography has also been used in studies to measure the mineral content of MIH lesions. This measure the mineral density (MD) of these defects compared to the normal range. In sound teeth, MD increases from the cemental-enamel junction (CEJ) to the cusp/incisal tip while it is the opposite in MIH enamel as the MD decreases from CEJ to the occlusal region and then increases again at the cusp tip. The MD was reported to be approximately 19% lower in MIH than sound enamel (Farah, RA, et al, 2010). The lower the mineral density, the higher the degree of staining which reflected the severity of MIH clinically (Farah, RA, et al). Hydroxyapetite density was reported to be reduced to around 1800 mg/cm3 in subsurface enamel in developmental hypomineralized teeth when measured using quantitative micro-CT (Schnabl et al., 2019).

Although ICON resin infiltration has been reported to produce considerably good results in masking white lesions, the evidence for clinical recommendation is still not strong and a need for further studies especially randomized controlled trials (RCT) with long-term follow-up is required (Borges, AB, Caneppele, TMF, Masterson, D. and Maia, LC., 2017). In addition, there has not been any study using the range of MIH-affected teeth to investigate the effect of ICON resin infiltration and micro-abrasion followed by CPP-ACP and compare their effects on the MIH-affected teeth on the colour, surface roughness and mineral density of these teeth prior and post-treatment and also compare the effects with the sound enamel as the control.

2.10 Research aim, objectives and hypotheses

2.10.1 Aim

The aim of this study is to evaluate the effect of two treatment methods; ICON resin infiltration and micro-abrasion followed by CPP-ACP application on colour, mineral density and surface roughness of MIH-affected teeth compared to sound enamel in vitro.

2.10.2 Objectives of the study

1- To assess the surface roughness of MIH enamel lesions and sound enamel before and after treatment of the MIH enamel lesions with ICON resin infiltration and micro-abrasion followed by CPP-ACP application in vitro

2- To compare the DIAGNOdent reading of the MIH enamel lesions and sound enamel before and after treatment of the MIH enamel lesions with ICON resin infiltration and micro-abrasion followed by CPP-ACP application in vitro

3- To assess and compare the effect of ICON resin infiltration and micro-abrasion followed by CPP-ACP application on the colour of the MIH enamel lesions and compare with the sound enamel in vitro

2.10.3 Null Hypotheses

Hypothesis 1- There is no difference in surface roughness of the MIH enamel lesions following treatment with ICON resin infiltration and micro-abrasion followed by CPP-ACP application and in comparison with the sound enamel in vitro

Hypothesis 2- There is no difference in DIAGNOdent readings of the MIH enamel lesions following treatment with ICON resin infiltration and Micro-abrasion followed by CPP-ACP application and in comparison with the sound enamel in vitro

Hypothesis 3- There is no difference in the colour of the MIH enamel lesions following treatment with ICON resin infiltration and micro-abrasion followed by CPP-ACP application and in comparison with the sound enamel in vitro

3 Materials and Methods

This study was a randomised, single-blinded (examiner) study in vitro. The methodology adopted in the present study is described in this chapter including preparation of the enamel samples, their treatment and analysis as well as the materials and equipment used.

The study was conducted in the bioengineering dental laboratory at Leeds school of dentistry and Welcome Trust Brenner building. Permanent human teeth (Sound and MIH first permanent molars) were requested from the Leeds School of Dentistry tissue bank for which tissue bank application was submitted with an application form (appendix 1).This application was approved (DREC ref: 040121/SA/314) (appendix 2) and the first permanent molars (sound and MIH) were then collected from the tissue bank.

3.1 Materials and Equipment

The following materials and equipment were used to complete this study:

3.1.1 Equipment

- Accutom-10 precision cut-off machine(300-3000rpm)(Struers, Denmark)
- Digital camera(D7500 Camera, Nikon, Japan)
- Laser Fluorescence-DIAGNOdent (DIAGNOdent™ Pen, KaVo, Biberach, Germany)
- Surface Profilometer(Proscan 2200, Scantron Industrial Products Limited, Somerset, England)
- Incubator (Gallenkamp, Netherlands)
- Magnetic stirrer(serial no 20559)
- Balance (Max .210 mg)
- Skyscan 1172 (Bruker micro-CT-Berlin, Germany)
- PH meter orion (model 900A,US)
- Balance (Max. 210mg)(HM-200, A&D Instruments Ltd. Abingdon, UK)
- Water Purelab Option-S
- Slow-speed handpiece

3.1.2 Materials

- Enamel (sound and MIH) slabs from extracted human first permanent molars collected from tissue bank after approval granted

- Impression compound (Kerr red wax)
- Multi-purpose tack (WHSmith batch: 28 D 20)
- Nail varnish(Passion red colour, Max Factor, England, UK)
- Medium toothbrush(colgate)
- Artificial saliva (night time saliva for storage)
- Di-ionized water
- ICON Proximal starter Kit- 2 treatments (PFD020(kit)
- Flour of Pumice Medium Grade (JPP001(500g))
- Unident Etchant Gel (FBG700)
- Tooth Mousse Assorted Intro Pack(PHG000)[10% CPP-ACP](Recaldent™)
- Container for storing sample and solution
- Thymol 0.05%
- Soflex discs (3M™ Sof-Lex™ XT)
- Rubber cap (vsDent)

3.2 Selection of teeth

Sound and MIH first permanent molars(FPMs) were obtained from a central reserve (Leeds School of Dentistry tissue bank) into which patients had consented and donated teeth that have been extracted due to either poor long term prognosis or are being extracted for orthodontic treatment. Application for use of Tissue from the School of Dentistry Skeletal Tissues Bank was approved by ethics committee and teeth were collected. Sample size was determined based on the availability of the extracted MIH teeth in the tissue bank. Initially 30 enamel slabs were prepared consisting of 10 sound enamel and 20 MIH-affected enamel (yellow-brown lesions). 20 further slabs were added subsequently (white-creamy lesions). Teeth were visually inspected prior to their recruitment in the study to ensure their clinical diagnosis attributed to the donor patient.

3.2.1 Control (sound FPMs) group

The inclusion criteria

- Sound first permanent molars extracted

The exclusion criteria

- Teeth with deep carious lesions/cracks
- Teeth with restorations
- Teeth with dental anomalies

3.2.2 Intervention (MIH FPMs) group

The inclusion criteria

- MIH-affected FPMs with white-creamy and yellow-brown opacities

The exclusion criteria

- MIH-affected FPMs presenting caries
- MIH-affected FPMs with post-eruptive breakdown (PEB)
- MIH-affected FPMs with restorations/cracks
- Other enamel defects in differential diagnosis with MIH such as fluorosis, amelogenesis imperfecta.

The samples obtained were inspected by the supervisor prior to their preparation.

3.3 Storage and cleaning of teeth

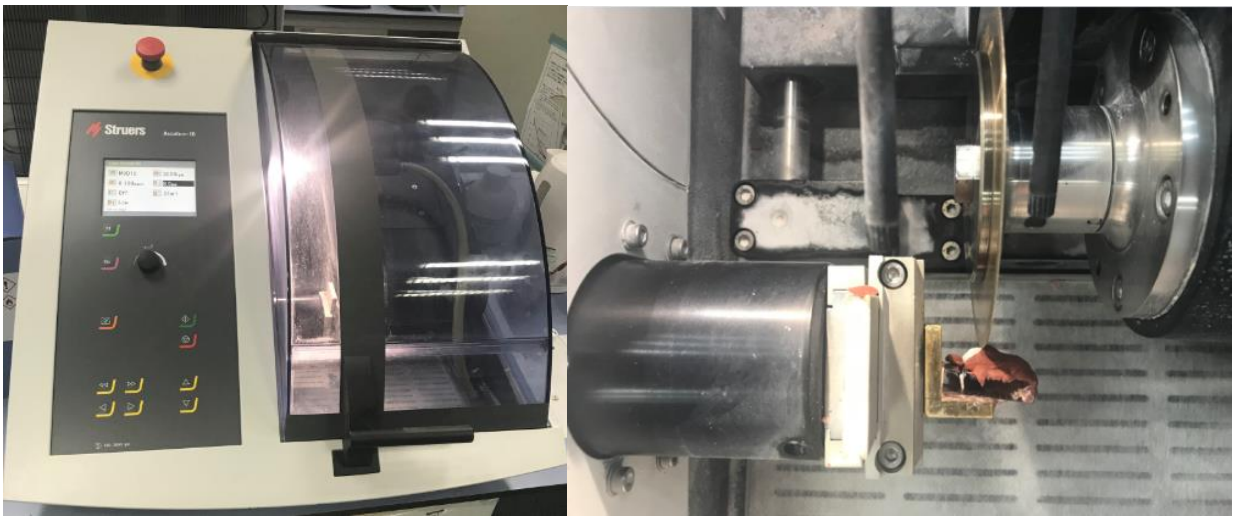
All teeth were stored in de-ionised water and 0.05% thymol at room temperature to be disinfected. Before sectioning, teeth were cleaned using a spoon excavator and a toothbrush with pumice powder to remove all soft tissue remnants. In addition they were again carefully checked by both the examiner and the supervisor for cracks, caries or restorations before their inclusion in the study. Lesions from buccal surfaces of the MIH-affected teeth were included and only one slab from each tooth was used however multiple slabs were obtained from the sound FPMs.

3.4 Preparation of enamel slabs

3.4.1 Cutting of the enamel slabs

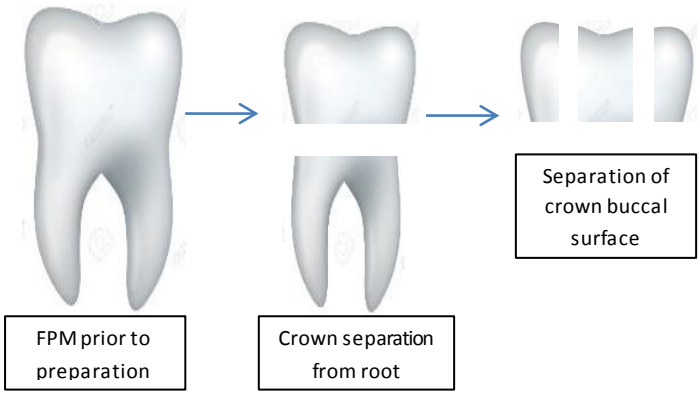
Each tooth was mounted using 'red stick' impression compound (Kerr, UK) on plates. The crowns were sectioned using "Accutom-10"cuttingg machine (Figure 1).

Figure 1: Accutom-10 cutting machine



Each tooth was de-coronated and then sectioned so buccal surfaces were obtained followed by additional sectioning to form slabs. Sectioned roots were discarded in the hazardous waste. The buccal and palatal surfaces of each crown were separated, and each buccal section was cut into three enamel slabs. The slabs with lesion were included in the study for MIH teeth whereas for the sound teeth, the middle slabs were selected. Figure 2 shows an illustration of enamel slab preparation

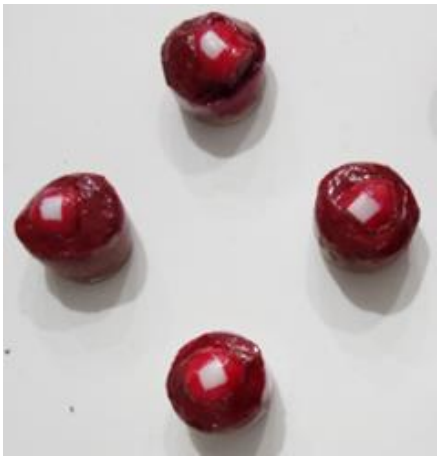
Figure 2: Illustration showing enamel slab preparation



3.4.2 Mounting of enamel slabs

To make handling of the slabs easy during treatment and analysis, the collected enamel slabs were mounted on a pipette dropper using impression compound and a "sticky wax". All samples were then coated with nail varnish (red colour, MaxFactor, England, UK); leaving a window of 3mm × 3 mm on the buccal surface (figure 3) and then they were immersed in artificial saliva (night time saliva) for 24 hours.

Figure 3: Enamel slabs after application of nail varnish



3.4.3 Storage of enamel slabs

Once the slabs had been prepared, they were kept hydrated by placing in night time artificial saliva (figure 4) for 24 hours to prevent dehydration of the slabs. The slabs were initially rinsed in de-ionised distilled water (pH 6.85±0.05) to remove traces of thymol before immersion in the artificial saliva.
The night time artificial saliva was a saturated solution that maintained the enamel condition. Its constituents are shown in table 1.

Table 1: Night time artificial saliva

Constituent	Concentration mmol/L
Calcium carbonate	0.5
Magnesium carbonate(hydrated basic)	0.225
Potassium di-hydrogen phosphate	0.5
HEPES buffer (acid form)	20.02
Potassium chloride	31.33

Figure 4: Enamel slab in night time artificial saliva

3.4.4 Slabs distribution

Several measurements were done at baseline and post treatment of the enamel slabs. Records were collected for each enamel slab using ImageJ (Fiji), DIAGNOdent (DIAGNOdent™ Pen, KaVo, Biberach, Germany), and Profilometry (Scantron Proscan 2200) whereas two slabs from each group were randomly chosen to be tested for micro-CT.

Initially, the slabs obtained from MIH teeth (intervention groups) were divided evenly according to DIAGNOdent measurements and colour of the lesions which was done visually to divide white-creamy and yellow-brown opacities evenly between groups. Later numbers were assigned to each slab and then randomized.

3.4.5 Randomisation and Blindness

Enamel slabs in intervention groups (Groups 2-5) were randomly allocated to each study group prior to their evaluation with other tests (Profilometry, image J and Micro-CT). For the slabs randomisation (https://www.randomizer.org/) was used. Four sets were generated (for groups 2-5) for 40 slabs (10 slabs for each group) and after randomization, slabs were then re-numbered.

3.4.5.1 Groups

After randomization of the slabs in the intervention groups, the slabs were grouped as follows:

3.4.5.1.1 Sound enamel

Group 1(SE): Permanent sound enamel slabs -No treatment -control group

3.4.5.1.2 White-creamy MIH lesions

Group 2(IRF): Permanent MIH enamel slabs – Treated with ICON resin Infiltration (intervention group)
Group 3(MCP): Permanent MIH enamel slabs – Treated with Micro-abrasion followed by CPP-ACP application (intervention group)

3.4.5.1.3 Yellow – brown MIH lesions

Group 4(IRF): Permanent MIH enamel slabs – Treated with ICON resin Infiltration (intervention group)
Group 5(MCP): Permanent MIH enamel slabs – Treated with Micro-abrasion followed by CPP-ACP application (intervention group)

Intervention groups 2-5 were coded (by the supervisor) 7-days following treatment of the MIH-affected enamel slabs prior to further data collection and the blindness was maintained until the end of the experiment. During scanning and data collection post-treatment (7 days, 1 month and 3 months), the investigator did not know to which group the enamel slabs belonged, making the data collection completely blind.

3.5 Test methods

Several measurements were performed and readings recorded at baseline, 7 days, 1month and 3 months collected for each enamel slab using ImageJ, DIAGNOdent pen, Profilometry whereas only 10% (two representative slabs) of the enamel slabs were randomly selected and tested for micro-CT. A summary of the tests performed at the different stages for the different groups is shown in figure 10

3.5.1 Photographic evaluation-Image J

Photographs were taken at baseline (T1) and 7-days (T2), 1-month (T3), 3-months (T4) following treatment. To standardize the photographic conditions, the camera was fixed at the same distance from the enamel slabs using camera (D7500 Camera, Nikon, Thailand), micro lens (AF105mm/1:2.8D) and flash trigger set at 1/4. The camera settings were as follows: shutter speed 1⁄160, F22, ISO 200, focal length 0.37m and Auto white balance and a photometer was used to maintain the intensity of light. The polarizing filters were equipped to minimize the light reflection.
In order to exclude the effect of moisture on the tooth colour, the enamel slabs were gently dried with a piece of gauze.

Data was extracted from photographs using Image J (Fiji) in order to assess the colour change of the MIH lesion and sound enamel at different time intervals. Colour change was analysed by changes in the different components such as lightness (L* values) and colour change (ΔE). The results of the colour obtained from Image J were quantified in terms of coordinates values L*, a * and b* established by the Commission International de l'Eclariage (CIE) (/, 1978, Joiner et al., 2008) which locates the object colour in three-dimensional colour space. From baseline photograph (T1) for every group, three readings were obtained as L1, a 1 and b1, similarly from 7-days photograph(T2), another readings were obtained as L2, a 2 and b2 and from 1-month (T3) and 3-months (T4), three readings were obtained as L3, a 3, b3 and L4, a 4, b4 respectively. Later changes in the reading of L*, a * and b*were calculated by taking the difference between L*, a * and b* values of 7-days, 1-month and 3-months with the baseline values to obtain ΔL, Δa and Δb. The difference in colour, ΔE given by Euclidean distance in CIELAB space, can be obtained by the following equation $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (Joiner et al., 2008) .

3.5.2 DIAGNOdent Reading (DD)

DIAGNOdent pen which is a battery operated version was used in this study (figure 5). The device offers a single reading on a 0 to 99 scale and operates at a wavelength of 655nm. It has been shown to strongly correlate with the older DIAGNOdent in terms of its validity and reliability (Aljehani et al., 2007). DIAGNOdent pen was used to assess the DIAGNOdent reading (DD) of MIH lesions and the sound enamel at baseline (T1) and 7-days (T2), 1-month (T3), 3-months (T4) following treatment of the MIH-affected enamel. After gently drying the samples with a piece of gauze, DIAGNOdent™ Pen, KaVo, Biberach, Germany was used to obtain the readings from the slabs. Before each reading the device was calibrated on a ceramic block according to the manufacturer's instructions and then the light emitting device was shone on each enamel slab and five readings were recorded from different area within the enamel lesion and sound enamel. Later the mean value of the readings was recorded. The device was recalibrated prior to each reading.

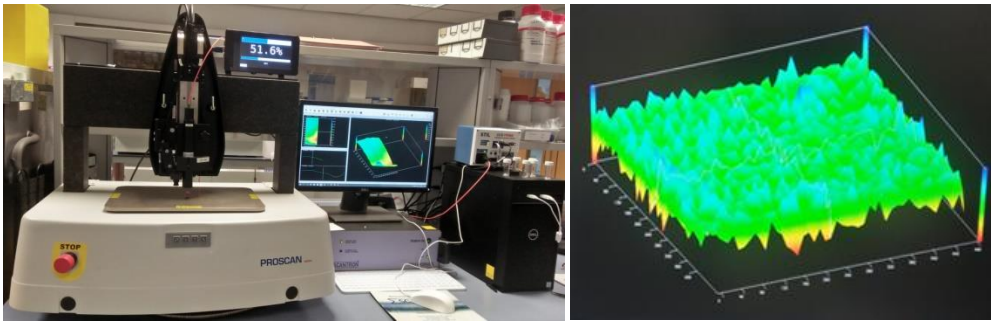
Figure 5: DIAGNOdent pen

3.5.3 Profilometry reading (Ra)

Measurements of the surface profile of the slabs were assessed using a surface profilometer (Proscan 2200, Scantron Iindustrial Products Limited, Somerset, England) before treatment (T1), 7-days (T2), 1-month (T3), and 3-months (T4) following treatment of the MIH-affected enamel. Surface roughness of sound enamel and MIH-affected enamel were recorded with a noncontact 3D optical profilometry using the arithmetic mean of the roughness profile values (Ra). The equipment gives a reading in nanometers (nm) which indicates difference between high and low points of the surface. The measurement was achieved by placing the sample on the key stage of the Scantron ProScan and using a 150 µm height of the sensor as standard. The measuring range was set to 150 µm in the Y axis and 70 µm in the X axis in

order to allow readings up to 150 µm up and 70 µm down from the start point. The resolution of the sensor was 3nm and the spot size 8 µm. A step size of 0.01 mm was used during scanning. Prior to scanning, a dark reference background check was performed each time to achieve optimum sensitivity during measurements. After scanning the reading was auto levelled, function interpolates x4 and warpage 1 to remove spikes from dust. A profile analysis where ISO Ra mean was measured (figure 6) and the measurements for mean X and Y were collected.

Figure 6: The non-contact surface Profilometry (Scantron Proscan 2200), scanned image and flat surface profile analysis for ISO Ra (Mean X and Mean Y)



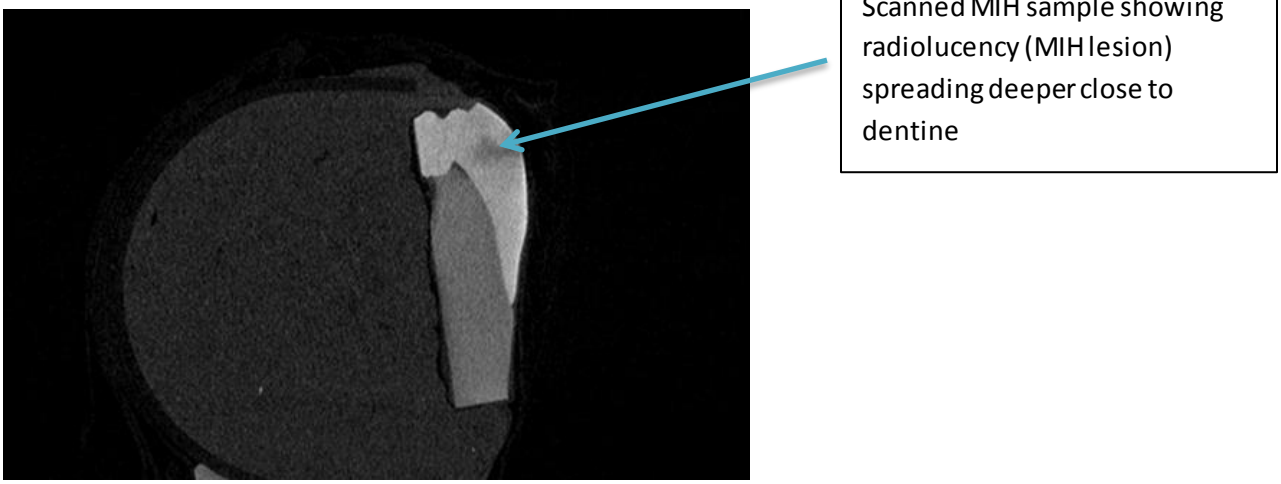
Function	Mean X	Min X	Max X	Count X	Mean Y	Min Y	Max Y	Count Y
ISO Ra	0.194	0.131	0.269	35	0.196	0.073	0.423	28
ISO Rz	0.674	0.458	0.948	35	0.582	0.216	1.355	28
ISO Rmax	0.858	0.593	1.177	35	0.822	0.231	2.047	28

3.5.4 Micro-tomography (Micro-CT)

Micro-CT was performed on two enamel slabs from each group to evaluate the mineral density before treatment (T1) and 7-days (T2), 1-month (T3), 3-months (T4) following treatment. A phantom with known densities of 0.25,0.75 and 2.9 g/cm3 was also scanned with each sample using Skyscan 1172 (Bruker-micro-CT, Berlin, Germany) with the following parameters: Voltage 100KV, source current of 100µA, image pixel size of 10.37mm, aluminium copper filter(0.5), rotation step of 0.4 over 180° rotation and frame averaging of 3. To correct the variations of the pixel sensitivity of the camera, flat field correction was used. The data was then reconstructed using NRecon software (Bruker-micro-CT, Kontich, Belgium) according to the following parameters namely BMP file type, ring artefact correction of 5% and 10% beam hardening correction. The target images (scanned at 7days, 1 and 3 months) were then positioned according to the reference image (scanned at baseline) using the

DataViewer v.1.5.6 software (Bruker-microCT) in the same way in the sagittal plane. The new database was created by removing any unnecessary background such as scans without any images, with slight images showing or only phantoms visible. Subsequently the mineral densities of the phantom were used for calibration and quantification of mineral density of enamel slabs using Image J (Fiji software). Mean mineral densities were obtained by positioning the post-treatment (7 days, 1 and 3 months) image relative to the baseline. The surface area of the determined experiment area was calculated at ten different times followed and the average mean mineral densities recorded. An example of micro-CT scanned section is shown in figure 7.

Figure 7: Micro-CT Scanned sample



3.6 Experimental protocol/regime

3.6.1 ICON Resin Infiltration (IRF)

Enamel slabs in group in group 2 and 4 were infiltrated with the low viscosity resin (ICON; DMG, Hamburg, Germany) as per the manufacturer's instructions (Mabrouk et al., 2020). The area exposed earlier with nail varnish was etched for 2 minutes using 15% hydrochloric acid gel ICON Etch, DMG. Subsequently, the etching gel was thoroughly washed for 30 seconds using a water spray and dried. Then the lesion was desiccated by applying ethanol ICON Dry DMG for 30 seconds and air-dried. Using the provided sponge applicator, ICON Infiltrant, DMG, was applied on the etched surfaces and left to work for 3 min. Excess material was removed using air from 3-in-1 and then light-cured for 60 seconds using a light emitting diode (LED) light curing unit. Again the infiltrant application was repeated,

allowed to penetrate for 1 minute to compensate for polymerisation shrinkage, and then light-cured. Following completion of treatment the slabs were returned to the night time saliva, which was changed twice daily for 7 days followed by weekly to prevent any contamination or bacterial growth. The slabs were kept in an incubator at 37.0°C at all times except during treatment (Figure 9).

3.6.2 Micro-abrasion followed by Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application (MCP)

Enamel slabs in group 3 and 5 were treated with micro-abrasion followed by 10% CPP-ACP (GC Tooth Mousse mint flavor – Recaldent ™) application as shown in figure 8. 37% phosphoric acid and pumice were mixed in equal proportion to form a homogeneous slurry (Sheoran et al., 2014). During the micro-abrasion, each slab was abraded under constant pressure using a contra-angle hand piece in slow rotation fitted with a synthetic rubber rotary application tip for 30 seconds and rinsed with distilled water for one minute. The same steps were repeated for 2 cycles and polished using all colours of soflex discs (Rodrigues et al., 2013).

After the micro-abrasion treatment, CPP-ACP was applied on the enamel slabs, left to work for 5 minutes (Sheoran et al., 2014) and returned to the night time saliva. Application of CPP-ACP was performed as described earlier twice daily, 6 hours apart for 7 days. Prior to changing the night time saliva, the containers used were rinsed with de-ionised water. This was done before returning the enamel slabs that have received the CPP-ACP treatment. The slabs were kept in an incubator at 37.0°C at all times except during treatment (figure 9). Artificial saliva was then changed every week to prevent any contamination or bacterial growth.

Figure 8: CPP-ACP application on MIH enamel slabs after micro-abrasion treatment

Figure 9: Sample placed in Incubator at 37.0°C

3.6.3 Data Collection

Following the completion of treatment, photographs were taken, DD readings collected, slabs were scanned with the profilometry and micro-CT as described earlier. These procedures were done after blinding of the intervention groups by the supervisor and then data collection was done after 7 days, 1 month and 3 months as described above in section 3.5. Figure 10 is the flow chart describing in brief the steps taken from sample collection to data collection.

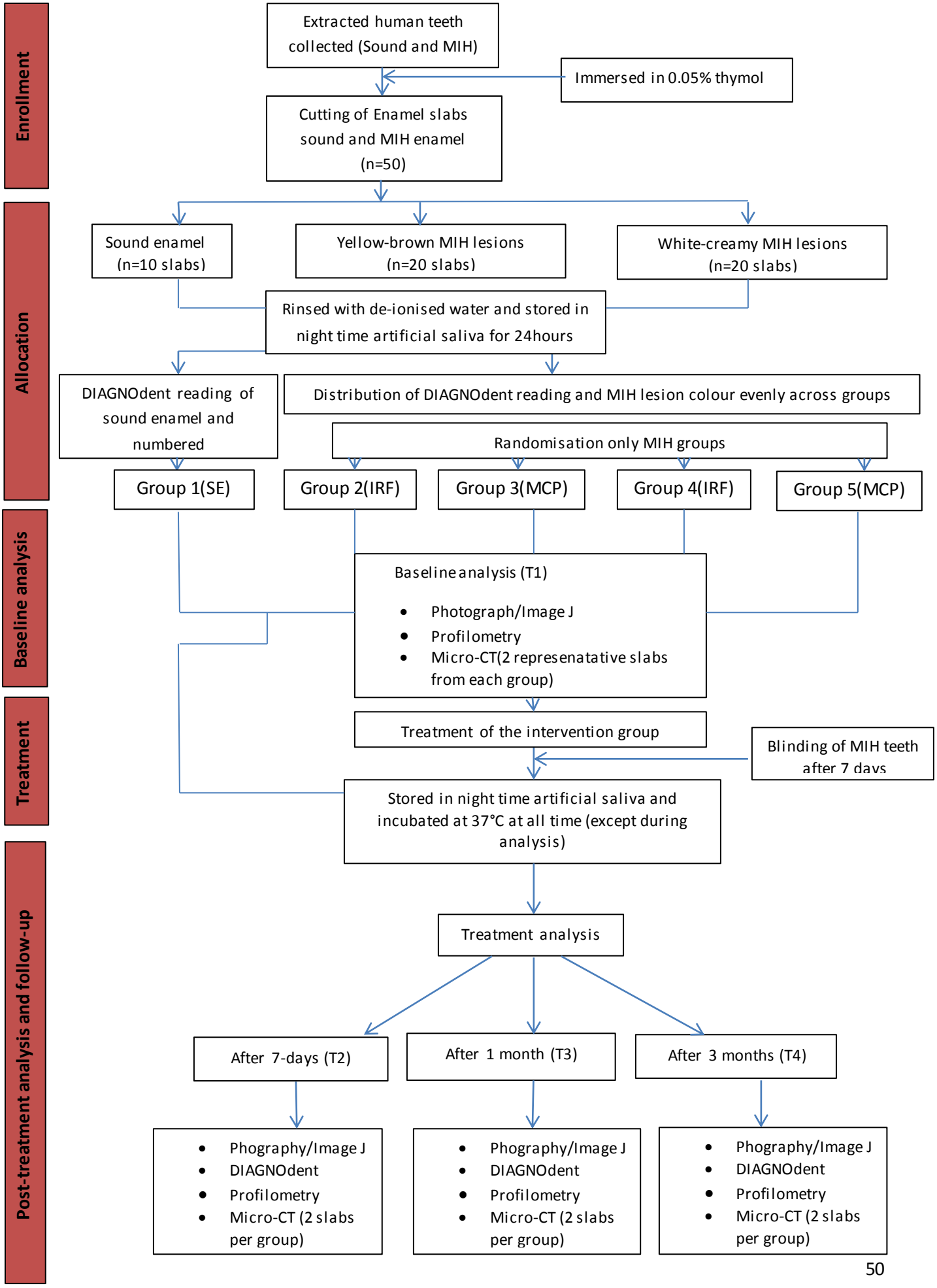


Figure 10: : In vitro procedure Flowchart

3.7 Statistical analysis

For the analysis, the data were uploaded in a SPSS Version 21. The normality of the data was tested using Shapiro-wilk test in order to proceed with the appropriate analysis. Significance level was set at 0.05 and a 95% confidence interval was reported.

Since some of the data were found to be normally distributed and some were not normally distributed, different tests were performed accordingly. Parametric tests (one-way repeated measure ANOVA test, post hoc tukey and Independent student t-test) and non-parametric tests (Friedman's ANOVA test, post hoc test and Mann-Whitney U-test) were used.

One-way ANOVA test with Bonferroni post hoc test and Friedman's ANOVA test, post hoc test w performed to assess the effect of the interventions within groups at different time intervals. On the other hand Independent student t-test and Mann-Whitney U-test were used to assess the effect and compare between groups at different time intervals.

3.8 Intra-examiner reliability

From all the enamel slabs measurements (n=50) 10 enamel slabs were chosen 2 from each group and they were reanalysed using Spearman's rho for DD (appendix 67), surface roughness(appendix 68) and colour(appendix 69)in order to allow assessment of intra-examiner reliability and found to be 0.97, 0.96 and 0.87 respectively indicating good reproducibility (Portney and Watkins, 2009)

4 Results

4.1 Overview

Overall there was a very little change in DIAGNOdent readings (DD) within the groups at different time interval. The lowest DD was seen in SE groups and the highest in both treatment protocols for yellow-brown MIH lesion compared to white-creamy MIH lesions. Surface roughness was higher in IRF for both white-creamy and yellow-brown MIH groups than in MCP groups whereas SE group exhibited the lowest surface roughness. Lightness however for IRF and MCP groups in white-creamy MIH lesions had a trend of initial significant improvement with relapse as time went where as in yellow-brown MIH lesions, lightness decreased for both IRF and MCP; IRF was more effective at masking the lightness than MCP. On the other hand, MCP was generally better at improving the colour change in both white-creamy and yellow-brown MIH lesions compared to IRF. In addition, MCP effectively masked the white-creamy lesions than yellow-brown lesions with yellow-brown lesion showed colour improvement initially and later relapsed as time went on.

4. 2 White-creamy MIH lesion versus Sound enamel [Groups: 1(SE), 2(IRF) and 3(MCP)]

4.2.1 Profilometry reading: Surface roughness (Ra-value)

4.2.1.1 Test of normality

Data was not normally distribution by the Shapiro-wilk test in group 1(SE) and group 3 (MCP) however group 2 (IRF) showed a normally distributed data. Therefore, Friedman's ANOVA test, post hoc test for pairwise comparison was used to assess the surface roughness (Ra-value) within the groups for group 1(SE) and group 3 (MCP) at different time intervals and one way repeated ANOVA and post hoc tukey test for pairwise comparison was used to assess the surface roughness within IRF group. Mann-Whitney U test was used to analyse the surface roughness between groups at different time intervals. Table 2 presents the normality for the group 1(SE), group 2(IRF) and group 3(MCP) at different time intervals.

Table 2: Normality test: surface roughness (Ra values in µm) at baseline, after 7days, 1 and 3 months for groups: 1(SE), 2(IRF) and 3(MCP)

	Groups	Shapiro-wilk		
		Statistic	df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.923	10	0.380
	MIH- Group 2 (IRF)	0.854	10	0.065
	MIH- Group 3 (MCP)	0.942	10	0.576
7-days	Sound Enamel-Group 1(SE)	0.767	10	**0.006**
	MIH- Group 2 (IRF)	0.909	10	0.272
	MIH- Group 3 (MCP)	0.816	10	**0.023**
1-month	Sound Enamel-Group 1(SE)	0.906	10	0.252
	MIH- Group 2 (IRF)	0.895	10	0.192
	MIH- Group 3 (MCP)	0.766	10	**0.006**
3-months	Sound Enamel-Group 1(SE)	0.902	10	0.229
	MIH- Group 2 (IRF)	0.926	10	0.407
	MIH- Group 3 (MCP)	0.737	10	**0.002**

4.2.1.2 Descriptive statistics

In the following table 3 are the results from descriptive statistics for enamel surface roughness (Ra in µm) for all three groups at different time intervals. Figures 11-13 are the boxplots for all groups at baseline, 7days, 1 month and 3 months.

Table 3: Descriptive statistics: surface roughness (Ra in µm) for the groups [1(SE), 2(IRF) and 3(MCP)] at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	Baseline	10	0.62	0.57	0.17	0.41	0.96	0.55	0.29
	7 days	10	0.49	0.49	0.09	0.42	0.73	0.30	0.09
	1 month	10	0.49	0.47	0.09	0.38	0.66	0.27	0.17
	3 months	10	0.59	0.61	0.17	0.31	0.77	0.46	0.34

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 2(IRF)	Baseline	10	0.85	0.79	0.32	0.52	1.61	1.09	0.36
	7 days	10	0.69	0.69	0.22	0.23	1.09	0.86	0.16
	1 month	10	0.96	0.95	0.29	0.39	1.58	1.19	0.19
	3 months	10	0.83	0.79	0.34	0.36	1.48	1.12	0.42

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 3(MCP)	Baseline	10	0.69	0.68	0.19	0.39	1.01	0.61	0.33
	7 days	10	0.51	0.45	0.21	0.29	1.01	0.72	0.21
	1 month	10	0.75	0.62	0.28	0.53	1.37	0.84	0.37
	3 months	10	0.62	0.52	0.29	0.38	1.37	0.98	0.29

Figure 11: Box plot of surface roughness (Ra) for group 1(SE)-control group

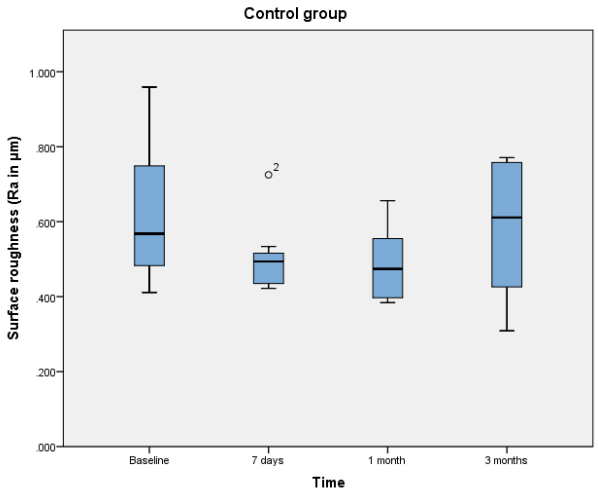
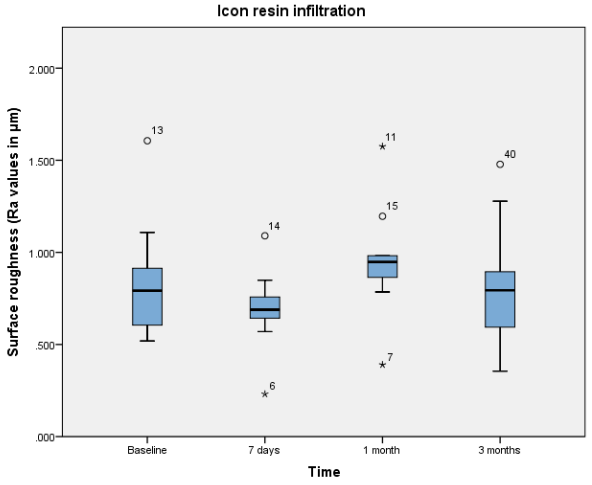


Figure 12: Box plot of surface roughness (Ra) for group 2(IRF)-ICON Resin Infiltration



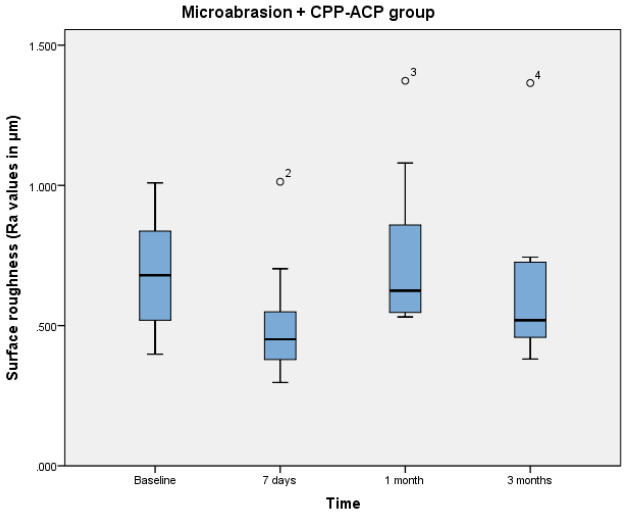


Figure 13: Box plot of surface roughness (Ra) for group 3(MCP) - Micro-abrasion followed by CPP-ACP application

4.2.1.3 Intragroup and intergroup comparison

Comparison within groups (Intragroup) on the enamel surface roughness (Ra values in µm) at baseline, 7-days, 1-month and 3 months (Table 4) were as follows:

- The median Ra values in SE group decreased gradually from baseline to 1 month followed by an increase at 3 months. However there was no statistically significant change in Ra values at all different time intervals with p>0.05(p=0.056)
- In IRF group, the mean Ra values decreased at 7 days, followed by an increase at 1 month and then reduced again at 3 months. However there was only a statistically significant increase in Ra values between 7 days and 1 month with p=0.05 (p=0.027)
- Similarly, in MCP group, the median Ra values decreased at 7 days, followed by an increase at 1 month and decreased later at 3 months. However there was only a statistically significant increase in Ra values between 7 days and 1 month with p=0.05 (p=0.006, adjusted p=0.034)
- The highest Ra values were seen in IRF group at different time intervals however the lowest Ra values were seen in MCP group at 7 days.

Table 4: Intragroup comparison: Median and Interquartile range (IQR) and statistical tests (statistics-Enamel surface roughness (Ra values in μm) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

	Ra values of Group 1(SE)	Ra values of Group 2(IRF)	Ra values of Group 3 (MCP)	Intragroup comparison		
	Median ± IQR	Mean ± SD	Median ± IQR	Group 1(SE)	Group 2(IRF)	Group 3 (MCP)
T1	0.57±0.29	0.85±0.32	0.68±0.33	T1 - T2, p=0.056	T1 - T2, p=0.564	T1 - T2, p=0.057 Adj p=0.340
T2	0.49±0.09	0.69±0.22	0.45±0.21	T2 - T3, p=0.056	T2 - T3, p=**0.027***	T2 - T3, p=**0.006*** Adj p=0.034
T3	0.47±0.17	0.96±0.29	0.62±0.37	T3 - T4, p=0.056	T3 - T4, p=1.000	T3 - T4, p=0.225 Adj p=1.000
T4	0.61±0.34	0.83±0.34	0.52±0.29	T1 - T4, p=0.056	T1 - T4, p=1.000	T1 - T4, p=0.729 Adj p=1.000

P at a significant value if p<0.05

The results of the comparison between groups (Intergroup) on the enamel surface roughness (Ra values in µm) at baseline, 7-days, 1-month and 3 months (Table 5) were:

- IRF group showed a higher Ra values compared to SE group after 7 days (p=0.041), after 1 month (p=0.010) and after 3 months (p=0.002). The overall Ra values from baseline to 3 months between the groups was also higher in IRF and was statistically significant (p=0.031).On the other hand, MCP groups showed a higher Ra values only from 1 month to 3 months which was statistically significant(p=0.008).
- Between the intervention groups, IRF group showed a statistically significant Ra values than MCP only from 7 days to 1 month with p=0.041.

Table 5: Intergroup comparison (statistics-Enamel surface roughness (Ra values in µm) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 2(IRF)	**Group 1(SE) versus Group 3(MCP)**	**Group 2(IRF) Versus Group 3(MCP)**
T1, **p=0.041***	T1, p=0.364	T1, p=0.173
T2, **p=0.010***	T2, p=0.450	T2, **p=0.041***
T3, **p=0.002***	T3, p=**0.008***	T3, p=0.070
T4**, p=0.031***	T4, p=0.705	T4, p=0.054

P at a significant value if p<0.05

4.2.2 DIAGNOdent reading: (DD)

4.2.2.1 Test of normality

Data presented normal distribution at different time intervals for the intervention [group 2(IRF) and group 3(MCP)] by the Shapiro-wilk test, however for the group 1 (SE), data was not normally distributed. Therefore the comparison of DD within groups were assessed using Friedman's ANOVA test followed by post hoc test and one way repeated ANOVA followed by bonferroni correction while intergroup comparison was performed using Mann Whitney u test and independent student t-test. Table 6 presents the normality for the three groups at different time intervals

Table 6: Normality test: DIAGNOdent reading (DD) at baseline, after 7days, 1 and 3 months for group 1(SE), group 2(IRF) and group 3(MCP)

	Groups	Shapiro-wilk		
		Statistic	df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.951	10	0.682
	MIH- Group 2 (IRF)	0.960	10	0.790
	MIH- Group 3 (MCP)	0.939	10	0.546
7-days	Sound Enamel-Group 1(SE)	0.965	10	0.837
	MIH- Group 2 (IRF)	0.881	10	0.134
	MIH- Group 3 (MCP)	0.900	10	0.219
1-month	Sound Enamel-Group 1(SE)	0.880	10	0.131
	MIH- Group 2 (IRF)	0.900	10	0.219
	MIH- Group 3 (MCP)	0.923	10	0.384
3-months	Sound Enamel-Group 1(SE)	0.830	10	**0.033**
	MIH- Group 2 (IRF)	0.918	10	0.341
	MIH- Group 3 (MCP)	0.910	10	0.284

4.2.2.2 Descriptive statistics

In the following table 7 are the results from descriptive statistics for DIAGNOdent reading (DD) for all the three groups at different time intervals.

Figures 14-16 are the boxplots for all groups at baseline, 7days, 1 month and 3 months.

Table 7: Descriptive statistics: DIAGNOdent reading (DD) for group 1(SE), group 2(IRF) and group 3(MCP) at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group1 (SE)	Baseline	10	6.30	6.20	1.26	4.60	8.60	4.00	1.85
	7 days	10	5.86	5.90	1.24	4.00	8.40	4.40	1.60
	1 month	10	6.14	6.00	1.54	4.20	9.80	5.60	1.70
	3 months	10	6.10	5.90	1.41	4.40	9.60	5.20	1.40

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 2(IRF)	Baseline	10	14.96	14.40	5.10	8.00	24.00	16.00	7.95
	7 days	10	13.02	12.10	4.12	8.20	18.60	10.40	9.10
	1 month	10	12.68	12.30	3.80	8.20	18.20	10.00	7.95
	3 months	10	13.56	12.50	5.54	7.60	25.00	17.40	8.50

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 3(MCP)	Baseline	10	14.56	14.10	6.63	6.60	27.60	21.00	11.20
	7 days	10	13.50	12.10	6.32	6.80	27.20	20.40	9.70
	1 month	10	13.66	12.20	5.78	6.60	25.60	19.00	8.80
	3 months	10	14.64	13.10	6.60	6.40	24.80	18.40	12.60

Figure 14: Box plot of DIAGNOdent reading (DD) for group 1(SE)-control group

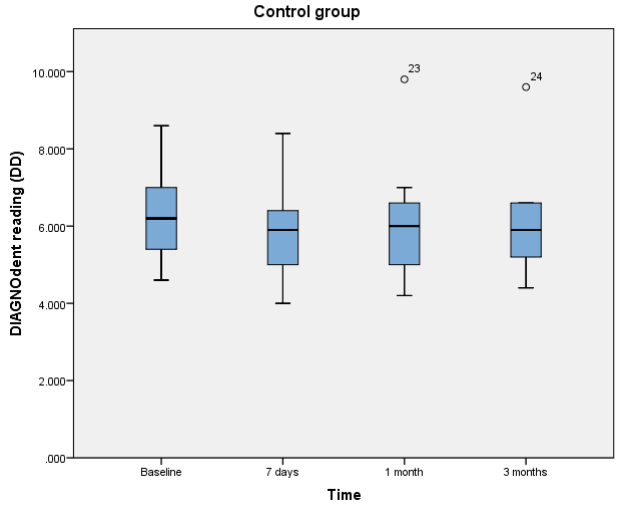
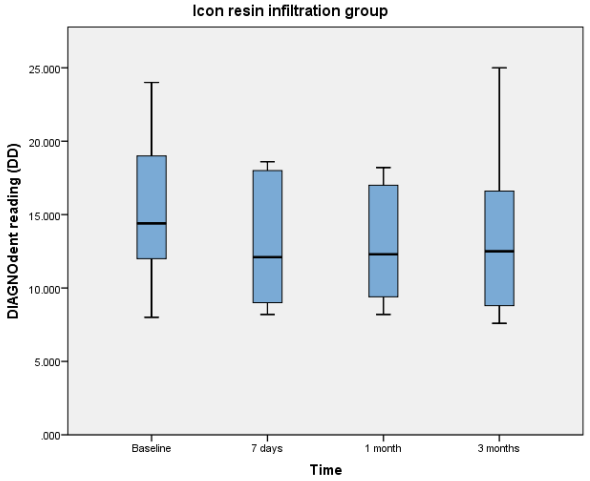


Figure 15: Box plot of DIAGNOdent reading (DD) for group 2(IRF)-ICON resin infiltration



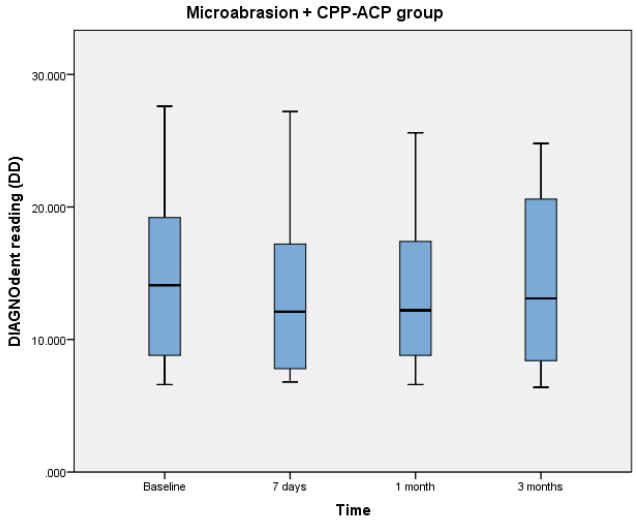


Figure 16: Box plot of DIAGNOdent reading (DD) for group 3(MCP) - Micro-abrasion followed by CPP-ACP application

4.2.2.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on the DIAGNOdent reading (DD) at baseline, 7-days, 1-month and 3 months (Table 8) showed the following:

- In SE group, the median DD decreased from baseline to 7 days followed by a slight increase at 1 month which again dropped at 3 months. The increase in DD was only significant from baseline to 7 days with p=0.002(adjusted p=0.011)
- In IRF group, the mean DD gradually decreased from baseline to 1 month and slightly increased at 3 months. However there was no statistically significant change in DD at different time intervals with p>0.05.
- On the other hand, mean DD in MCP group also decreased slightly from baseline to 7 days followed by a gradual increase up to 3 months. However there was also no statistically significant change in DD at different time intervals with p>0.05.
- The lowest DD were seen in SE group at different time intervals especially at 7 days (Mean= 5.90, SD=1.60) and 3 months (Mean= 5.90, SD=1.40) while the highest DD was seen in IRF group at baseline (Mean= 14.96, SD=5.10).

Table 8: Intragroup comparison: Mean, Median Standard deviation(SD), Interquartile range(IQR) and statistical tests (statistics- DIAGNOdent reading (DD) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

	DIAGNOdent reading (DD) of Group 1(SE)	DIAGNOdent reading (DD) of Group 2(IRF)	DIAGNOdent reading (DD) of Group 3 (MCP)	Intragroup comparison		
	Median ± IQR	Mean ± SD	Mean ± SD	Group 1(SE)	Group 2(IRF)	Group 3 (MCP)
T1	6.20±1.85	14.96±5.10	14.56±6.63	T1 - T2, p=**0.002*** Adj p= 0.011	T1 - T2, p=0.390	T1 - T2, p=1.000
T2	5.90±1.60	13.02±4.12	13.50±6.32	T2 - T3, p=0.057 Adj p=0.340	T2 - T3, p=0.675	T2 - T3, p=1.000
T3	6.00±1.70	12.68±3.80	13.66±5.78	T3 - T4, p=1.000 Adj p= 1.000	T3 - T4, p=1.000	T3 - T4, p=1.000
T4	5.90±1.40	13.56±5.54	14.64±6.60	T1 - T4, p=0.225 Adj p=1.000	T1 - T4, p=0.397	T1 - T4, p=1.000

P at a significant value if p<0.05

The results of the comparison between different groups (Intergroup) on the DIAGNOdent reading (DD) at baseline, 7-days, 1-month and 3 months (Table 9) were as follows:

- Comparing between SE and intervention groups, DD in intervention group were higher compared with the SE group and was statistically significant at different time intervals [SE versus IRF group (p=0.000) and SE versus MCP group (p=0.001)]. when intervention groups were compared, MCP group had higher DD than IRF group at 7 days, 1 month and 3 months while IRF had higher DD at baseline only however there was no statistically significant increase in DD at all-time points (p>0.05)

Table 9: Intergroup comparison (statistics: DIAGNOdent reading (DD) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 2(IRF)	**Group 1(SE) versus Group 3(MCP)**	**Group 2(IRF) Versus Group 3(MCP)**
T1, **p=0.000****	T1, **p=0.001***	T1, p=0.882
T2, **p=0.000****	T2, **p=0.001***	T2, p=0.843
T3, **p=0.000****	T3, **p=0.001***	T3, p=0.660
T4, **p=0.000****	T4, **p=0.001***	T4, p=0.697

P at a significant value if $p<0.05$

4.2.3 Photographic parameter: L* Values (Surface Lightness)

4.2.3.1 Test of normality

Data in the L* values presented a normally distribution for group 1(SE) and intervention groups [2(IRF) and 3(MCP)]. Therefore the intragroup comparison was analysed using one way repeated anova with a bonferroni correction and intergroup analysed using Independent student t test. Table 10 presents the normality for the groups at different time intervals.

Table 10: Normality test of enamel surface lightness (L* values) of different groups at baseline, 7-days, 1 month and 3 months

	Groups	Shapiro-wilk		
		Statistic	df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.925	10	0.403
	MIH- Group 2 (IRF)	0.895	10	0.194
	MIH- Group 3 (MCP)	0.958	10	0.765
Day 7	Sound Enamel-Group 1(SE)	0.906	10	0.256
	MIH- Group 2 (IRF)	0.954	10	0.722
	MIH- Group 3 (MCP)	0.965	10	0.839
1 month	Sound Enamel-Group 1(SE)	0.907	10	0.261
	MIH- Group 2 (IRF)	0.863	10	0.083
	MIH- Group 3 (MCP)	0.956	10	0.745
3 months	Sound Enamel-Group 1(SE)	0.882	10	0.137
	MIH- Group 2 (IRF)	0.921	10	0.363
	MIH- Group 3 (MCP)	0.956	10	0.745

4.2.3.2 Descriptive statistics

In the following table 11 are the results from descriptive statistics for enamel surface lightness (L* values) for the groups at different time intervals. Figures 17-19 are the boxplots for all groups baseline (T1), 7days (T2), 1-month (T3) and 3months (T4)

Table 11: Descriptive statistics: Lightness (L*values) for group 1(SE), group 2(IRF) and group 3(MCP) at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	Baseline	10	54.04	53.81	5.27	43.29	63.23	19.93	5.18
	7 days	10	40.11	39.59	4.69	34.73	47.46	12.73	8.56
	1 month	10	43.35	44.01	4.57	33.39	48.84	15.44	5.07
	3 months	10	39.61	40.55	3.96	31.45	43.52	12.07	5.65

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 2(IRF)	Baseline	10	35.45	34.83	5.59	28.59	43.02	14.43	11.91
	7 days	10	50.75	48.98	7.17	39.55	61.48	21.93	13.45
	1 month	10	43.29	40.74	6.18	34.47	53.83	19.35	10.39
	3 months	10	37.52	36.87	5.16	30.77	46.39	15.62	7.60

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 3(MCP)	Baseline	10	33.67	33.16	6.34	22.81	46.62	23.81	6.32
	7 days	10	55.37	53.78	8.15	42.49	67.66	25.18	12.14
	1 month	10	44.39	43.27	9.04	28.55	57.05	28.50	16.86
	3 months	10	36.46	36.09	7.03	23.43	48.37	24.95	7.66

Figure 17: Box plot of lightness (L* values) for group 1(SE) - control group

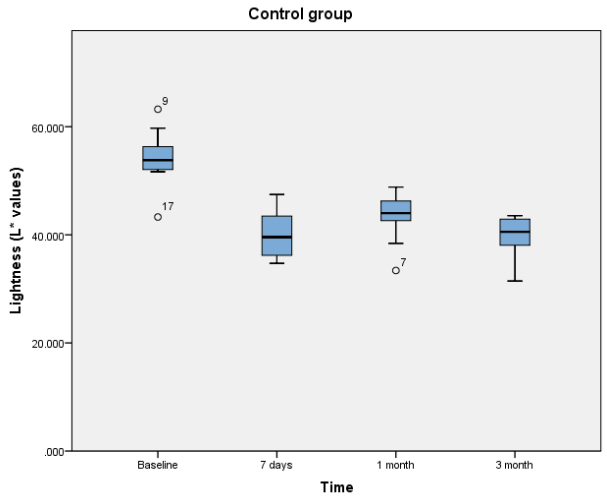
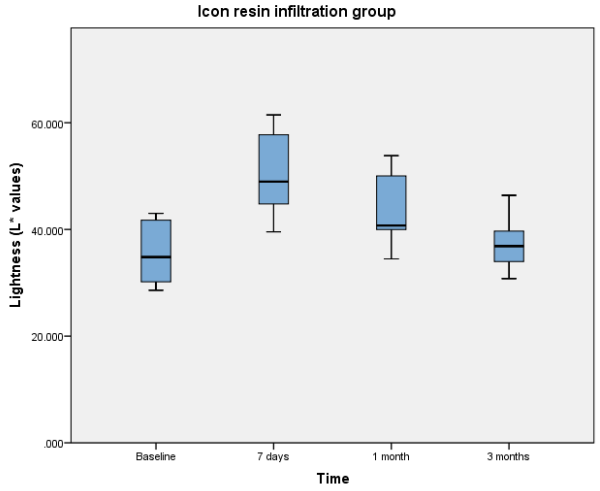


Figure 18: Figure 16: Box plot of lightness (L* values) for group 2(IRF): ICON resin infiltration



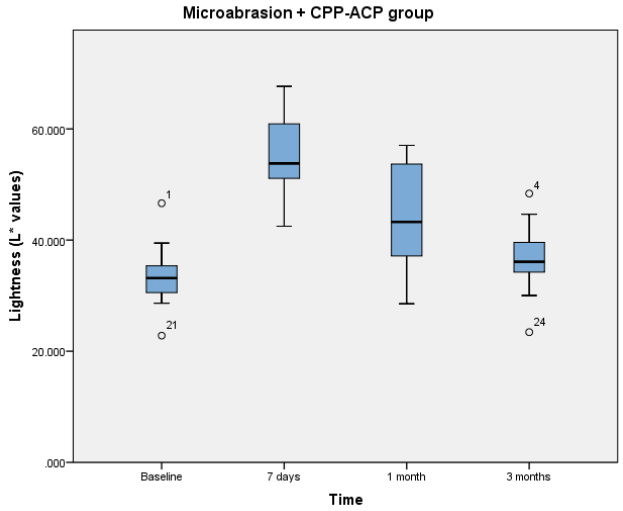


Figure 17: Box plot of lightness (L* values) for group 3(MCP) - Micro-abrasion followed by CPP-ACP application

4.2.3.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on enamel surface lightness (L* values) at baseline, 7-days, 1-month and 3 months (Table 12) showed the following:

- Mean L* values in the SE group decreased from baseline to 7 days and was statistically significant with $p<0.05$($p=0.000$). After 1 month, the mean L* value increased slightly was not statistically significant ($p=0.059$). However after 3 months, the mean L* values decreased significantly ($p=0.006$). The overall mean L* values decreased from baseline to 3 months with a significance of $p=0.000$
- In IRF group, mean L* values increased from baseline to 7 days with significance of $p=0.000$ followed by a gradual decrease at 1 month and 3 months with $p=0.001$ and $p=0.004$ respectively. Overall, mean L* values increased from baseline to 3 months (T1-T4) but this was not statistically significant($p=1.000$)
- Similarly, in MCP group, mean L* values increased from baseline to 7 days with significance of $p=0.000$ followed by a gradual decrease at 1 month with $p=0.000$ and 3 months with $p=0.002$. The. Overall, mean L* values increased from baseline to 3 months but this was not statistically significant($p=0.071$)

Table 12: Intragroup comparison: Mean, Median Standard deviation(SD), Interquartile range(IQR) and statistical tests (statistics: Enamel surface lightness (L* values) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

	L* values of Group 1(SE)	L* values of Group 2(IRF)	L* values of Group 3 (MCP)	Intragroup comparison		
	Mean ± SD	Mean ± SD	Mean ± SD	Group 1(SE)	Group 2(IRF)	Group 3 (MCP)
T1	54.04±5.27	35.45±5.59	33.67±6.34	T1 - T2, **p=0.000****	T1 - T2, **p=0.000****	T1 - T2, **p=0.000****
T2	40.11±4.69	50.75±7.17	53.78±8.15	T2 - T3, p=0.059	T2 - T3, **p=0.001***	T2 - T3, **p=0.000****
T3	43.35±4.57	43.29±6.18	44.39±9.04	T3 - T4, **p=0.006***	T3 - T4, **p=0.004***	T3 - T4, **p=0.002***
T4	39.61±3.96	37.52±5.16	36.46±7.03	T1 - T4, **p=0.000****	T1 - T4, p=1.000	T1 - T4, p=0.071

P at a significant value if $p<0.05$

The results for the comparison between different groups (Intergroup) on enamel surface lightness (L* values) at baseline, 7-days, 1-month and 3 months (Table 13) were as follows:

- There was statistically significant higher mean L* values in SE group than intervention groups at baseline (T1, p=0.000) whereas the opposite was seen at 7 days (T2) where the mean L* values were higher in the intervention groups compared to the SE group and was statistically significant with p=0.001(SE versus IRF) and p= 0.000 (SE versus MCP application).
- At 1 month (T3), mean L* values was higher in MCP than SE and IRF however at 3 months (T4), SE group had higher mean L* values compared to intervention groups but there was no statistically significant difference seen between groups at 1(T3) and 3 months (T4).

- On the other hand, mean L* values was higher in IRF group at baseline(T1) and 3 months(T3) whereas at 7 days (T2)and 1 month(T3), MCP group exhibited higher L* values than IRF group however there was not statistically significant between the intervention groups at different time intervals(p>0.05)

Table 13: Intergroup comparison (statistics-Enamel surface lightness (L* values) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 2(IRF)	**Group 1(SE) versus Group 3(MCP)**	**Group 2(IRF) Versus Group 3(MCP)**
T1, **p=0.000****	T1, **p=0.000****	T1, p=0.513
T2, **p=0.001***	T2, **p=0.000****	T2, p=0.195
T3 , p=0.984	T3, p=0.747	T3, p=0.754
T4, p=0.324	T4, p=0.142	T4, p=0.051

P at a significant value if p<0.05

4.2.4 Colour change (ΔE)

4.2.4.1 Test of normality

Data presented a normally distribution for the SE and intervention groups. Therefore the intragroup comparison was analysed using one way repeated anova followed by bonferroni comparison and intergroup analysed using Independent student t test. Table 14 presents the normality for group 1(SE), group 2(IRF) and group 3(MCP) at different time intervals.

Table 14: Normality test of a colour change (ΔE) of different groups from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

	Groups	Shapiro-Wilk		
		Statistics	Df	Sig.
ΔE_1(T1-T2)	Sound Enamel-Group 1(SE)	0.891	10	0.173
	MIH- Group 2 (IRF)	0.875	10	0.115
	MIH- Group 3 (MCP)	0.979	10	0.957
ΔE_2(T1-T3)	Sound Enamel-Group 1(SE)	0.905	10	0.246
	MIH- Group 2 (IRF)	0.897	10	0.201
	MIH- Group 3 (MCP)	0.986	10	0.990
ΔE_3(T1-T4)	Sound Enamel-Group 1(SE)	0.935	10	0.495
	MIH- Group 2 (IRF)	0.916	10	0.323
	MIH- Group 3 (MCP)	0.939	10	0.541

4.2.4.2 Descriptive statistics

In the following table 15 are the results from descriptive statistics for colour change (ΔE values) for the groups at different time intervals. Figures 20-22 are the boxplots for all groups' baseline-7 days (T1-T2), baseline-1month (T1-T3) and baseline-3months (T1-T4)

Table 15: Descriptive statistics: Colour change (ΔE- values) for group 1(SE), group 2(IRF) and group 3(MCP) from baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3). (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	ΔE_1 (T1-T2)	10	17.03	16.59	2.19	13.62	21.39	7.76	1.95
	ΔE_2 (T1-T3)	10	12.69	12.47	4.00	7.74	21.36	13.62	3.98
	ΔE_3 (T1-T4)	10	17.56	17.36	3.72	13.02	23.99	10.97	6.51

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 2(IRF)	ΔE_1 (T1-T2)	10	17.71	18.01	4.59	11.98	28.16	16.18	5.34
	ΔE_2 (T1-T3)	10	10.91	11.68	1.59	7.72	12.87	5.15	2.35
	ΔE_3 (T1-T4)	10	7.70	7.91	2.11	5.14	12.25	7.11	3.22

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group3 (MCP)	ΔE_1 (T1-T2)	10	22.33	22.21	3.52	16.56	27.81	11.25	6.07
	ΔE_2 (T1-T3)	10	11.84	11.05	4.87	3.56	19.87	16.31	7.60
	ΔE_3 (T1-T4)	10	5.61	5.56	2.32	2.83	9.87	7.04	4.03

Figure 20: Box plot of the colour change (ΔE values) for group 1(SE) - control group

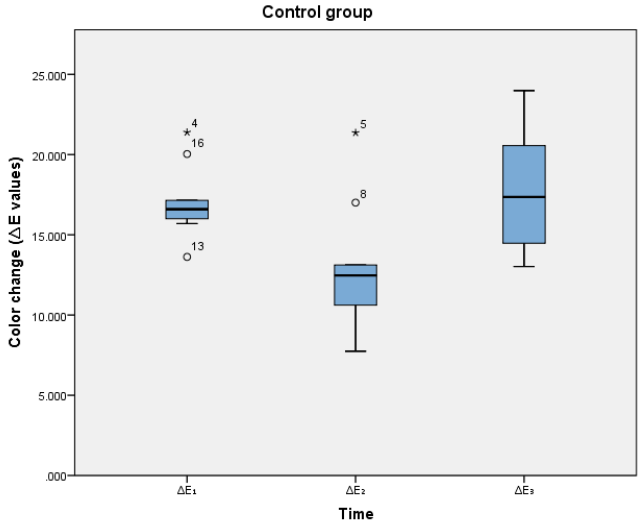
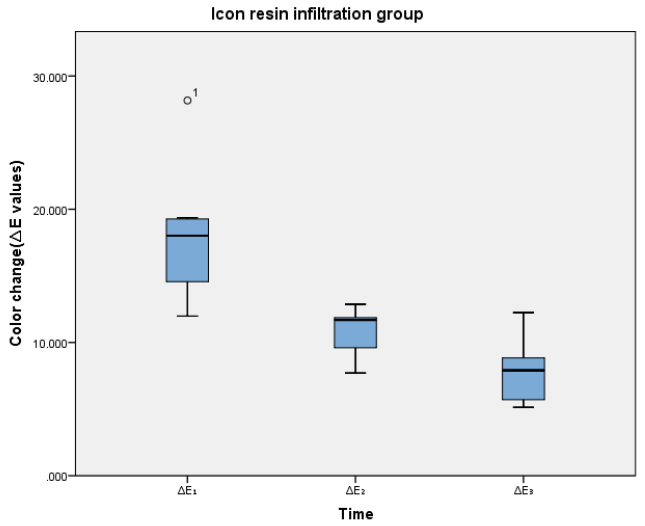


Figure 21: Box plot of the colour change (ΔE values) for group 2(IRF) - ICON resin infiltration



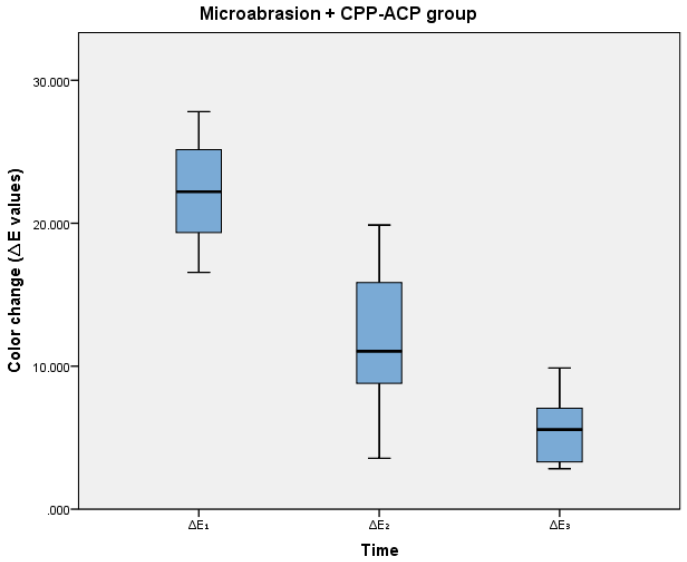


Figure 18: Box plot of the colour change (ΔE values) for group 3(MCP) - Micro-abrasion followed by CPP-ACP application

4.2.4.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on colour change (ΔE values) at baseline, 7-days, 1-month and 3 months (Table 16) showed the following:

- The mean ΔE values decreased significantly for all the groups from ΔE_1 - ΔE_2 (baseline- 7 days and baseline- 1 month) p=0.002 (SE group), p=0.002 (IRF group) and p= 0.000 (MCP group).The mean ΔE values increased from ΔE_2 - ΔE_3 (baseline-1 month and baseline- 3 months) which was also statistically significant with p=0.000. However, there no statistically significant difference (p=1.000) in the mean ΔE values from ΔE_1 - ΔE_3 (baseline to 7 days and baseline to 3 months) for the SE group.
- On the other hand, the mean ΔE values decreased significantly for both the intervention groups from ΔE_2 - ΔE_3 (baseline-1 month and baseline- 3 months) with p= 0.004 for IRF group and p=0.003 for MCP. There was also statistically significant decreased in the mean ΔE values for the intervention groups from ΔE_1 - ΔE_3 (baseline to 7 days and baseline to 3 months) with p=0.001 for IRF and p=0.000 for MCP.
- The significant decrease in the mean ΔE values from baseline to 3 months was seen in MCP group (74.9%) followed by IRF group (56.5%). However SE group showed a very slight increase in the mean ΔE values from baseline to 3 month (3%)

Table 16: Intragroup comparison: Mean, Standard deviation(SD) and statistical tests (statistics- colour change(ΔE values)for group 1(SE), group 2(IRF) and group 3(MCP) baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3).

	ΔE of Group 1(SE)	**ΔE of Group 2(IRF)**	**ΔE of Group 3 (MCP)**	**Intragroup comparison**		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Group 1(SE)**	**Group 2(IRF)**	**Group 3 (MCP)**
ΔE_1	17.03±2.19	17.71±4.59	22.33±3.52	ΔE_1 - ΔE_2, **p=0.002***	ΔE_1 - ΔE_2, **p=0.002***	ΔE_1 - ΔE_2, **p=0.000****
ΔE_2	12.69±4.00	10.91±1.59	11.84±4.87	ΔE_2 - ΔE_3, **p=0.000****	ΔE_2 - ΔE_3, **p=0.004***	ΔE_2 - ΔE_3, **p=0.003***
ΔE_3	17.56±3.72	7.70±2.11	5.61±2.32	ΔE_1 - ΔE_3, p=1.000	ΔE_1 - ΔE_3, **p=0.001***	ΔE_1 - ΔE_3, **p=0.000****

P at a significant value if p<0.05

The results of the comparison between groups (Intergroup) on colour change (ΔE values) for group 1(SE), group 2(IRF) and group 3(MCP) from baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3) (Table 17)

- IRF group showed higher mean ΔE values than SE group from baseline to 7 days(ΔE_1) however this was no statistically significant with p=0.676. However the opposite was observed from baseline- 1 month(ΔE_2) and baseline- 3 months (ΔE_3) where SE group had higher mean ΔE values than IRF and was only statistically significant from baseline – 3months(ΔE_3) p=0.000
- The mean ΔE value for MCP group was higher than the SE group from baseline to 7 days (ΔE_1) with significance p= 0.001. On the other hand mean ΔE values for SE group were higher than the MCP from baseline-1 month (ΔE_2) and baseline-3 months (ΔE_3), however the mean ΔE value was only statistically significant between the groups from baseline-3 months (ΔE_3) with p=0.000 .Intergroup comparison between intervention groups showed a statistically significant higher mean ΔE value in MCP than IRF group from baseline to 7 days with p=0.021. Figure 23 are the photographic images of the sound enamel at different time intervals. Figure 24 are the photographs of the MIH-affected sample before and after treatment with IRF illustrating the colour of the white-creamy MIH lesion partially masked after 3

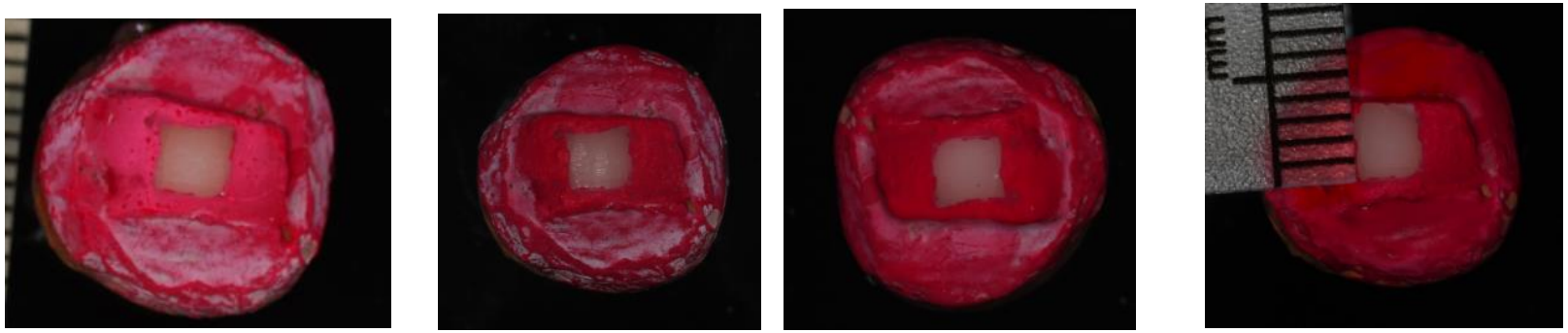
months and in figure 25 are the photographs of the MIH-affected sample before and after treatment with MCP illustrating the colour masking of white-creamy MIH lesion (almost completely masked after 3 months)

Table 17: Intergroup comparison: statistics colour change (ΔE value) of different groups from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 2(IRF)	**Group 1(SE) versus Group 3(MCP)**	**Group 2(IRF) Versus Group 3(MCP)**
ΔE_1 (T1-T2), p=0.676	ΔE_1 (T1-T2), **p=0.001***	ΔE_1 (T1-T2), **p=0.021***
ΔE_2 (T1-T3), p=0.208	ΔE_2 (T1-T3), p=0.674	ΔE_2 (T1-T3), p=0.579
ΔE_3 (T1-T4), **p=0.000****	ΔE_3 (T1-T4), **p=0.000****	ΔE_3 (T1-T4), **p=0.049***

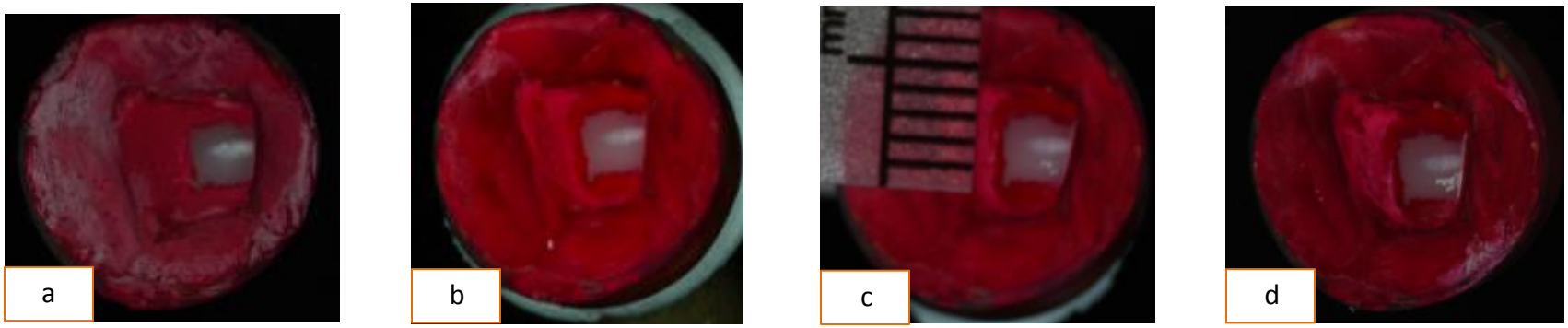
P at a significant value if p<0.05

Figure 19: Photographs of the sound enamel at different time intervals (baseline, 7 days, 1 month and 3 months)



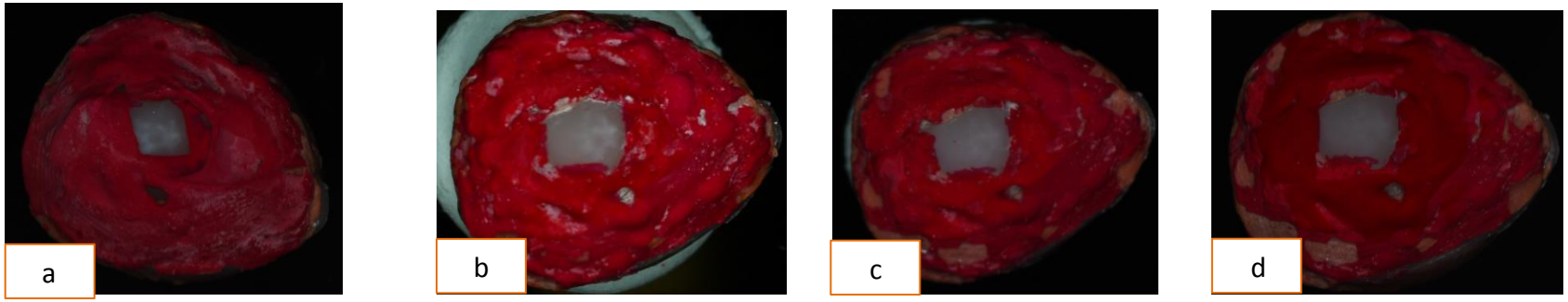
(a): Baseline (T1) **(b):** 7 days (T2) **(c):** 1 month (T3) **(d):** 3 months (T4)

Figure 20: Photograph of hypomineralised enamel (white-creamy MIH lesion) before and after treatment with IRF



(a): Baseline (T1) **(b):** 7 days (T2) **(c):** 1 month (T3) **(d):** 3 months (T4)

Figure 21: Photograph of hypomineralised enamel (white-creamy MIH lesion) before and after treatment with MCP



(a): Baseline (T1) **(b):** 7 days (T2) **(c):** 1 month (T3) **(d):** 3 months (T4)

4.2.5 Micro-CT readings (Mineral densities-MD in g/cm3)

4.2.5.1 Descriptive statistics for all groups

Table 18 shows the mean and standard deviation of mineral densities for only two samples from group 1(SE), group 2(IRF) and group 3(MCP). The mean mineral densities were the highest for SE group and the lowest for MCP.

Table 18: Descriptive statistics Mean Mineral densities (MD in g/cm3) of different groups at baseline, 7-days, 1 month and 3 months (N=2 for each group)

	Samples	Baseline		7 days		1 month		3 months	
		Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD
Sound Enamel- Group 1(SE)	1	2.535	0.312	2.429	0.261	2.398	0.246	2.406	0.251
	2	2.226	0.232	2.292	0.279	2.362	0.247	2.414	0.242
MIH- Group 2 (IRF)	21	2.364	0.322	2.282	0.247	1.310	0.173	1.918	0.266
	24	2.298	0.206	2.402	0.176	1.676	0.123	2.437	0.161
MIH- Group 3 (MCP)	22	2.195	0.251	2.169	0.339	1.506	0.112	2.283	0.161
	23	2.491	0.268	2.374	0.223	2.406	0.257	2.463	0.200

For the SE group, mean mineral density in sample 1 decreased gradually from baseline to 1 month (4.2% from baseline to 7 days and 1.3% from 7 days to 1 month) followed by a very slightly increase of 0.3% at 3 months. Overall, there was a decrease of 5 % mineral density from baseline to 3 months. On the other hand, mean mineral density in sample 2 increased gradually at different time intervals (2.9% from baseline to 7 days, 3% from 7 days to 1 month and 2.2% from 1 month to 3 months). Overall, mean mineral density increased from baseline to 3 months was 7.8%.

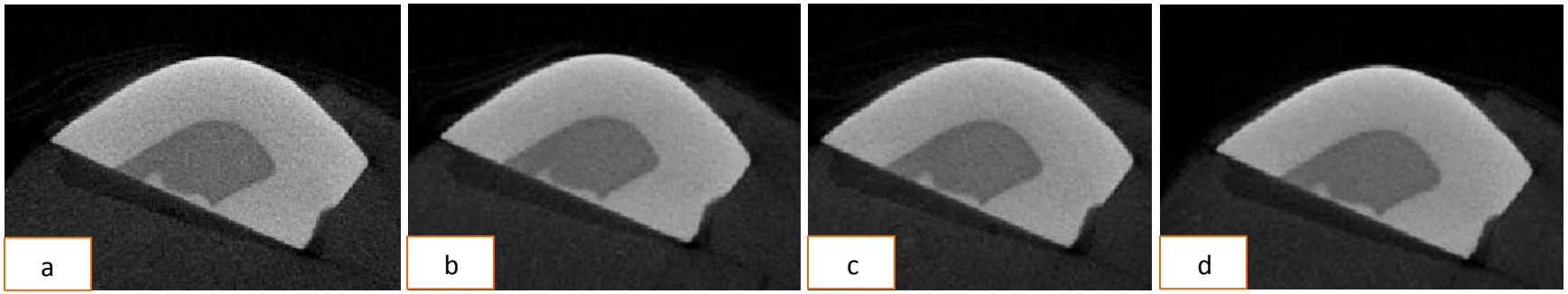
Mean mineral densities in the IRF group (sample 21)decreased slightly from baseline to 7 days by 3.5% followed by a further decrease after 1 month by 42.6% which later increased by 31.7% at 3 months. Overall, there was a decrease of 18.9% in mean mineral density from baseline to 3 months post treatment. On the other hand in sample 24, mean mineral density increased slightly from baseline to 7 days by 4.3% followed by a

decrease of 30.2% in mean mineral density after 1 month. Later at 3 months, there was an increase of 31.2% in mean mineral density. Overall there was 5.7% increase in mean mineral density from baseline to 3 months.

Sample 22 in MCP group, mean mineral density also decreased gradually from baseline to 1 month and was significant decrease of 30.6% from 7 days to 1 month with a later increase of 30% after 3 months. Overall there was an increase of 3.9% mean mineral density from baseline to 3 months. Similarly, sample 23 decreased by 4.7% after 7 days followed by a gradual increase up to 3 month by 3.6%. Overall the re was a decrease of 1.1% mean mineral density from baseline to 3 months.

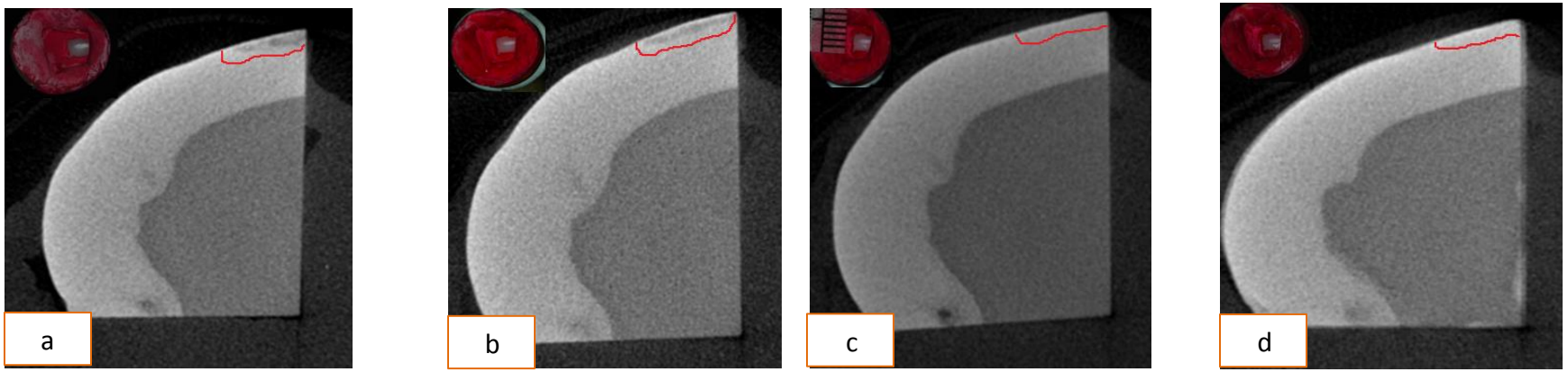
Comparing the mineral densities within the intervention groups, sample 23 from MCP group had the highest mean mineral density at baseline while sample 21 from IRF group had the lowest mean mineral density at 1 month. Figure 26 is the micro-CT image of the sample from sound enamel at different time intervals and figure 27 and 28 are the micro-CT images of the MIH samples before and after IRF and MCP treatment respectively. The region of interest are illustrated by the outline in figure 27 which show the radiolucency (MIH lesion) reduces overtime following IRF treatment and arrows in figure 28 show the region of interest before and after MCP treatment.

Figure 22: Micro-CT images of a sample (sound/SE) at baseline, 7 days, 1 month and 3 months



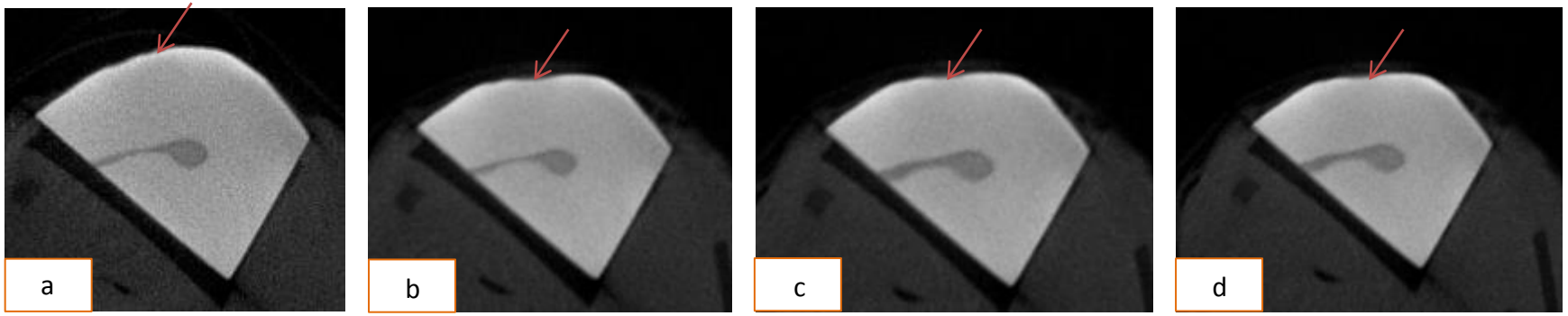
a) Baseline (T1) MD: 2.535 **g/cm3** (b) 7 days (T2) MD: 2.429 **g/cm3** (c) 1 month (T3) MD: 2.398 **g/cm3** (d) 3 months (T4) MD: 2.406 **g/cm3**

Figure 23: Micro-CT image of MIH-affected enamel sample (white-creamy lesion) before and after IRF treatment



(a) Baseline (T1) MD: 2.298 g/cm3 g/cm3 **(b)** 7 days (T2) MD: 2.402 g/cm3 **(c)** 1 month (T3) MD: 1.676 g/cm3 **(d)** 3 months (T4) MD: 2.437

Figure 24: Figure 28: Micro-CT image of MIH-affected enamel sample (white-creamy lesion) before and after MCP treatment



(a) Baseline (T1) MD: 2.195 g/cm3] **(b)** 7 days (T2) MD: 2.169 g/cm3] **(c)** 1 month (T3) MD: 1.506 g/cm3 **(d)** 3 months (T4) MD: 2.283 g/cm3

4.3 Yellow-brown MIH lesion versus Sound enamel [Groups: 1(SE), 4(IRF) and 5(MCP)]

4.3.1 Profilometry reading: Surface roughness (Ra in µm)

4.3.1.1 Test of normality

Data was not normally distributed by the Shapiro-wilk test in all groups. Since most of the data showed a not-normal distribution, Friedman's ANOVA test, post hoc test was used to assess the surface roughness (Ra in µm) within the groups at different time intervals while Mann-Whitney U test was used to analyse the surface roughness(Ra in µm) between groups at different time intervals. Table 19 presents the normality for the three groups at different time intervals.

Table 19: Normality test: Surface roughness (Ra in µm) at baseline, after 7days, 1 and 3 months for group 1(SE), group 4(IRF) and group 5(MCP)

Days	Groups	Shapiro-Wilk test		
		Statistic	df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.923	10	0.380
	MIH- Group 4 (IRF)	0.862	10	0.081
	MIH- Group 5 (MCP)	0.659	10	**0.000**
Day 7	Sound Enamel-Group 1(SE)	0.767	10	**0.006**
	MIH- Group 4 (IRF)	0.967	10	0.864
	MIH- Group 5 (MCP)	0.922	10	0.370
1 month	Sound Enamel-Group 1(SE)	0.906	10	0.252
	MIH- Group 4 (IRF)	0659	10	**0.000**
	MIH- Group 5 (MCP)	0.915	10	0.320
3 months	Sound Enamel-Group 1(SE)	0.902	10	0.229
	MIH- Group 4 (IRF)	0.678	10	**0.000**
	MIH- Group 5 (MCP)	0.854	10	0.065

4.3.1.2 Descriptive statistics

In the following table 20 are the results from descriptive statistics for surface roughness (Ra in µm) for the three groups at different time intervals. Figures 29-31 are the boxplots for all groups at baseline, 7days, 1 month and 3 months.

Table 20: Descriptive statistics: Surface roughness (Ra in µm) for group 1(SE), group 4(IRF) and group 5(MCP) at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1 (SE)	Baseline	10	0.62	0.57	0.17	0.41	0.96	0.55	0.29
	7 days	10	0.49	0.49	0.09	0.42	0.73	0.30	0.09
	1 month	10	0.49	0.47	0.09	0.38	0.66	0.27	0.17
	3 months	10	0.59	0.61	0.17	0.31	0.77	0.46	0.34

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 4(IRF)	Baseline	10	0.74	0.62	0.25	0.44	1.11	0.68	0.48
	7 days	10	0.96	0.96	0.24	0.63	1.42	0.79	0.35
	1 month	10	1.03	0.89	0.58	0.61	2.59	1.99	0.41
	3 months	10	0.92	0.81	0.43	0.56	2.08	1.52	0.24

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 5(MCP)	Baseline	10	0.74	0.58	0.44	0.44	1.91	1.47	0.32
	7 days	10	0.89	0.86	0.27	0.52	1.33	0.82	0.45
	1 month	10	0.79	0.80	0.16	0.43	0.99	0.57	0.17
	3 months	10	0.68	0.72	0.11	0.52	0.79	0.28	0.21

Figure 29: Box plot of surface roughness (Ra) for group 1(SE) - control

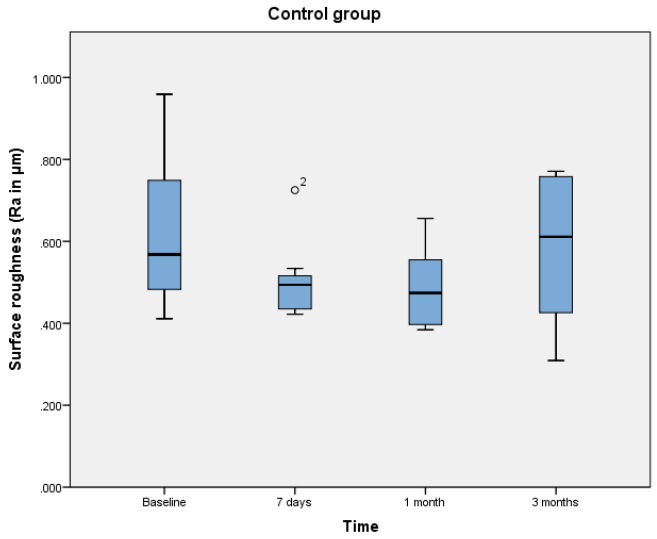
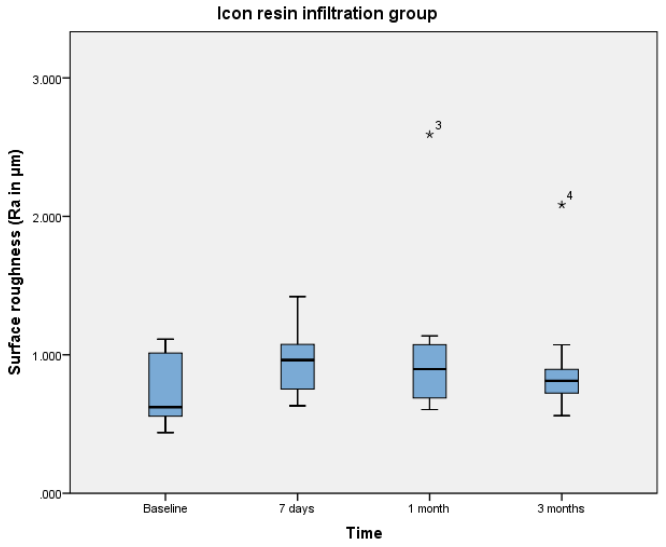


Figure 30: Box plot of surface roughness (Ra) for group 4(IRF) - ICON resin infiltration



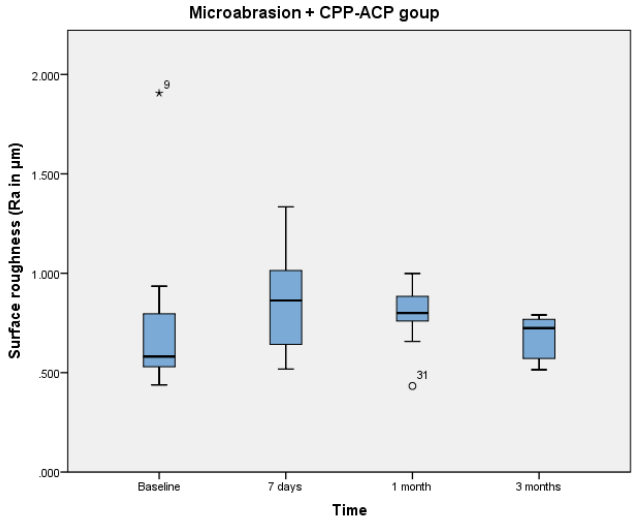


Figure 25: Box plot of surface roughness (Ra) for group 5(MCP) - Micro-abrasion followed by CPP-ACP application

4.3.1.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on surface roughness (Ra values) at baseline, 7-days, 1-month and 3 months (Table 21) showed the following:

- The median Ra values in SE group decreased gradually from baseline to 1 month and later increased after 3 months. However there was no statistically significant change in Ra values at all different time intervals with $p>0.05$($p=0.056$)
- The median Ra values in both the intervention groups increased from baseline to 7 days. Later the Ra values decreased gradually for IRF and MCP group from 7days to 3 months. Median Ra values were not statistically significant in IRF However, in MCP group, the decrease in median Ra values were statistically significant from 1 month to 3 months with $p<0.05$ ($p=0.024$)

Table 21: Intragroup comparison: Median and Interquartile range (IQR) and statistical tests: surface roughness (Ra values in µm) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

	Ra values of Group 1(SE)	Ra values of Group 4(IRF)	Ra values of Group 5 (MCP)	Intragroup comparison		
	Median ± IQR	Median ± IQR	Median ± IQR	Group 1(SE)	Group 4(IRF)	Group 5 (MCP)
T1	0.57±0.29	0.62±0.48	0.58±0.32	T1 - T2, p=0.056	T1 - T2, p=0.229	T1 - T2, p=0.057 Adj p=0.340
T2	0.49±0.09	0.96±0.35	0.86±0.45	T2 - T3, p=0.056	T2 - T3, p=0.229	T2 - T3, p=1.000
T3	0.47±0.17	0.89±0.41	0.80±0.17	T3 - T4, p=0.056	T3 - T4, p=0.229	**T3 - T4, p=0.024*** Adj p=0.146
T4	0.61±0.34	0.81±0.24	0.72±0.21	T1 - T4, p=0.056	T1 - T4, p=0.229	T1 - T4, p=0.729 Adj p=1.000

P at a significant value if p<0.05

The results of the comparison between groups (Intergroup) on surface roughness (Ra in µm) at baseline, 7-days, 1-month and 3 months (Table 22):

- IRF showed a statistically significant increase in Ra values from 7 days to 1 month (p=0.000), from 1 month to 3 months (p=0.000) and from baseline to 3 months (p=0.010) than SE group. Comparing between SE and MCP, MCP showed statistically significant increase in Ra values from 7 days to 1 month (p=0.001) and 1 month to 3 months (p=0.001) only.
- Between the intervention groups, IRF group showed a statistically significant overall increase in Ra values from baseline to 3 months than MCP with p=0.038

Table 22: Intergroup comparison (statistics-surface roughness (Ra in µm) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 4(IRF)	**Group 1(SE) versus Group 5(MCP)**	**Group 4(IRF) Versus Group 5(MCP)**
T1, p=0.226	T1, p=0.762	T1, p=0.384
T2, **p=0.000****	T2, **p=0.001***	T2, p=0.545
T3, **p=0.000****	T3, **p=0.001***	T3, p=0.364
T4, **p=0.010***	T4, p=0.273	T4, **p=0.038***

P at a significant value if p<0.05

4.3.2 DIAGNOdent reading: (DD)

4.3.2.1 Test of normality

Data presented normal distribution at different time intervals for the intervention groups [4(IRF) and 5(MCP) groups] by the Shapiro-wilk test, however for the group 1 (SE), data was not normally distributed. Therefore the comparison of DD within groups was assessed using Friedman's ANOVA test followed by post hoc test for pairwise comparison and One way repeated ANOVA followed by bonferroni correction while intergroup comparison was performed using Mann whitney u test and independent student t-test. Table 23 presents the normality for the groups at different time intervals.

Table 23: Normality test: DIAGNOdent reading (DD) of different groups at baseline, after 7days, 1 and 3 months

Day	Groups	Shapiro-wilk		
		Statistic	df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.951	10	0.682
	MIH- Group 4 (IRF)	0.877	10	0.121
	MIH- Group 5 (MCP)	0.949	10	0.657
Day 7	Sound Enamel-Group 1(SE)	0.965	10	0.837
	MIH- Group 4 (IRF)	0.873	10	0.108
	MIH- Group 5 (MCP)	0.934	10	0.485
1 month	Sound Enamel-Group 1(SE)	0.880	10	0.131
	MIH- Group 4 (IRF)	0.877	10	0.121
	MIH- Group 5 (MCP)	0.967	10	0.860
3 months	Sound Enamel-Group 1(SE)	0.830	10	**0.033**
	MIH- Group 4 (IRF)	0.880	10	0.132
	MIH- Group 5 (MCP)	0.956	10	0.737

4.3.2.2 Descriptive statistics

In the following table 24 are the results from descriptive statistics for DD for the groups at different time intervals. Figure 32-34 are the boxplots for groups at baseline, 7days, 1 month and 3 months.

Table 24: Descriptive statistics: DIAGNOdent reading (DD) for group 1(SE), group 4(IRF) and group 5(MCP) at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	Baseline	10	6.30	6.20	1.26	4.60	8.60	4.00	1.85
	7 days	10	5.86	5.90	1.24	4.00	8.40	4.40	1.60
	1 month	10	6.14	6.00	1.54	4.20	9.80	5.60	1.70
	3 months	10	6.10	5.90	1.41	4.40	9.60	5.20	1.40

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 4(IRF)	Baseline	10	46.72	33.00	25.63	16.00	91.40	75.40	41.30
	7 days	10	46.44	50.50	22.81	18.20	74.40	56.20	46.20
	1 month	10	45.12	41.80	21.79	20.00	77.00	57.00	44.10
	3 months	10	46.66	42.50	21.14	22.40	80.60	58.20	37.40

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 5(MCP)	Baseline	10	57.46	51.60	23.89	22.00	92.00	70.00	37.40
	7 days	10	57.48	61.40	22.70	25.00	91.60	66.60	39.00
	1 month	10	59.68	59.00	19.43	29.00	89.60	60.60	31.75
	3 months	10	58.80	62.80	18.23	28.00	88.00	60.00	29.25

Figure 32: Box plot of DIAGNOdent (DD) for group 1(SE)-Control group

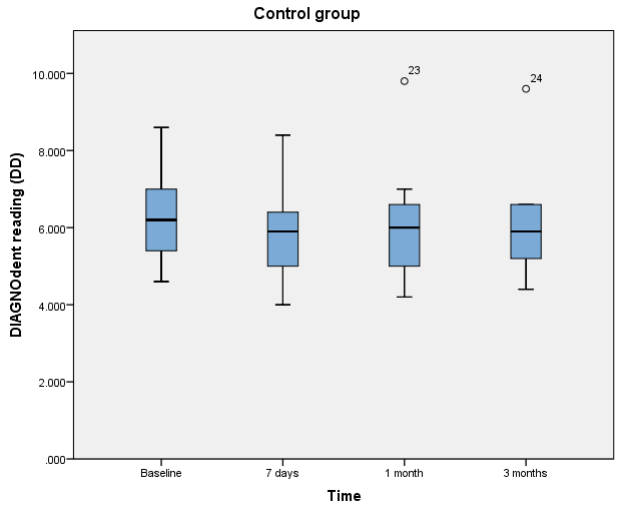
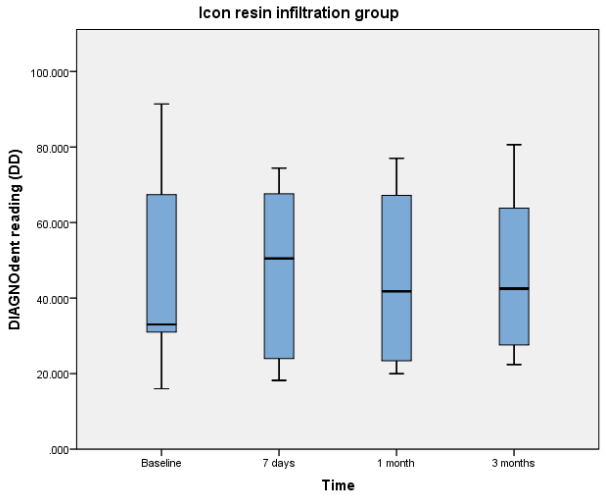


Figure 33: Box plot of DIAGNOdent (DD) for group 4(IRF)-ICON resin infiltration



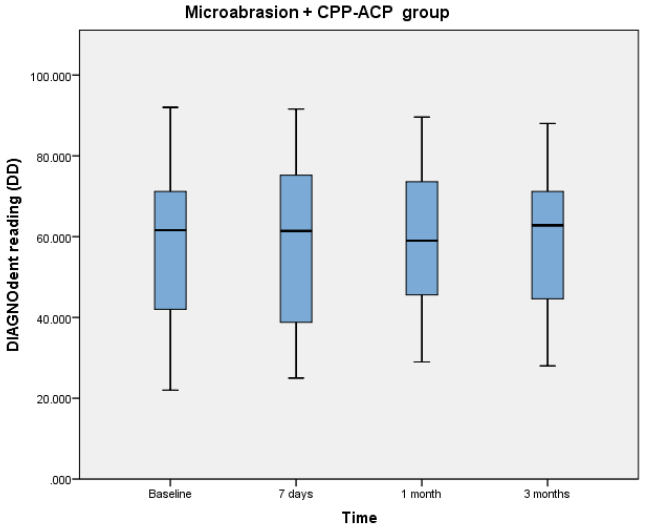


Figure 26: Box plot of DIAGNOdent (DD) for group 5(MCP) -Micro-abrasion followed by CPP-ACP application

4.3.2.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on DIAGNOdent reading (DD) at baseline, 7-days, 1-month and 3 months (Table 25) showed the following:

- In SE group, the median DD decreased from baseline to 7 days and later increased slightly at 1 month followed by another decrease at 3 months. The increase in DD was only significant from baseline to 7 days with p=0.002(adjusted p=0.011)
- In IRF group, the mean DD decreased gradually from baseline to 1 month. After 3 months, the DD increased slightly. However there was no significant change in DD at all-time points with p=1.000
- On the other hand, in MCP group, the mean DD increased gradually from baseline to 1 month and later dropped after 3 months. However there was no significant change in DD at all-time points with p=1.000

Table 25: Intragroup comparison: Mean, Median Standard deviation(SD), Interquartile range(IQR) and statistical tests (statistics- DIAGNOdent reading (DD) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

	DIAGNOdent reading (DD) of Group 1(SE)	DIAGNOdent reading (DD) of Group 4(IRF)	DIAGNOdent reading (DD) of Group 5 (MCP)	Intragroup comparison		
	Median ± IQR	Mean ± SD	Mean ± SD	Group 1(SE)	Group 4(IRF)	Group 5 (MCP)
T1	6.20±1.85	46.72±25.63	57.46±23.89	T1 - T2, **p=0.002*** Adj p= 0.011	T1 - T2, p=1.000	T1 - T2, p=1.000
T2	5.90±1.60	46.44±22.81	57.48±22.70	T2 - T3, p=0.057 Adj p=0.340	T2 -T3, p=1.000	T2 - T3, p=1.000
T3	6.00±1.70	45.12±21.79	59.68±19.43	T3 - T4, p=1.000 Adj p= 1.000	T3 -T4, p=1.000	T3 - T4, p=1.000
T4	5.90±1.40	46.66±21.14	58.80±18.23	T1 -T4, p=0.225 Adj p=1.000	T1 - T4, p=1.000	T1 - T4, p=1.000

P at a significant value if p<0.05

The results for the comparison between different groups (Intergroup) on the DIAGNOdent reading (DD) at baseline, 7-days, 1-month and 3 months (Table 26) are as follows:

- Comparing between SE and intervention groups (SE versus IRF and SE versus MCP group), DD in intervention group was higher compared with the SE group and was statistically significant at different time intervals (p=0.000). However when intervention groups were compared, MCP group had higher DD than IRF resin infiltration group but was not statistically significant at all-time points (p>0.05)

Table 26: Intergroup comparison (statistics: DIAGNOdent reading (DD) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 4(IRF)	**Group 1(SE) versus Group 5(MCP)**	**Group 4(IRF) Versus Group 5(MCP)**
T1, **p=0.000****	T1, **p=0.000****	T1, p=0.345
T2, **p=0.000****	T2, **p=0.000****	T2, p=0.292
T3, **p=0.000****	T3, **p=0.000****	T3, p=0.132
T4, **p=0.000****	T4, **p=0.000****	T4, p=0.186

P at a significant value if $p<0.05$

4.3.3 Photographic parameter: Lightness (L* Values)

4.3.3.1 Test of normality

Data presented a normally distributed for both group 1(SE) and group 4 (IRF) however, in group 5 (MCP) data was found to be not normally distributed at 1 month. Table 27 presents the normality for the groups at different time intervals. Therefore, one way repeated measures anova with a bonferroni correction for group 1(SE) and group 4 (IRF) and Friedman followed by post hoc test for group 5 (MCP) were used for comparison within the groups while independent student t-test and Mann-whitney U-test was used for comparison between the groups.

Table 27: Normality test of enamel surface lightness (L* values) of different groups at baseline, 7-days, 1 month and 3 months

Days	Groups	Shapiro-Wilk		
		Statistics	Df	Sig.
Baseline	Sound Enamel-Group 1(SE)	0.925	10	0.403
	MIH- Group 4 (IRF)	0.977	10	0.948
	MIH- Group 5 (MCP)	0.884	10	0.143
Day 7	Sound Enamel-Group 1(SE)	0.906	10	0.256
	MIH- Group 4 (IRF)	0.922	10	0.374
	MIH- Group 5 (MCP)	0.931	10	0.455
1 month	Sound Enamel-Group 1(SE)	0.907	10	0.261
	MIH- Group 4 (IRF)	0.890	10	0.169
	MIH- Group 5 (MCP)	0.839	10	**0.043**
3 months	Sound Enamel-Group 1(SE)	0.882	10	0.137
	MIH- Group 4 (IRF)	0.909	10	0.275
	MIH- Group 5 (MCP)	0.859	10	0.074

4.3.3.2 Descriptive statistics

In the following table 28 are the results from descriptive statistics for enamel surface lightness (L* values) for the groups at different time intervals. Figures 35-37 are the boxplots for all groups' baseline, 7 days, 1 month and 3 months.

Table 28: Descriptive statistics: Lightness (L-values) for group 1(SE), group 4(IRF) and group 5(MCP) at baseline, 7days, 1-month and 3months (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	Baseline	10	54.04	53.81	5.27	43.29	63.23	19.93	5.18
	7 days	10	40.11	39.59	4.69	34.73	47.46	12.73	8.56
	1 month	10	43.35	44.01	4.57	33.39	48.84	15.44	5.07
	3 months	10	39.61	40.55	3.96	31.45	43.52	12.07	5.65

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 4 (IRF)	Baseline	10	61.78	61.20	4.69	54.77	70.16	15.39	7.49
	7 days	10	45.81	46.02	5.56	39.07	54.58	15.52	11.18
	1 month	10	52.58	51.79	5.61	46.08	60.67	14.59	11.37
	3 months	10	41.54	40.26	4.85	35.62	50.58	14.96	8.04

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 5(MCP)	Baseline	10	63.10	65.19	5.69	51.82	68.91	17.09	8.43
	7 days	10	53.69	54.86	5.59	45.10	60.75	15.65	10.35
	1 month	10	59.56	62.41	6.89	48.72	66.23	17.51	13.24
	3 months	10	46.52	48.42	5.65	39.25	53.54	14.29	10.85

Figure 35: Box plot of lightness (L* values) for group 1(SE) - control group

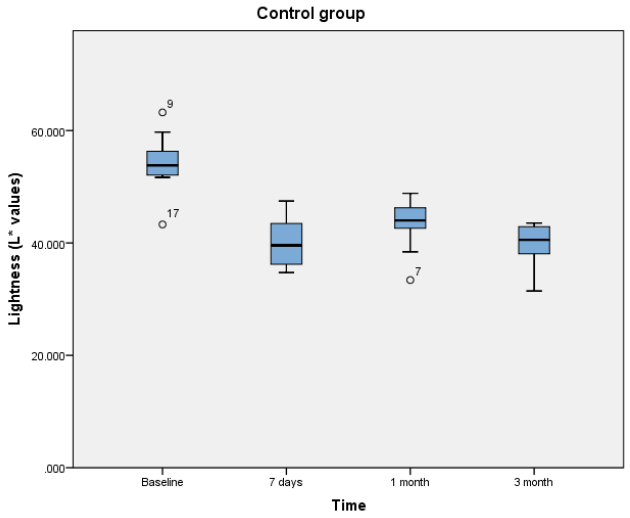
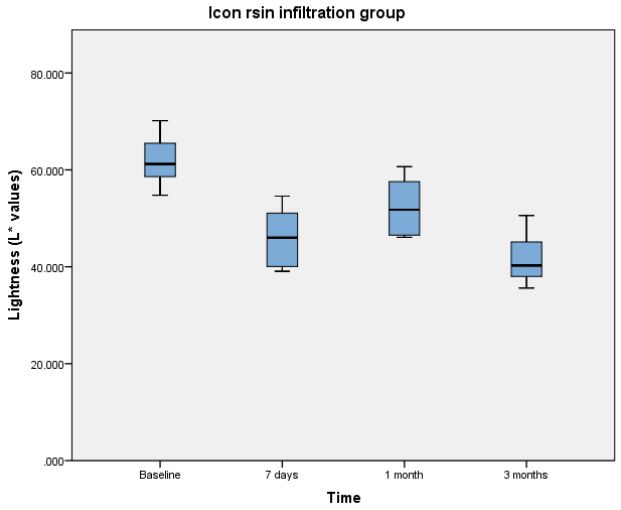


Figure 36: Box plot of lightness (L* values) for group 4(IRF) - ICON resin infiltration



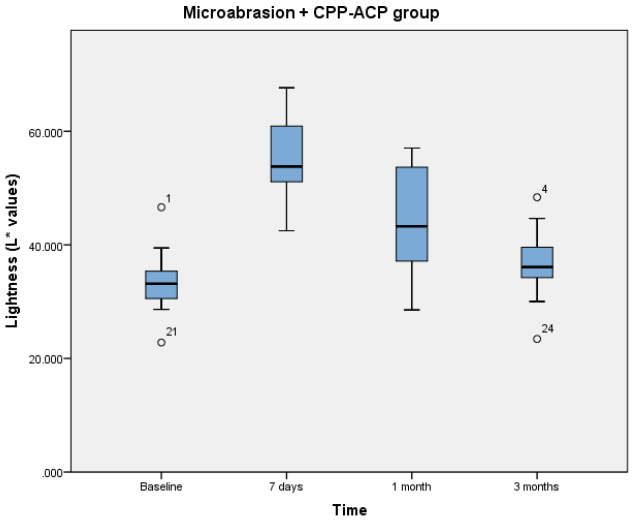


Figure 27: Box plot of lightness (L* values) for group 5(MCP) - Micro-abrasion followed by CPP-ACP

4.3.3.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on enamel surface lightness (L* values) at baseline, 7-days, 1-month and 3 months (Table 29) showed the following:

- Mean L* values in the SE group decreased from baseline to 7 days and was statistically significant with $p<0.05$($p=0.000$). After 1 month, the mean lightness value increased slightly however, this was not statistically significant ($p=0.059$). After 3 months, the mean lightness decreased significantly ($p=0.006$) and the overall decrease in mean lightness from baseline to 3 months was also statistically significant ($p=0.000$)
- Mean L* values in IRF group decreased from baseline to 7 days significance $p=0.000$ followed by an increase after 1 month, which was also statistically significant ($p=0.000$). The mean lightness value later again after 3 months decreased with a significance ($p=0.000$). Overall, the mean lightness value decreased significantly ($p=0.000$) from baseline to 3 months.
- Likewise in MCP, median lightness value decreased after 7 days which was statistically significant ($p=, 0.009$, adjusted $p=0.056$). However after 1 month, there was an increase in median lightness value which was also statistically significant ($p=0.038$, adjusted $p=0.226$). Later after 3 months, the median lightness value significantly decreased ($p=0.000$). The overall median lightness value dropped significantly ($p=0.000$) from baseline to 3 months.
- In all the groups, the lowest L* values at after 3 months were seen in SE group followed by IRF group. However the overall decreased in L* values from baseline to 3 months was seen in IRF group followed by SE groups.

Table 29: Intragroup comparison: Mean, Median Standard deviation(SD), Interquartile range(IQR) and statistical tests: enamel surface lightness (L* values) of different groups at baseline(T1), 7 days(T2), 1 month(T3) and 3 months(T4)

	L* values of Group 1(SE)	L* values of Group 4(IRF)	L* values of Group 5 (MCP)	Intragroup comparison		
	Mean ± SD	Mean ± SD	Median ± IQR	Group 1(SE)	Group 4(IRF)	Group 5 (MCP)
T1	54.04±5.27	61.78±4.69	65.19±8.43	T1 - T2, **p=0.000****	T1 - T2, **p=0.000****	T1 - T2, **p=0.009*** Adjusted p=0.056
T2	40.11±4.69	45.81±5.56	54.86±10.35	T2 - T3, p=0.059	T2 - T3, **p=0.000****	T2 - T3, **p=0.038*** Adjusted p=0.226
T3	43.35±4.57	52.58±5.61	62.41±6.89	T3 - T4, **p=0.006***	T3 - T4, **p=0.000****	T3 - T4, **p=0.000**** Adjusted p=0.000
T4	39.61±3.96	41.54±4.85	48.42±10.85	T1 - T4, **p=0.000****	T1 - T4, **p=0.000****	T1 - T4, **p=0.000**** Adjusted p=0.000

P at a significant value if p<0.05

The results of the comparison between different groups (Intergroup) on enamel surface lightness (L* values) at baseline, 7-days, 1-month and 3 months (Table 30) are as follows:

- IRF showed higher L* values compared with SE group at different time intervals, which was statistically significant at baseline, 7 days and 1 month(p,0.05) whereas MCP had higher L* values compared to SE group and was statistically significant at all-time points(p=0.05)
- At all-time points, MCP showed higher L* values compared to IRF however, this was statistically significant at 7 days, 1month and 3 months (p<0.05

Table 30: Intergroup comparison (statistics-Enamel surface lightness(L* values) of different groups at baseline (T1), 7 days (T2), 1 month (T3) and 3 months (T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 4(IRF)	**Group 1(SE) versus Group 5(MCP)**	**Group 4(IRF) Versus Group 5(MCP)**
T1, **p=0.003***	T1, **p=0.005***	T1, p=0.450
T2, **p=0.023***	T2, **p=0.000****	T2, **p=0.016***
T3, **p=0.001***	T3, **p=0.000****	T3, **p=0.023***
T4, p=0.341	T4, **p=0.023***	T4, **p=0.038***

P at a significant value if p<0.05

4.3.4 Colour change (ΔE)

4.3.4.1 Test of normality

Data presented a normally distribution for group 1(SE), group 4(IRF) and group 5(MCP). Therefore the intragroup comparison was analysed using one way repeated anova, post hoc tukey test and intergroup analysed using Independent student t test. Table 31 presents the normality for the three at different time intervals.

Table 31: Normality test of a colour change (ΔE) of different groups from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

	Groups	Shapiro-Wilk		
		Statistics	df	Sig.
ΔE_1(T1-T2)	Sound Enamel-Group 1(SE)	0.891	10	0.173
	MIH- Group 4 (IRF)	0.901	10	0.226
	MIH- Group 5 (MCP)	0.957	10	0.754
ΔE_2(T1-T3)	Sound Enamel-Group 1(SE)	0.905	10	0.246
	MIH- Group 4 (IRF)	0.925	10	0.404
	MIH- Group 5 (MCP)	0.929	10	0.441
ΔE_3(T1-T4)	Sound Enamel-Group 1(SE)	0.935	10	0.495
	MIH- Group 4 (IRF)	0.902	10	0.230
	MIH- Group 5 (MCP)	0.942	10	0.573

4.3.4.2 Descriptive statistics

In the following table 32 are the results from descriptive statistics for colour change (ΔE) for the groups at different time intervals. Figures 38-40 are the boxplots for all groups' baseline-7 days (T1-T2), baseline-1month (T1-T3) and baseline-3months (T1-T4)

Table 32: Descriptive statistics: (ΔE) values for group 1(SE), group 4(IRF) and group 5(MCP) baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3). (N=10 for each group)

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
Sound Enamel-Group 1(SE)	ΔE_1 (T1-T2)	10	17.03	16.59	2.19	13.62	21.39	7.76	1.95
	ΔE_2 (T1-T3)	10	12.69	12.47	4.00	7.74	21.36	13.62	3.98
	ΔE_3 (T1-T4)	10	17.56	17.36	3.72	13.02	23.99	10.97	6.51

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 4(IRF)	ΔE_1 (T1-T2)	10	19.64	20.39	2.31	15.89	22.27	6.38	4.71
	ΔE_2 (T1-T3)	10	12.83	12.63	2.54	9.66	17.18	7.52	4.04
	ΔE_3 (T1-T4)	10	21.61	21.69	3.47	13.62	26.78	13.16	3.13

Groups	Days	Number of slabs	Mean	Median	Std. deviation	Minimum	Maximum	Range	Interquartile range
MIH-Group 5(MCP)	ΔE_1 (T1-T2)	10	22.82	22.75	2.99	17.29	26.72	9.43	5.39
	ΔE_2 (T1-T3)	10	20.38	18.94	4.18	14.51	27.01	12.50	7.28
	ΔE_3 (T1-T4)	10	21.77	20.84	4.92	15.32	30.18	14.87	7.64

Figure 28: Box plot of the colour change (ΔE values) for group 1(SE) - control group

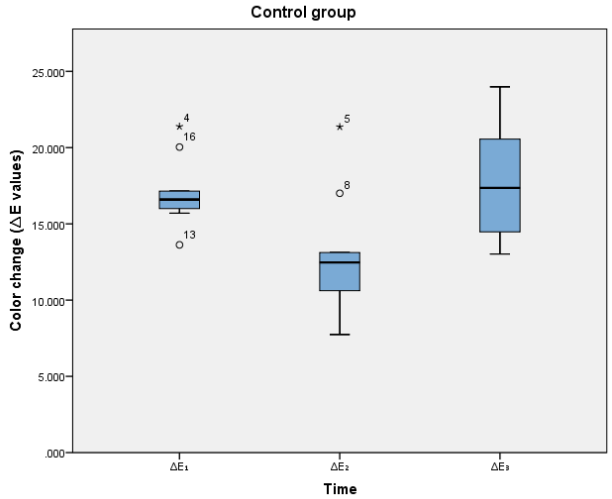
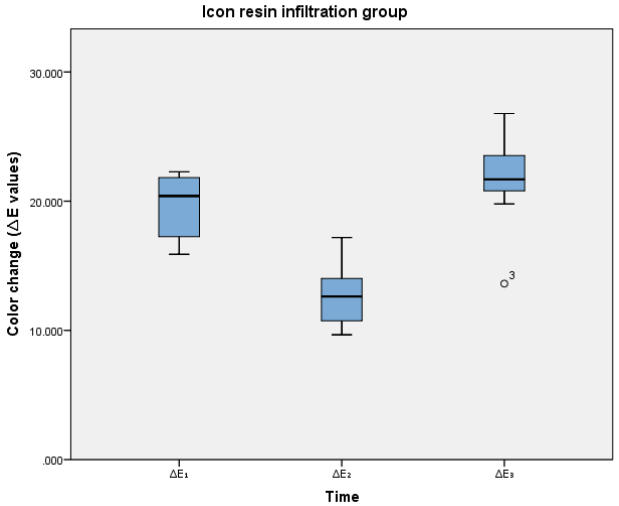


Figure 29: Box plot of the colour change (ΔE values) for group 4(IRF) - ICON resin infiltration



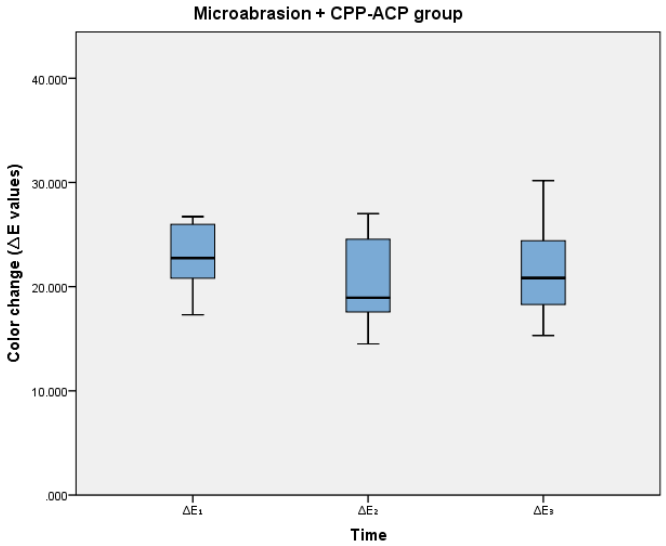


Figure 30: Box plot of the colour change (ΔE values) for group 5(MCP) - Micro-abrasion followed by CPP-ACP application

4.3.4.3 Intragroup and Intergroup comparison

Intragroup and intergroup comparisons were performed at different time intervals for all three groups. The results of the comparison within groups (Intragroup) on colour change (ΔE values) at baseline, 7-days, 1-month and 3 months (Table 33) showed the following:

Mean of difference in colour change signifies colour change from baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3).

- The mean ΔE values decreased significantly for all the groups from ΔE_1 - ΔE_2 (baseline- 7 days and baseline- 1 month) p=0.002 (SE group), p=0.000 (IRF group) and p= 0.024 (MCP group).The mean ΔE values increased from ΔE_2 - ΔE_3 (baseline-1 month and baseline- 3 months) but was also only statistically significant in SE and IRF groups with p=0.000. However, there no statistically significant difference (p=1.000) in the mean ΔE values from ΔE_1 - ΔE_3 (baseline to 7 days and baseline to 3 months) for the MCP group.
- Overall, overall mean ΔE values increase from ΔE_1 - ΔE_3 (baseline -7 days and baseline-3 months) for SE and IRF groups and decreased slightly for MCP group, however there was no statistically significant change in all groups (p=1.000).

Table 33: Intragroup comparison: Mean, Standard deviation(SD) and statistical tests (statistics- Colour change(ΔE values) for group 1(SE), group 4(IRF) and group 5(MCP)groups baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3)

	ΔE of Group 1(SE)	ΔE of Group 4(IRF)	ΔE of Group 5 (MCP)	Intragroup comparison		
	Mean ± SD	Mean ± SD	Mean ± SD	Group 1(SE)	Group 4(IRF)	Group 5 (MCP)
ΔE_1	17.03±2.19	19.64±2.31	22.82±2.99	ΔE_1 - ΔE_2, **p=0.002***	ΔE_1 - ΔE_2, **p=0.000****	ΔE_1 - ΔE_2, **p=0.024***
ΔE_2	12.69±4.00	12.83±2.54	20.38±4.18	ΔE_2 - ΔE_3, **p=0.000****	ΔE_2 - ΔE_3, **p=0.000****	ΔE_2 - ΔE_3, p=1.000
ΔE_3	17.56±3.72	21.61±3.47	21.77±4.92	ΔE_1 - ΔE_3, p=1.000	ΔE_1 - ΔE_3, p=0.219	ΔE_1 - ΔE_3, p=1.000

P at a significant value if p<0.05

The results for the comparison between groups (Intergroup) on colour change (ΔE values)for SE, IRF and MCP group from baseline to 7 days (ΔE_1), baseline to 1 month (ΔE_2) and baseline to 3 months (ΔE_3) (Table 34)

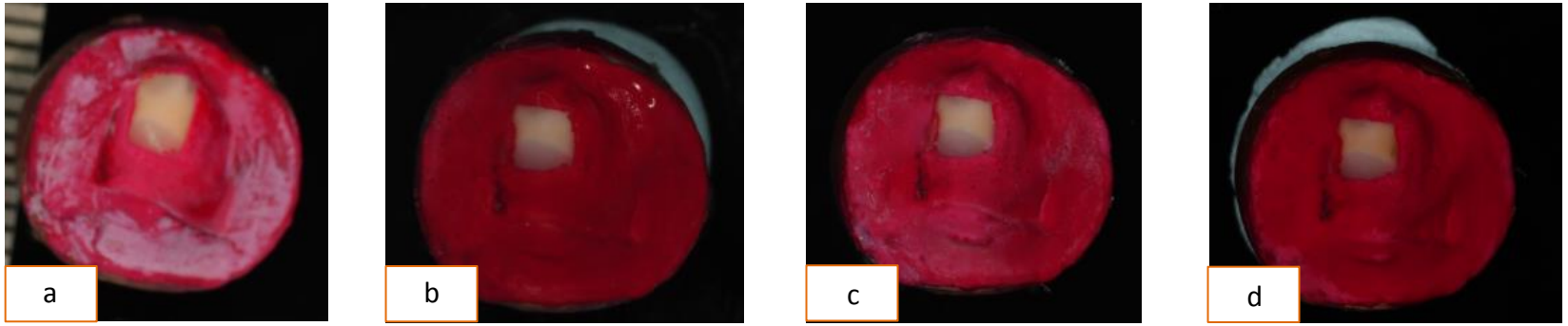
- IRF showed a statistically significant colour change value from baseline to 7 days (ΔE_1, p=0.018) and baseline to 3 months (ΔE_3, p= 0.022) than SE group. Similarly, MCP group showed a statistically significant colour change value from baseline to 7 days (ΔE_1, p=0.018), baseline to 1 month (ΔE_3 , p=0.045) and baseline to 3 months (ΔE_3, p= 0.022) than SE group
- Between the intervention groups, MCP showed a statistically significant colour change value from baseline to 7 days (ΔE_1, p=0.016) and baseline to 1 month (ΔE_3, p=0.000) than IRF group. Figures 41 and 42 are the photographs of the samples from IRF and MCP taken at different time intervals.

Table 34: Intergroup comparison: statistics colour change (ΔE) of different groups from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Intergroup comparison (Statistical significance)		
Group 1(SE) versus Group 4(IRF)	**Group 1(SE) versus Group 5(MCP)**	**Group 4(IRF) Versus Group 5(MCP)**
ΔE_1 (T1-T2**), p=0.018***	ΔE_1 (T1-T2), **p=0.000****	ΔE_1 (T1-T2), **p=0.016***
ΔE_2 (T1-T3), p=0.929	ΔE_2 (T1-T3), **p=0.001***	ΔE_2 (T1-T3), **p=0.000****
ΔE_3 (T1-T4), **p=0.022***	ΔE_3 (T1-T4), **p=0.045***	ΔE_3 (T1-T4), p=0.933

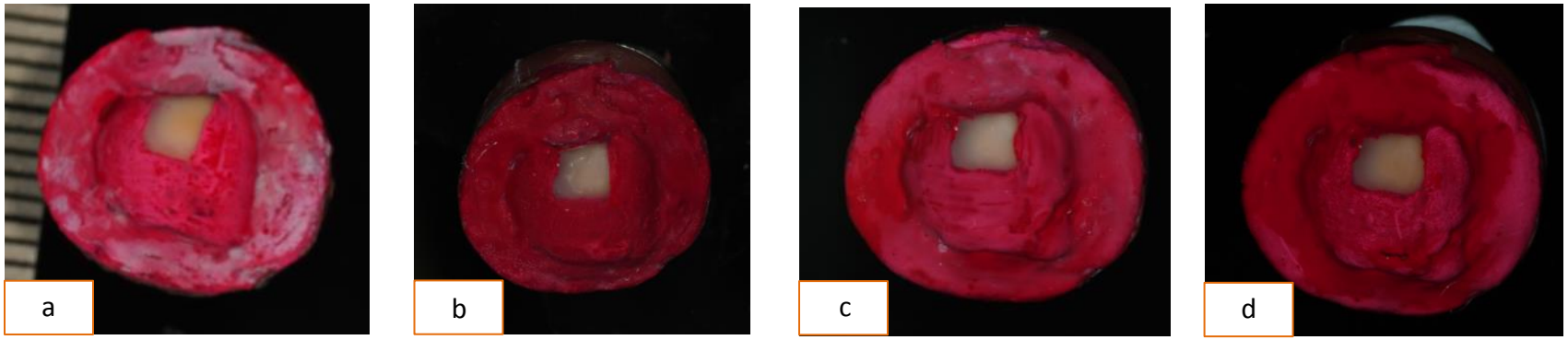
P at a significant value if p<0.05

Figure 31: Photograph of hypomineralised enamel at different time intervals before and after IRF treatment



(a): Baseline (T1) **(b):** 7 days (T2) **(c):** 1 month (T3) **(d):** 3 months (T4)

Figure 32: Photograph of hypomineralised enamel at different time intervals before and after MCP treatment



(a): Baseline (T1) **(b):** 7 days (T2) **(c):** 1 month (T3) **(d):** 3 months (T4)

4.3.5 Micro-CT readings (Mineral densities-MD in g/cm3)

4.3.5.1 Descriptive statistics for all groups

Table 35 shows the mean and standard deviation of mineral densities for only two samples from group 1(SE), group 4(IRF) and group 5(MCP)

Table 35: Descriptive statistics Mineral densities (MD in g/cm3) of different groups at baseline, 7-days, 1 month and 3 months (N=2 for each group)

	Samples	Baseline		7 days		1 month		3 months	
		Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD	Mean MD (g/cm3)	SD
Sound Enamel-Group 1(SE)	1	2.535	0.312	2.429	0.261	2.398	0.246	2.406	0.251
	2	2.226	0.232	2.292	0.279	2.362	0.247	2.414	0.242
MIH-Group 4(IRF)	2	2.003	0.267	2.523	0.332	2.238	0.243	2.018	0.265
	5	1.843	0.206	2.021	0.224	2.216	0.208	2.236	0.208
MIH-Group 5(MCP)	1	1.966	0.197	2.122	0.155	2.231	0.228	2.255	0.143
	3	1.859	0.193	1.815	0.246	1.768	0.141	1.636	0.142

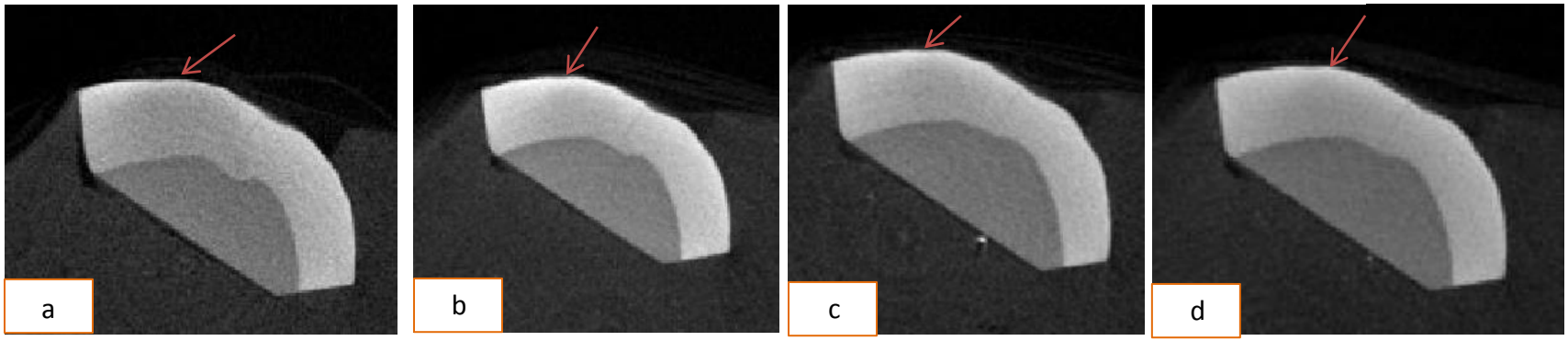
The mean mineral densities were the highest for SE group and the lowest for MCP group. For the SE group, mean mineral density in sample 1 decreased gradually from baseline to 1 month (4.2% from baseline to 7 days and 1.3% from 7 days to 1 month) followed by a very slightly increase of 0.3% at 3 months. Overall, there was a decrease of 5 % mean mineral density from baseline to 3 months. On the other hand, mean mineral density in sample 2 increased gradually at different time intervals (2.9% from baseline to 7 days, 3% from 7 days to 1 month and 2.2% from 1 month to 3 months). Overall, mean mineral density increased from baseline to 3 months was 7.8%.

Mineral density in sample 2 in IRF group increases significantly from baseline to 7 days by 20.6% and later reduced gradually at 1 month by 11.3% and 3 months by 9.8%. The overall mean mineral density increased from baseline to 3 months by 0.7% whereas sample 5 increased gradually from baseline to 3 months by 8.8%(baseline to 7 days),8.8%(7 days to 1 month) and 0.9% (1 month to 3 months).Overall mean mineral density increased from baseline to 3 months by 17.5%.

The Mean mineral density in MCP group(sample 1), also increased gradually from baseline to 3 months(7.4% from baseline to 7 days, 4.9% from 7 days to 1 month and 1.1% from 1 month to 3 months) with 12.8% overall increase in mean mineral density from baseline to 3 months. However in sample 3, the opposite was seen with mean mineral density decreased gradually from baseline to 3 months by 2.3% from baseline to 7 days, 2.6% from 7 days to 1 month and 7.5% from 1 month to 3 months. Overall, mean mineral density decreased by 11.9% from baseline to 3 months.

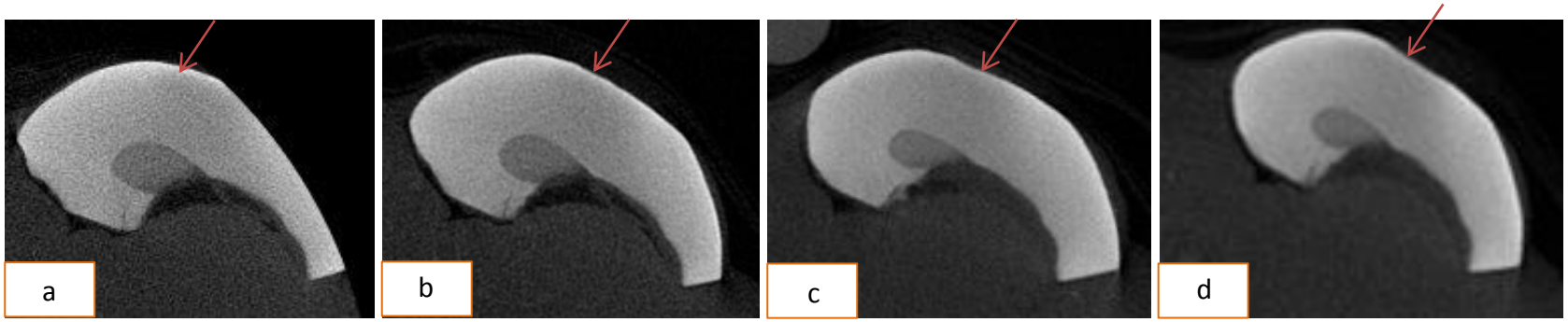
Comparing the mean mineral densities within the intervention groups, IRF had the highest overall increase in the mean mineral densities which was seen in sample 5. In addition to that the mean mineral density at 7 days (sample 2) came very close to sample 1 mean mineral density in the SE group at baseline. Figure 43 and 44 shows the micro-CT images of a hypomineralised sample 2 and sample 3 at baseline and following IRF and MCP treatment respectively after 7 days, 1month and 3 months. The arrows represent the region of interest and showing how the radiolucency (MIH lesion) spreading deeper close to dentine

Figure 33: Micro-CT images of MIH-affected enamel sample (yellow-brown lesion) before and after IRF treatment



(a) Baseline (T1) MD: 2.003 g/cm3 **(b)** 7-days (T2) MD: 2.523 g/cm3 **(c)** 1 month (T3) MD: 2.238 g/cm3 **(d)** 3 months (T4) MD: 2.018 g/cm3

Figure 34: Micro-CT images of MIH-affected enamel sample (yellow-brown lesion) before and after MCP treatment



(a) Baseline (T1) MD: 1.859 g/cm3 **(b)** 7 days (T2) MD: 1.815 g/cm3 **(c)** 1 month (T3) MD: 1.768 g/cm3 **(d)** 3 months (T4) MD: 1.636 g/cm3

5 Discussion

5.1 Justification of study aim

There are many studies conducted on MIH teeth in the last decade. The aetiology of the condition is multifactorial and the management of these teeth depends on multiple factors including severity. Children with MIH teeth often have aesthetic concerns especially when incisors are affected and treatment of these affected teeth by masking the opacities have been reported to have a positive impact on these children (Hasmun et al., 2018). Different treatment approaches have been reported for the affected incisors including micro-abrasion, IRF , tooth whitening (using hydrogen peroxide-containing products) and resin composite restoration (Hanlin et al., 2015, Pini et al., 2015, Borges et al., 2017).

Several studies evaluating these two micro-invasive treatments (IRF and micro-abrasion) on MIH teeth have been performed to date (Gençer and Kirzioğlu, 2019, Ozgur et al., 2023, Bhandari et al., 2018, Bhandari et al., 2019). IRF concept was developed in Germany (ICON; DMG, Hamburg, Germany) with the primary focus of treating incipient carious lesions by allowing the low viscosity resin to fill, reinforce and stabilize demineralized lesions as well as mask the opacities, this treatment was also suggested as a potential method to manage MIH affected teeth.

In previous in-vitro studies, IRF was used to evaluate the penetration potential of the material into the MIH lesions as well as assess the micro-hardness of the enamel post infiltration (Crombie et al., 2014). Other studies used different pre-treatment protocols prior to infiltrating the MIH lesion with resin infiltration to assess the penetrability of resin and found these protocols to improve penetration of resin into the body of the lesion (Natarajan et al., 2015). Additionally, in-vivo studies looked at the structural integrity of MIH teeth post infiltration with IRF (Nogueira et al., 2021) and found IRF treatment maintains the structural integrity of these lesions post treatment. The ability to conceal white opaque appearance of Grade 1 MIH lesion and improve aesthetic (Bhandari et al., 2018, Mabrouk et al., 2020, Saccucci et al., 2022) has also been studied and reported to mask the opacities and improve aesthetic and patient perception (dos Santos Athayde et al., 2022). Furthermore, surface roughness properties were also evaluated, IRF was reported to improve surface roughness after 1 week post infiltration however surface roughness increased progressively in comparison to sound enamel (Luppieri et al., 2022).

Few studies have looked at remineralisation of MIH teeth to increase mineral contents of enamel and prevent post-eruptive breakdown by using complex of casein phosphopeptides and amorphous calcium phosphate (CPP-ACP) that acts as a reservoir of calcium and phosphate, stabilizing high concentrations of these ions (Reynolds, 2008). Patients who used CPP-ACP and CPP-ACFP had a positive effect in reducing hypomineralisation on enamel surfaces of MIH-diagnosed teeth for up to one month post treatment (Bakkal et al., 2017b). In 2011, Baroni and Marchionni observed an improvement in mineralization, morphology, and porosities of MIH-affected enamel following a continued use of a cream containing 10% CPP-ACP (Baroni and Marchionni, 2011). A combination of microabrasion followed with the application of CPP-ACPF in an in vivo study by Bhandari in 2019 reported reduction in whiteness of the grade 1 MIH lesion with considerable colour change and improve aesthetics of MIH teeth (Bhandari et al., 2019).

Another in-vivo studies compared between IRF and MCP treatment for treatment of developmental defects on colour and found that IRF had successful aesthetic results in both fluorosis and hypomineralised lesions (Gençer and Kirzioğlu, 2019).

To date, no studies have been published regarding the comparison of the effects of the MCP and IRF treatment on different surface properties such as colour, surface roughness, DD and mineral density. In the present study the effect of both treatment (IRF and MCP) on the range of MIH teeth (white-creamy, Yellow-brown lesions) were evaluated. Surface properties including colour change and surface roughness was evaluated as well assessment of the DD and mineral density using DIAGNOdent and Micro-CT.

5.2 Appraisal of methodology

The present study used an in vitro model to compare the effects of IRF and MCP on a range of MIH teeth, following that the treatment outcomes were evaluated in terms colour masking, surface roughness, DIAGNOdent reading and mineral density. 50 samples were evaluated and were located to 5 groups as follows: group 1(SE) of 10 sound enamel and 4 intervention groups [(white-creamy group2 (IRF) and group 3(MCP)) and yellow-brown group 4(IRF) and group 5(MCP)] to receive two treatment protocols (IRF and MCP). MIH-affected enamel slabs were randomly allocated using an online randomization engine. Intergroup and intragroup comparisons were made at baseline, 7 days, 1 and 3 months post treatment.

In-vitro studies are very important for clinical decision making as this helps the clinician to compare dental material's physical and chemical properties prior to their application clinically. The model also helps clinician to understand biological properties of dental hard or soft tissues and therefore carries a major proportion of the published articles in dentistry. The key advantage of in vitro studies enables the researchers to perform single-variable experiments under controlled conditions(White, 1995) and can be conducted easily under controlled environment thus reducing the risk of bias (Krithikadatta et al., 2014).

In this study micro-abrasion technique used was a combination of 37% phosphoric acid gel with pumice in equal proportions as proposed by Kamp (Kamp, 1989), the advantage of using 37% phosphoric acid is its availability and commonly used in practice. After micro-abrasion procedure, the samples were polished with soflex disc followed by application of CPP-ACP for 5 minutes (Sheoran et al., 2014). The other treatment groups using IRF method was performed as per manufacturer instructions.

Previous in vivo and in vitro studies used various methods of management of MIH including application of CPP-ACP up to a month (Bhandari et al., 2019, Pasini et al., 2017, Bakkal et al., 2017c). In this study CPP-ACP was applied twice daily for 7 days, it was applied on the samples and left undisturbed to act on the lesion for 5 min and later stored in night time artificial saliva for 6 hours followed by a second application after 6 hours. Samples were then stored in a night time artificial saliva under 37.0°C in an incubator at all times except during treatment of the slabs. Other storage media were reported in the literature including hanks banks solution (Bakkal et al., 2017c, Cardoso-Martins et al., 2022).Since the purpose of the study was to evaluate the effect of the two treatments(IRF and MCP), using only night time artificial saliva was ideal to reduce the risk of introducing confounding factor since it has lower amount of minerals.

5.2.1 Enamel slabs preparation

The enamel slabs were obtained from the buccal surfaces of MIH FPMs. Prior to abtaining the enamel slabs, FPMs were stored in distilled de-ionised water and 0.05% thymol for 7 days prior to experiment in order to inhibit growth of bacteria and rehydrate the teeth. According to Shellis, the teeth should be kept moist up to the time of experiment with any chosen disinfected agent to achieve the greatest effect against a prion disease which is transmissible from infected human or bovine tissues (Shellis et al., 2011).The ideal germicide should disinfect and hydrate extracted teeth during storage without further altering tissue structure. Thymol is a commonly used storage and disinfection media in in-vitro studies ;

a naturally occurring biocide with strong antimicrobial attributes (Stappert et al., 2006, Shafiei et al., 2014). The principal mode of action of thymol is the ability to penetrate the cell membrane and subsequently destroy the pathogens that may grow on the teeth (Shafiei et al., 2014). In a study by Vejay reported that teeth which were stored in 0.05% thymol had a flexural strength similar to teeth stored in the control solution (Hanks banks solution) and that sodium hypochlorite resulted in lower flexural strength. Another study reported no change in micro or nanohardness when stored in thymol for several days (Shellis et al., 2011).

The MIH slabs were distributed evenly into four groups. Due to limited availability of MIH-affected teeth in tissue bank during national lockdown, only yellow-brown lesions were obtained initially. Few months later more samples (white-creamy lesions) were recruited. In order to divide the samples evenly into treatment protocols, visual inspection as well as DD readings were used to ensure the distribution is even across both treatments for both white-creamy and yellow-brown lesions.

5.4 Evaluation of the results

5.4.1 Treatment of the MIH teeth

Four groups of 40 samples were treated with IRF and MCP. The 20 samples from white-creamy MIH lesions were divided into group 2(IRF) and group 3(MCP), similarly 20 yellow-brown MIH lesions were divided into group 4(IRF) and group 5(MCP). The treatment of the intervention groups was performed then by one investigator.

IRF is a non- invasive treatment which infiltrates the porous carious enamel with low viscosity resins occluding the pathways of acids into enamel thereby arresting the progression of the lesion (Paris et al., 2007), improve the translucency, optical properties and overall appearance of teeth (Crombie et al., 2014). Owing to its low viscosity nature, IRF has been reported to be able to penetrate 600µm into carious enamel lesions clinically (Meyer-Lueckel and Paris, 2010). On the other hand ,penetration of IRF in MIH lesion has been reported to be inconsistent and not as extensive as reported in carious lesions due to different lesion characteristics and protein contents (Crombie et al., 2014) and that infiltration of unprepared enamel does not reach a depth of 300 µm.

Micro-abrasion treatment is another conservative approach which has been reported to improve aesthetic appearance of enamel when the discolouration is limited to its outer layer. Most widely techniques used are micro-abrasion using a combination of 18% hydrochloric acid(HCL) and pumice (Croll, 1989b), HCL(6.6% and 10%) with silica carbide (Croll, 1989b) and 37% phosphoric acid gel with pumice in equal proportions (Rodrigues et al., 2013) to remove around 100–200µm of enamel when used appropriately (Lygidakis et al., 2022),hence this approach may not be suitable for deeper opacities (Wong and Winter, 2002).Micro-abrasion followed by remineralising agent such as CPP-ACP has been reported to remineralise the subsurface enamel crystals and result in aesthetic improvement of the Grade I MIH lesion (Bhandari et al., 2019). CPP-ACP, naturally occurring molecules, are able to bind calcium and phosphate ions and stabilize amorphous calcium phosphate in metastable solution (Walsh, 2000) It maintains the pores in enamel surface, which consequently reduces bacterial adherence. When CPP-ACP is applied in the oral environment, it will bind to biofilms, plaque, bacteria, hydroxyapatite and soft tissue, localizing bio available calcium and phosphate ions. It will thus maintain a state of super saturation with respect to tooth enamel, reducing demineralization and enhancing remineralization (Walsh, 2000).

5.4.1.1 Evaluation of enamel surface roughness (Ra)

In the present study, non-contact surface profilometry was used to check the surface roughness by obtaining Ra values. Ra is the most reported surface roughness parameter in dental literature (Field et al., 2010) and is defined as arithmetic average height of roughness from the mean line measured within the sample length. The surface roughness of intraoral hard surfaces is very important in the process of bacteria adhesion as bacterial adhesion and proliferation on dental hard tissue have been reported to occur when the surface roughness of the hard dental tissue is above the critical value of 0.2µm, increasing the risk of dental caries and periodontal inflammation (Bollen et al., 1996, Quirynen et al., 1996, Bollenl et al., 1997).

It has been reported that the restorations with smooth surfaces have reduced risk for the development of secondary caries and stainings resulting from accumulation of dental plaque (Weitman and Eames, 1975, Herrgott et al., 1989). In a study by Weitman and colleague, it was reported that plaque can accumulate on the composite treated surface with surface roughness of 0.7-1.44µm (Weitman and Eames, 1975) and polishing the surfaces following restoration resulted in smooth surfaces with reduced surface roughness values. Different polishing systems have been evaluated in the literature and several studies reported that aluminium oxide discs were the best (Dennis T,Zoltie T,Wood D and Altaie A, 2021)

(Wilson et al., 1990, Chen et al., 1988). Another study by Chung reported that polishing procedures could result in superficial surface roughness reduction ranging from 26% to 74% (Chung, 1994). In our study, MIH-affected samples were polished using soflex disc immediately after micro-abrasion (prior to CPP-ACP application).

The SE group had the lowest median surface roughness compared to the intervention groups and did not show any significant change in surface roughness at different time intervals($p>0.05$). However the median surface roughness remained above the critical value of 0.2µm.

The white-creamy MIH lesion in IRF group, median surface roughness decreased after 7 days (Median=0.69, IQR=0.22) following treatment with IRF. This is in agreement with the results observed in a study by Luppieri (Luppieri et al., 2022) whose study was conducted on MIH-affected FPMs of children aged 6 to 18 years and a range of MIH lesion were included. The decrease in surface roughness 7 days following IRF treatment indicated that resin infiltration occluded the porous enamel surface reducing the surface roughness (Soveral et al., 2021) producing a less plaque retentive surfaces thus reducing the risk of bacteria adhesion and dental diseases. However, in the present study, the decrease in median surface roughness after 7 days was not statistically significant ($p>0.05$). On the other hand, an increase in median surface roughness was observed after 1 month (Mean=0.96, SD=0.29) following IRF which was statistically significant ($p<0.05$). This may suggest a poor resin adhesion to hypomineralised enamel (Elhennawy et al., 2017). For yellow-brown MIH lesions, mean surface roughness increased after 7 days followed by a reduction up until 3 months, however there was no statistically significant change observed at different time($p>0.05$)

In MCP groups, the increase in median surface roughness after 1 month in white-creamy MIH lesions(Median=0.62, IQR=0.37) and after 7 days(Median=0.86, IQR=0.45) in yellow-brown MIH lesions following MCP could be due to the 37% phosphoric acid used in the process of micro-abrasion. In a study by Melreles and collegues, bovine teeth were used to evaluate surface roughness and surface loss following micro-abrasion techniques using either HCL or 37% phosphoric acid and found that both HCL and 37% phosphoric acid produced surface roughness however higher surface roughness was reported in micro-abrasion technique using 37% phosphoric acid (Meireles et al., 2009). They suggested the increased in surface roughness could be due to less aggressive nature of the acid used to decalcify the enamel surface resulting in a selective conditioning pattern on the surface while leaving a more granular and irregular surface (Silverstone et al., 1975). The variations in patterns produced following treatment with acids not only highlights the chemical composition of the acid but also structural variation which

can occur in enamel not only from different teeth but also surfaces or site (Silverstone et al., 1975). In this study, micro-abrasion treatment was performed using a mixture of 37% phosphoric acid with pumice on MIH-affected teeth and an increase in surface roughness was observed after 7 days in yellow-brown MIH lesion, but this was not statistically significant($p>0.05$).However, a statistically increase after 1 month in white-creamy MIH lesions was seen($p<0.05$).On the other, the median surface roughness decrease after MCP treatment of yellow-brown MIH lesions was statistically significant after 3 month (Median=0.72, IQR=0.21, $p<0.05$). The median surface roughness reduction after 3 months could be as a result of polishing following micro-abrasion treatment and CPP-ACP application (Mathias et al., 2009). Fragoso et al found improved surface hardness and smoother enamel surfaces when bovine enamel was treated with micro-abrasion and polishing followed by immersion in artificial saliva for 24hours (Fragoso et al., 2011). They explained that saliva also plays a role in re-establishing the characteristic of enamel subjected to treatment with micro-abrasion. Furthermore, a study by Pini et al, reported that, micro-abrasion technique caused significant changes in surface roughness of the bovine enamel however, they found surface roughness reduction when enamel surfaces were exposed to saliva for 7 days (Pini et al., 2014)which could be due to mineral reposition on the eroded surface.

Surface roughness comparison between the SE and IRF (white-creamy MIH enamel) groups, showed a statistical significant difference at all-time intervals ($p<0.05$) with the highest surface roughness values detected in the IRF group. On the other hand, the surface roughness in MCP group was statistically higher after 1 month compared to the SE group ($p<0.05$).Between the intervention groups, surface roughness was higher in IRF group compared to MCP group at all-time however the surface roughness was statistically significant higher after 7 days ($p<0.05$). Whereas comparing the SE and IRF (yellow/brown MIH enamel), IRF had higher surface roughness than the SE group after 7days, 1 and 3 months ($p<0.05$) and MCP showed statistically significant higher surface roughness after 7 days and 1 month ($p<0.05$).Between the intervention groups, IRF showed a statistically significant higher surface roughness than the MCP group after 3 months ($p<0.05$). The higher surface roughness in the intervention groups in comparison to SE group could be related to the surface porosity of the MIH-affected area (Crombie et al., 2013) whereas the lower surface roughness observed in MCP group in comparison to IRF group suggests the micro-abrasion technique which removed the outer porous surface of hypomineralised enamel and the higher surface roughness in IRF group suggested the poor infiltration of resin into these lesion due to the presence of high protein content (Elhennawy et al., 2017). It has been reported that infiltration of resin without deproteination using pre-treatment protocol like sodium hypochlorite prior to IRF may result in little or no infiltration of resin into the MIH

lesion (Natarajan et al., 2015). Furthermore in this study, no further polishing of the surfaces was performed after application of IRF; therefore it is worth considering additional polishing step after IRF in future studies.

5.4.1.2 Evaluation of DIAGNOdent reading (DD)

DIAGNOdent (DIAGNOdent, KaVo, Biberach, Germany) was commercially introduced in 1990 as an adjunct method for dental caries diagnosis. A good correlation of severity of MIH teeth and DIAGNOdent reading was reported in a study by Farah and colleagues indicating the a role of DIAGNOdent in identifying the severity of the condition(Farah et al., 2010a). DIAGNOdent pen measures the fluorescence of the tooth caused by its fluorescent components that are organic in nature (Mendes et al., 2003, Shi et al., 2001a, Shi et al., 2001b). In dental caries, the fluorophores (fluorescent components) are bacteria metabolites called porphyrins (Hibst, 1999) which results in increased DIAGNOdent reading. However in MIH teeth, increased DIAGNOdent reading could be due to altered enamel proteins which are present in high content in hypomineralised enamel and the altered prismatic nature of enamel and stains in darker hypomineralised enamel (Farah et al., 2010b).It has also been reported that, sound enamel show very low DIAGNOdent reading(fluorescence) which correlates to its low organic content (Hibst et al., 2001)

DD for the SE group were below 10 at different time intervals suggesting a healthy enamel (Khatri et al., 2022)DIAGNOdent reading in SE group reduced from baseline(Median=6.20,IQR=1.85) to 7 days(Median=5.90, IQR= 1.60)and was statistically significant $p<0.05$ which later slightly increased at 1 month(Median=6.00, IQR=1.70) and then dropped again after 3 months(Median=5.90, IQR=1.40) however, there was no statistically significant change after 1 and 3 months ($p>0.05$). The higher DD reading at baseline could be related to dehydration of the sample as the samples were stored dried prior to the experiment and a decrease after 7 days suggests rehydration of the samples as a result of the samples being stored in artificial saliva. Mendes and colleagues found a statistically increase in DD values in teeth dried for 15seconds and those dehydrated for 24 hours (Mendes et al., 2004)

For white-creamy and yellow-brown lesion, DD in IRF group decreased gradually from baseline- 1 month followed by a slight increase after 3 months, however this was not statistically significant($p>0.05$). The decrease in DD following treatment with IRF was also observed in a study by Altan who reported that the DD readings were higher at baseline compared to post treatment and that the values obtained following treatment remained stable for 6 months. The reason for the decrease could be due to the low viscosity resins penetrating the porous hypomineralised enamel, filling the pores resulting in the

reduction of micro-porous structure as reported by Altan and the unchanged values after 6 month suggested a homogeneous infiltration of resin into the lesion (Altan and Yılmaz, 2022), however in our study no statistically significant decrease was observed, suggesting that resin may have not infiltrated to the deep sub-layers of the enamel lesions.

Similarly for white-creamy lesions in MCP group, DD decreased from baseline followed by a gradual increase up to 3 months. On the other hand, in yellow-brown lesions, DD increased gradually from baseline to 1 month followed by slight reduction after 3 months. However, there was no statistically significant change of DD values at all-time intervals ($p>0.05$). The decrease in DD reading in white-creamy lesion is in agreement with the study by Olgen who observed the decrease during the follow up periods for both white-creamy and yellow-brown lesions however in our study; yellow-brown lesions increased DD in the follow up period compared to baseline without presenting any significant differences($p>0.05$). The decrease in white-creamy lesions after 7 days suggested that CPP-ACP had resulted remineralisation of these lesions as observed by Olgen (Olgen et al., 2022). Similarly another study reported a decrease in DD reading of MIH teeth after being exposed to different remineralising paste (10% CPP-ACP and 10% CPP-ACP with0.2% NaF) for 1 month and found significant decrease in DD reading after treatment (Bakkal et al., 2017c). In the present study CPP-ACP was applied twice daily for 7 days (to make up-14 applications) following micro-abrasion of MIH-affected enamel, this was following a similar protocol followed by Singh SK and colleagues (Singh et al., 2021). In their study, CPP-ACP was used once daily after tooth brushing with fluoridated dentifrices for 15 days prior to the assessment of the teeth using DIAGNOdent device and found a significant decrease in DD reading(LF values) 15 days after application. Even though the number of CPP-ACP applications was almost similar to the study by Singh SK, fluoridated dentifrice was not used in our study. Additionally, the study conducted by Singh SK suggested that saliva might have also contributed to the decrease in DD reading.

Comparing between SE and the intervention groups for both white-creamy and yellow-brown lesions showed that the intervention groups had higher DD reading than SE at all-time with significance of $p<0.05$. This is in agreement with the study by Altan who reported DD reading to be higher in anterior MIH-affected teeth when compared with healthy teeth (Altan and Yılmaz, 2022) and the reason for higher DD reading in hypomineralised teeth has been reported by Farah et al to be due to high protein content and the scattering of light by the non-homogeneous enamel surface (Farah et al., 2010b).

A further change in DD reading in the intervention groups could have been found if pre-treatment of the MIH-affected teeth with pre-treatment protocols such as sodium hypochlorite (NaOCl) or use of other

remineralising agents were considered. Natarajan AK et al (Natarajan et al., 2015) found that pre-treatment protocol with either hydrochloric acid (HCl), sodium hypochlorite (NaOCl) and hydrogen peroxide (H2O2) improved penetration depths of resin and that the structure of MIH lesion post treatment came very close to that of the sound enamel suggesting decreased protein content in MIH lesion. Furthermore, studies reported a decreased in DD readings after use of CPP-ACP and CPP-ACPF on MIH teeth for one 1 month suggesting a positive effect in reducing hypomineralisation (Bakkal et al., 2017a).

5.4.1.3 Evaluation of colour

In this study, a standardized photography was used for quantifying the colour of the MIH enamel lesion. This technique has been reported and recommended by other studies to be objective, reproducible and reliable method if the camera and light source are positioned in the same orientation (Benson et al., 2000, Willmot et al., 2000, Cochran et al., 2004). Following images capture, results were then evaluated using Image J software to obtain the RGB values which were then converted to coordinates values L*, *a* * and b* using the Commission Internationale de l'E' clairage (CIE) L* *a** b*scoring method, which is the most preferred method(Johnston and Reisbick, 1997). The colour change was then calculated using the formula $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})$ ½.

L*represents the lightness value which refers to the brightness of the colour, *a* * value represents the location of the colour on the red/green axis and b* value, location of the colour on the yellow/blue axis. The ΔE value refers to the colour change that occurs in the tooth at different time intervals however it does not provide information about the direction of the colour change. In that case the ΔE and L* values were evaluated together in this study. An increase in L* values in the teeth with MIH lesions indicates an increase in brightness/lightness of the lesion area while the decrease refers to masking of the MIH lesion which appears closer to the healthy enamel (Torres et al., 2011).

ΔE value is the value used to quantify the difference between two colours with a ΔE difference of 3.7ΔE values is considered a clinical indicator for mismatching colours or colour change (Johnston and Kao, 1989) with ΔE values higher than 3.7 units indicating the clinical change (Johnston and Kao, 1989, Paul et al., 2002). In the present study, the comparison between the tooth surfaces to get the ΔE values did not

include the comparison between the MIH lesion and the healthy enamel but aimed to evaluate the changes overtime by comparing with the baseline values for each group.

In the SE group, the L* values dropped from baseline to 7 days (Mean=40.11, SD=4.69) and slightly increased after 1 month (Mean=43.35, SD=4.57) followed by another decrease after 3 month (Mean=39.61, SD=3.96). There was statistically significant decrease of L* values at all-time intervals ($p<0.05$) except for the increase observed from 7 days to 1 month. Similarly the ΔE values were significantly lowest after 1 month (Mean=12.69, SD=4.00) and highest after 3 months (Mean=17.56, SD=3.72) ($p<0.05$) however the overall ΔE values from baseline to 3 months was not statistically significant ($p>0.05$). The higher L* values at baseline could be related to dehydration of the teeth as the teeth were stored dry prior to the experiment which could be due to dehydration of the enamel prisms. This led to the lower translucency causing more reflection which masked the underlying dentine shade, hence the lighter appearance indicated by higher L*values. Similarly, the ΔE values were higher from baseline to 7 days(Mean= 17.03, SD=2.19) which explains the effect of dehydration of enamel as observed in a study by Burki and colleagues (Burki et al., 2013) who found the ΔE values to be higher in the teeth after being isolated with rubber dam and kept dehydrated for 30 min. The decrease in both L* and ΔE values observed later after being stored in artificial saliva demonstrated the rehydration of the teeth (Burki et al., 2013).In our study, L* values decreased significantly up to 3 months while ΔE values decreased up until 1 month and later increased after 3 months with the values returning and slightly higher to those of the baseline(Mean=17.56, SD=3.72), however, the overall increase in ΔE values from baseline to 3 months was not statistically significant ($p>0.05$) suggesting the colour at 3 months almost returned to be close to that of baseline.

L* values in white-creamy MIH lesions for both the intervention groups increased significantly from baseline to 7 days (IRF: Mean =50.75, SD=7.17, MCP: Mean=53.78, SD=8.15) and came close to the L * values of the SE group at baseline (Mean=54.04, SD=5.27). The L* values later decreased significantly up until 3 months for both the intervention groups (IRF: Mean =37.52, SD=5.16, MCP: Mean=36.46, SD=7.03) ($p<0.05$). Overall L* values increased for both the groups from baseline to 3 months, however this was not statistically significant ($p>0.05$). Similarly the ΔE values were higher in both the intervention groups from baseline until 7 days (IRF: Mean =17.71, SD=4.59, MCP: Mean=22.33, SD=3.52) with the highest ΔE values observed in MCP group. The ΔE values later decreased up until 3 months with a

marked reduction observed in MCP(IRF: Mean =7.70, SD=2.11, MCP: Mean=5.61, SD=2.32) and significance of $p<0.05$ bringing the desired results after 3 months.

The decrease of L* values in IRF in this study could be related to the infiltration of resin into the micro-pores and support the prism structure reinforcing the enamel and resulting in refractive index which is similar to that of the sound enamel. Similarly, Bhandari et al(Bhandari et al., 2018) observed the reduction in both whiteness (L* values) and colour change (ΔE values) of MIH teeth in children aged 7-16 years with grade I MIH immediately after treatment with IRF and 6 months following treatment, however in the present study, an increase in L* values and higher ΔE values were observed after 7 days followed by a decrease up until 3 months.

The increase in L* values 7 days following MCP could be as a result of removal of the superficial layer exposing the body of the lesion. On the other hand, a reduction in both L* values and ΔE values in MCP could be due to remineralising effect of CPP-ACP after micro-abrasion treatment to remove the hypermineralised surface and exposing the subsurface layer to the remineralising agent. Similar results were observed by Bhandari et al and reported a decrease in L* values and ΔE values immediately after micro-abrasion treatment of the grade 1 MIH teeth of 7-16 years aged children followed by a remineralisation by CPP-ACP up until 6 months(Bhandari et al., 2019). White-creamy MIH lesions which are less porous and variable depth have been reported to respond to micro-abrasion followed by CPP-ACP application however the procedure is not recommended to lesion deeper than 100µm-200µm(Lygidakis et al., 2022).

Comparing between SE and intervention groups, intervention groups had significantly lower L* values at baseline compared to SE group ($p<0.05$) and after 7 days, the L* values in the intervention group increased and were higher compared to the SE group but came close to the L* values of the SE at baseline ($p<0.05$). The reduction in L* values in IRF group suggest the effectiveness of the treatment on the white-creamy lesions which resulted in increasing the refractive index close to that of the sound enamel. The refractive index of the sound enamel ranges from 1.62-1.65 while hypomineralised enamel presents a refractive of 1.33 when rehydrated (Kidd and Fejerskov, 2004) and dropped down to a refractive index of 1.00 (Meng et al., 2009)when dehydrated and filled with air causing more light to scatter and result in a whitish , chalky and opaque appearance . When the microporosities are filled with infiltrant resin, the refractive indices increase to 1.52. Since the refractive index of IRF is 1.46

(Spagopoulos et al., 2017) which is similar to the healthy enamel, treatment of the MIH lesions with IRF results in the aesthetic improvement of the lesion. Kim observed 25% of developmental white defects to be masked completely concluding that the masking of the lesions is better when the lesion is active, in younger state with thin surface layer and not so deep (Kim et al., 2011). L* values in MCP were lower compared to those of the SE at baseline. The L* values later increased after 7 days following micro-abrasion and CPP-ACP application and came very close to the L* values of SE at baseline. This could be due to the micro-abrasion procedure which removed the hypermineralised layer exposing the deeper enamel layers for remineralisation with CPP-ACP. The marked reduction of L* values later could be as a result of remineralisation of the subsurface porosity by CPP-ACP as reported by Bhandari et al (Bhandari et al., 2019).Similarly the ΔE values reduced significantly in MCP up until 3 months indicating its effectiveness in masking the white-creamy MIH lesions.

On the other hand, the L* values in yellow-brown MIH lesions, dropped after 7 days for both the intervention groups (IRF: Mean =45.81, SD=5.56, MCP: Mean=54.86, SD=10.35) and then increased after 1 month((IRF: Mean =52.58, SD=5.61, MCP: Mean=62.41, SD=6.89) followed by another decrease after 3 months(IRF: Mean =41.54, SD=4.85, MCP: Mean=48.42, SD=10.85). On the other hand, the ΔE values decreased until after 1 month for both the groups ((IRF: Mean =12.69, SD=4.00, MCP: Mean=12.83, SD=2.54). The decrease in ΔE values was statistically significant up until 1 month ($p<0.05$). ΔE values increased again after 3 months for both the groups (IRF: Mean =21.61, SD=3.47, MCP: Mean=21.77, SD=4.92), suggesting relapse of the colour. The increase in ΔE values after 3 month in IRF group could also be related to the poor adhesion of the material to hypomineralised enamel and inability to penetrate deeper into the lesion body due to thicker surface layer which might have resulted in unstable results in the colour change. The changes in the L* and ΔE values in IRF group is believed to be dependent on the depth and activity of the lesion. The masking potential of IRF can be little or have no masking effect in deeper lesions. The material cannot infiltrate to deeper extent or/and inactive old lesion which has thicker surface layer which makes the removal of the thicker layer by surface erosion incomplete thereby blocking the penetration of resin into the lesion (Kim et al., 2011). Kim observed masking effect in only 60% of developmental defects treated with IRF (Kim et al., 2011).

Similarly, the decrease in L* values in MCP group are in agreement with the results observed in a study by Bhandari et al, who reported a decrease in whiteness(L* values) immediately after treatment with micro-abrasion and observed an increase in whiteness after 6 months (Bhandari et al., 2019). The

reduction of the L* and lowest ΔE values after MCP treatment explains the ability of micro-abrasion to remove the superficial layer of stain. Since these defects are diffuse and extend deeper into the enamel (Wong and Winter, 2002), micro-abrasion could only remove the surface layer and expose the deeper lesion with slight aesthetic improvement. Furthermore, remineralisation could not be accomplished to the full extent due to the extensive nature of the yellow-brown MIH lesions. Micro-abrasion could remove the stains favorably when they are confined to enamel layer no more than 100µm(Croll, 1989a, Bishara et al., 1987) and when stains are deeper, micro-abrasion would not be effective and stains will be left visible clinically as observed in the present study that the ΔE values in MCP group decreased after micro-abrasion and CPP-ACP up until after 1 month with significance($p<0.05$) indicating improvement in colour however, after 3 months the ΔE values increased .L*values in this group was also not stable with a decrease after7 days post treatment with MCP which increased again after 1 month and then decreased after 3 months. Overall, L* values decreased significantly from baseline to 3 months ($P<0.05$) suggesting ability in reducing the white spot lesions in MIH enamel.

Comparing between the groups, L* values for both intervention groups in yellow-brown MIH lesions were significantly higher than the SE group at different time intervals ($p<0.05$) except after 3 months where L* values in IRF were not statistically significantly compared to SE group ($p>0.05$). The higher L* values in the intervention groups suggests that both the treatment could not completely mask MIH lesions. Between intervention groups, L* values were higher in MCP group and was statistically significant at all-time intervals ($p<0.05$) except from baseline to 7 days ($p>0.05$).

Similarly, the ΔE values were statistically significant higher in both the intervention groups compared to SE group at all-time intervals except between IRF and SE group after 1 month ($p>0.05$). Comparing the ΔE values between the intervention groups in yellow-brown lesions, ΔE values were significantly higher in MCP than IRF at all-time ($p<0.05$) except after 3 months ($p>0.05$). The higher L* values in MCP group at different time may have been due to the exposure of the lesion body after micro-abrasion procedure and the inability of CPP-ACP to remineralise the entire lesion. However, the higher ΔE values (colour change) indicate the effectiveness of micro-abrasion and CPP-ACP treatment in the aesthetic improvement of yellow-brown lesions compared to IRF group due to the change in ΔE values being higher in this group.

The ΔE value is determined to be the accepted threshold value and when the ΔE values between two different time frames is more than 3.7, it indicates a clinical change (Paul et al., 2002) and according to

Johnson and Kao, a match for compared teeth in the oral cavity reported an average of 3.7 ΔE units (Johnston and Kao, 1989). In our study, ΔE values were >3.7 for all intervention groups in yellow-brown MIH lesions and IRF group in white-creamy MIH lesions indicating clinical change and potential for the treatment to mask the MIH lesions. Interestingly, for micro-abrasion followed by CPP-ACP group in white-creamy MIH lesions, 20% of lesions had ΔE values <3.7 indicating MIH lesion in this group almost completely masked and clinically invisible. When the intervention groups were evaluated with each other, the highest ΔE value after the treatment in both white-creamy and yellow-brown lesions was observed in MCP suggesting that MCP group effective in both white-creamy and yellow-brown MIH lesions because ΔE value (colour change) was the highest.

5.4.1.4 Evaluation of mineral density

When considering treatment of MIH-affected teeth, severity of the MIH is one of the major determinant factors for the choice of treatment. Teeth affected by MIH are characterized by a decreased mineral contents, lower mineral density and lower hardness and modulus of elasticity (Elhennawy et al., 2017)compared to healthy teeth. Lower mineral contents lead to lower strength and hardness of the enamel (Kodaka et al., 1992)which in turn results in enamel porosities causing increased tooth sensitivity to food, drinks and thermal changes (Elhennawy et al., 2017).

Micro-CT, a non-invasive and commonly accepted technique was used in this study to evaluate the mineral density of MIH- affected teeth. 2 samples from each group were evaluated at different time intervals (Baseline, 7 days, 1 month and 3 months).

Sample 1 in SE group had the highest mean mineral density (Mean= 2.535, SD= 0.312) at baseline and the lowest mean mineral density was observed from yellow-brown lesion (sample 5) in IRF group (mean= 1.843, SD=0.206). The SE group and white-creamy lesion in both the intervention groups had mean mineral densities above 2g/cm3 whereas the mean mineral densities in yellow-brown lesions were $\leq$ 2g/cm3. The findings in the present study are in agreement with the study by Neboda and collegues , found lowest tooth mineral density in brown lesions while white lesions had no significant different mineral density in comparison to the unaffected enamel (Neboda et al., 2017, Kurihara et al., 2017). They also found the tooth mineral density of unaffected enamel to range from 2.34 to 2.56 g/cm3, whereas in the present study, one sample had mean mineral density lower than 2.34 g/cm3(2.226 g/cm3).The lowest mineral densities in brown lesions explains why these lesions result in post-eruptive breakdown and atypical restorations compared to white lesions (Costa-Silva et al. 2011).

The mineral densities in IRF in both white-creamy and yellow-brown lesions fluctuated at different time intervals. 3 out 4 had an increase in the mean mineral density from baseline to 7 days following treatment with IRF. In addition, the overall mean mineral density increase for the same 3 samples from baseline to 3 months.

The mean mineral density for MCP groups in both white-creamy and yellow-brown lesions fluctuated at different time intervals showing increased and decreased mean mineral densities. Overall, there was an increase in one sample and a decrease in another in the mean mineral density from both white-creamy and yellow-brown lesions from baseline to 3 months after treatment with MCP. Furthermore, 3 out of 4 samples from both white-creamy and yellow-brown lesions showed a decrease in mean mineral density after 7 days post-treatment with MCP. This could be due to micro-abrasion procedure which removed the outer surface of enamel reported to have higher mineral density than inner enamel (He et al., 2011) as a result of constant exposure to saliva compared to the inner enamel (Kurihara et al., 2017)

Miyahira et al (Miyahira et al., 2017) used different dentrifices such as fluoride, CPP-ACP, and CPP-ACPF in their study on demineralized enamel to evaluate their remineralising effect using micro-CT and found that fluoride and CPP-ACPF exhibited similar results with least mineral loss, and CPP-ACP increased the enamel mineral density and decreased the depths of lesions, they concluded that, both CPP-ACP and CPP-ACPF had increased mineral density when five applications of the pastes were performed on the enamel.

Other factor which may have contributed to the results found in all groups is a role of artificial saliva, even though the night time artificial saliva had lower minerals, the pH-balanced artificial saliva formulation contained $CaCl_2$ and KH_2PO_4 to better reproduce the oral environment, a certain degree of remineralisation caused by exposure of the lesions to this solution may have occurred. It was reported that length of study could increase remineralization of the demineralized enamel when exposed to saliva (Oliveira et al., 2014) and that artificial saliva has higher mineral contents allow higher mineral deposition onto enamel than human saliva (Damian et al., 2022). Another study reported that pH of artificial saliva played a role in mineral migration between enamel and artificial saliva with released from tooth enamel to artificial saliva when the pH of artificial saliva is 5.5 and reported that immersion of specimen in artificial saliva more than 2 days with the same saliva could have significant changes in the Calcium concentration before and after immersion (Kurihara et al., 2017). In the present study, the night time artificial saliva with pH of 7 was changed every 7 days which could also have effect in the increased or decrease in mineral densities observed for all groups at different time interval.

5.5 Study limitations and challenges encountered

Small sample size is one of the limitations of this study. 50 samples were used in this study as this was the maximum amount of teeth that could be obtained during Covid-19 national lockdown. The generalisability and statistical power of the data is influenced by sample size (Polit and Beck, 2010), Therefore it is recognised that this study could be at risk of being underpowered and -have increased risk of Type II error (false negative). However in future, this study data can be used as basis for power calculation for further studies using the same methodology.

This in-vitro study was conducted to simulate a few intra-oral conditions to evaluate the effects of treatment on range of MIH-affected teeth in comparison with the healthy enamel. Night time artificial saliva was used in this study and was changed weekly, thus the replication of the complex oral environment including the effect of remineralising potential of artificial saliva is limited.

Micro-abrasion procedure was challenging since the lesions were initially exposed using nail varnish and in order to avoid contaminating the lesions with nail varnish particles during the procedure, the whole sample was covered with the laboratory parafilm.

The application of CPP-ACP following micro-abrasion treatment was performed twice daily for 7 days which might have not yielded results. Considering the busy clinical schedule, four different equipments used, their availability and their different location as well as the time frame for completion of study, it was decided that the application of CPP-ACP twice daily for 7 days could suffice. Use of CPP-ACP for at least a month would have yielded better results.

Photographs were taken using standardized parameters, such as exposure, white balance, film speed, aperture, light source, and resolution. Other tools used were cross-polarizing filters and a white balance reference card to reduce reflection and improve the accuracy in recording colour images. However, some images were darker and others lighter. This could be due to the camera battery and the influence of nail varnish might have affected the quality of the image.

5.6 Recommendations for future research

The results of this research have yielded interesting findings and further questions for future research including:

The sample size in this study was small and cannot be generalizable. Similarly, the results of the mineral density analysis using Micro-CT were used to analyse the data, could be tested for results significance therefore doubling the number of slabs used is needed to see any significance in the results of the mineral density before and after treatment with IRF and MCP. A repetition of the experiment using more MIH-affected teeth may be beneficial.

The current study was performed under in vitro conditions. Only night time artificial saliva was used as storage media in order to allow the effects of treatment to be evaluated without introducing any confounding factors. However using both night time and day time artificial saliva changed daily as an alternative to natural human saliva to replicate oral environment may yield more clinically relevant results.

The recommended etch time in IRF technique is based on that required to penetrate the hypermineralised surface layer of carious lesions. MIH-affected teeth may have different properties and the standard etching may not be sufficient to allow infiltration of the low viscosity resin fully into the lesion. Use of different pre-treatment protocols such as sodium hypochlorite to remove the protein-rich enamel prior to IRF could result in improved resin penetration into the enamel. Furthermore, fluoride treatment protocols could also be incorporated to evaluate the effect of fluoride to the affected teeth.

Micro-abrasion was performed on MIH lesions which are of varying depth. Micro-abrasion exposes these lesions for remineralization. However some of these lesions were much deeper to get remineralized to full extent. In this study, CPP-ACP was applied to the teeth twice daily for 7 days only which could not have yielded desired results as seen when DIAGNODent pen and micro-CT were used. Therefore additional studies like clinical studies may be useful to investigate the remineralizing potential of CPP-ACP with longer use on different range of MIH teeth as well as assess the clinical outcome measures such as sensitivity, aesthetic and function.

5.7 Null hypotheses outcome

From the results demonstrated in this study, it can be concluded that the null hypotheses that were made initially are either rejected or not rejected as follows:

Hypothesis 1(Rejected): There was a significant difference in surface roughness of the MIH lesions following treatment with IRF and MCP and in comparison with the SE.

In white-creamy lesions, intragroup comparison showed a significant increase in surface roughness in both the intervention groups from 7 days to 1 month with $p<0.05$ while in yellow-brown lesions, only MCP group exhibited a significant decrease in surface roughness from 1 month to 3 months ($p<0.05$). The SE group on the other hand was not statistically significant at different time intervals.

There was also statistically significant difference in surface roughness between the groups. Both white-creamy and yellow-brown MIH lesions showed a statistically higher surface roughness compared to the SE. The higher surface roughness was observed at all-time intervals between white-creamy MIH lesions and SE while yellow-brown MIH lesions exhibited higher surface roughness after 7 days, 1 and 3 months following treatment. When intervention groups were compared, IRF exhibited higher surface roughness compared to MCP after 7 days (white-creamy lesions) and 1 month (yellow-brown lesions).

Hypothesis 2 (Rejected): There was a significant difference in the DD of the MIH lesions following treatment with IRF and MCP and in comparison with the SE.

SE showed a significant decrease in DD from baseline to 7 days ($p<0.05$). On the other hand, there was no statistically significant difference in DD within the intervention groups for both white-creamy and yellow-brown lesions ($p>0.05$).

Intergroup comparison showed a statistically significant higher DD at all-time intervals in both intervention groups for both white-creamy and yellow-brown lesions in comparison to SE however, there was no statistically significant difference in DD between the intervention groups for both white-creamy and yellow-brown MIH lesions ($p>0.05$)

Hypothesis 3 (Rejected): There was a difference in colour (Lightness and colour change) of the MIH-affected teeth treated with IRF and MCP in comparison with the SE.

There was significant difference in colour for the SE with surface lightness decreasing from baseline to 3 months. There was a decrease in surface lightness in the intervention groups for yellow-brown lesions from baseline to 7 days, 1 month to 3 months and overall from baseline to 3 months. A significant increase in surface lightness (L* values) however, was seen from days to 1 month ($p<0.05$). Comparison between the groups showed a significant increase in surface lightness (L* values) in both intervention groups from baseline to 1month. In addition, MCP group showed an increase in surface lightness (L* values) even after 3 months. On the other hand, both the intervention groups in white-creamy lesions had an increase in surface lightness (L* values) from baseline to 7 days followed by a decrease up until 3 months. Intergroup comparison between SE and white-creamy MIH lesions showed a significant higher L* values (surface Lightness) at baseline compared to MIH lesions whereas the opposite was seen after 7 days with an increase in surface lightness (L* values) in white-creamy MIH lesions compared to sound enamel. No difference in surface lightness was observed between the intervention groups in both white-creamy and yellow-brown lesions.

Furthermore, the colour change(mean ΔE values)significantly decrease for both SE and yellow-brown lesion treated with IRF up until 3 month while colour change for yellow-brown lesions treated with MCP significantly decreased up to 1 month. Similarly, a significant difference was observed in colour change between the groups with IRF exhibiting higher colour change from baseline to 7 days as well as overall from baseline to 3 months than SE. While yellow-brown MIH lesions treated with MCP showed higher colour change than SE at different time intervals. A significant colour change was also observed from baseline up until 1 month between the intervention groups with higher colour change in MIH-lesions treated with MCP than the lesions treated with IRF.

For white-creamy MIH lesions, both intervention groups showed a significant decrease in colour change (mean ΔE values) at different time intervals ($p<0.05$).On the other hand, a significant higher change in colour was seen from baseline to 3months whereas an additional higher colour change was observed from baseline to 7 days when SE was compared with MIH lesion treated with MCP. Between intervention groups, a significant higher colour change was observed for MIH lesions treated with MCP than lesions treated with IRF from baseline to 7 days and from baseline to 3 months.

6 Conclusions

Based on this study's results, the following conclusion can be formulated:

1- Both IRF and MCP have the potential to reduce the surface roughness of white-creamy MIH enamel lesions 7-days following treatment whereas MCP was beneficial in surface roughness reduction of yellow-brown MIH enamel after 3 months. IRF exhibited higher surface roughness compared to MCP which was significant after 3 months for yellow-brown MIH enamel lesions and 7 days for white-creamy MIH enamel lesions. SE exhibited the lowest surface roughness compared to the intervention groups.

2- DD in both IRF and MCP groups indicated there was no difference in benefits between both minimally invasive treatments in reducing the DD of the MIH enamel lesions (both white-creamy and yellow-brown). Furthermore, dehydration of the enamel can cause an increase in the DD and rehydration of the enamel as found in the SE group led to a significant decrease in DD as observed 7-days after SE immersion in artificial saliva. SE had the lowest DD compared to intervention groups for both white-creamy and yellow-brown MIH lesions.

3- Treatment of both white-creamy and yellow-brown MIH enamel lesion with IRF and MCP were evaluated and the significant difference in the colour of MIH enamel lesion was achieved as a result of both treatments.

In this study, we found that the ΔE values calculated immediately after both IRF and MCP treatment and those calculated 1 and 3 months later were on an average, greater by a factor of ΔE 3.7 indicating that the colour change was clinically perceptible after both treatment as reported in the literature. At different time intervals, MCP resulted in the highest change in ΔE values for both white-creamy and yellow-brown MIH enamel lesion which was determined to be beneficial and effective treatment for both white-creamy and yellow-brown MIH enamel lesion than IRF. On the other hand, L* values were significantly lower in IRF compared to MCP in yellow-brown MIH lesions at all-time indicating the effectiveness of IRF in reducing the lightness/whiteness of yellow-brown MIH lesions compared to MCP. The decrease in L* values implied that the colour of the MIH enamel lesions had darkened and approached the natural tooth colour.

Sound enamel stored dry can appear lighter in colour due to dehydration as observed in SE group with L* values higher at baseline and significantly reduced up until 3 months as a result of immersion of the samples in night time artificial saliva. Similarly, ΔE values (colour change) were higher at baseline and reduced significantly up until 3 months indicating rehydration of the enamel.

4- Mean Mineral densities were the lowest for yellow-brown MIH enamel lesions while the mean mineral densities for white-creamy MIH enamel lesions were almost similar to the SE. Mean mineral densities increasing and decreasing in the intervention groups as well as SE group may suggest different contributing factors such as the surface properties of enamel in MIH and sound enamel, increased porosity of the MIH enamel, mineral transfer between enamel and artificial saliva, the effect of remineralizing agent (CPP-ACP) and micro-abrasion procedure. To confirm this more research is required with larger sample size.

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8 Lists of Appendices

Appendix 1: Application to tissue bank

Condition of teeth:
10 sound teeth and 10 MIH teeth

[X] Sound [X] Other

Provide further details here if required:
1st permanent molars as described above

[ ] Fresh [X] Dry

If fresh teeth are required, please justify why this is necessary:

Part of tooth required:

[ ] Pulp [ ] Root [X] Crown

Will any part of the teeth requested be useable by other researchers?:

Roots could be used but usually roots are destroyed

Provide the time period which tissue will be required? (e.g. numbers required per week/month/year):

It is anticipated teeth will be needed from late January 2021 onwards

Brief summary of your project in lay terms:

Aim:
1- To assess and compare the effect of Icon Resin infiltrate and micro-abrasion with CPP-ACP on the enamel surface properties and colour of 1st permanent molars teeth effected by MIH
2- To compare the mineral content of the hypomineralised areas and intact enamel of 1st permanent molars affected by MIH before and after treatment with Icon and micro-abrasion with CPP-ACP
3- To assess the surface roughness of intact enamel of 1st permanent molars and permanent molars affected by MIH before and after treatment with Icon and micro-abrasion with CPP-ACP

Inclusion criteria:
-Extracted MIH/first permanent molars with intact enamel surfaces (range of lesions: white and yellow)

Teeth sectioning:
Before sectioning, teeth will be cleaned using a toothbrush to remove any soft tissue remnants and mounted using green stick impression compound. Roots of each tooth will then be sectioned at the neck of the tooth and separated from the crowns using a water-cooled diamond wire saw or a microtome and disposed in accordance with the Human Tissue Research Banks Code of Practice. The coronal part of each tooth will be mounted using green stick impression compound

The crown of the tooth will be further sectioned into different sections using a water cooled, diamond wire saw, cutting-machine or a microtome. The buccal and palatal or lingual surface of each crown will be separated, and slabs containing a hypomineralised lesion and sound enamel will be prepared from these surfaces. The enamel slabs will then be mounted with green stick impression compound

Application form and proposed Standard Operating Procedure for use of tissue stored in the School of Dentistry Skeletal Tissues Bank

1. Application for use of Tissue from the School of Dentistry Skeletal Tissues Bank

Title of Project: Comparative evaluation of Icon (resin infiltration) and micro-abrasion with CPP-ACP on colour, mineral content and surface roughness of MIH teeth in vitro

Application number:

Lead applicant name: Salama Humaid Khalfan Al mahrugi

Co-applicant name(s) including supervisor(s):
Dr Richard Balmer, Professor David Wood, Dr Asmaa Al-Tale, Dr Jianhua Wu

Can animal samples be used for this research project: No

If yes, you are not required to complete an application for the Tissue Bank and must use animal samples

If no, please justify why human samples are necessary for the study:
Molar Incisor hypomineralisation is limited to human first permanent molars and incisors. This study will investigate two treatment modalities for MIH lesions on teeth that have been extracted due to either poor long term prognosis or are being extracted for orthodontic treatment.

Type of tissue samples required:

[ ] Bone [X] Teeth [ ] Other (please specify) _____

Type of teeth and numbers required: 1st permanent molars with MIH

Primary teeth (please insert number required in the appropriate box(es) below):

[ ] Any [ ] Molar [ ] Incisor [ ] Canine

Permanent teeth (please insert number required in the appropriate box(es) below):

20 Molar [ ] Premolar [ ] Canine [ ] Incisor [ ] Any

Provide further details here if your request is more specific, e.g. third molar teeth:

Figure: The process of enamel slab preparation in the laboratory

Baseline records will be collected for each enamel slab. The measurements will use DIAGNOdent to record fluorescence, Micro-CT to evaluate mineral content and surface topography, Profilometry to assess surface roughness and photographic evaluation to assess colour. Each slab will record two measurements - from defective enamel and from sound enamel.

The slabs will be randomly divided into two groups according to DIAGNOdent measurements and colour of the lesions prior to treatment with Icon or micro-abrasion and CPP-ACP. One group will be treated with ICON and the other group with microabrasion and CPP-ACP.

After treatment the enamel slabs will be evaluated again as described above to compare the changes that occur with the two treatments.

Storage of Enamel Slabs:
The enamel slabs will be immersed in artificial saliva after the treatments for one week before completing the post treatment evaluations to enable any further mineralisation. The slabs will be kept in an incubator at 37.0C. The artificial saliva will be changed daily to prevent contamination and bacteria growth.

Is this work part of an UG project? If yes, give details:
No

Is this work part of a PG project? If yes, give details and title of degree:

Yes. This is part of the requirements for the DRaed Dent

Is it intended that any tissue or parts of tissue (including cells grown on from primary tissue samples) will be sent outside of University of Leeds/Teaching Hospitals NHS Trust? If yes provide a contact name and address (remember to enclose a copy of the signed Tissue Transfer Agreement form if appropriate):

No

2. Standard Operating procedure for use, storage and disposal of tissue in this project

Where will the work be carried out? (e.g. Department and lab name/room number):

The work will be carried out in the Dental Histology Laboratory at the School of Dentistry. Level 6, Worsley building, University of Leeds

How will the tissue be stored, including exact location and labelling details (it is expected that all samples are suitably labelled with investigators name, date etc):
The samples will be stored in the Dental Research Laboratory at the School of Dentistry, Level 6, Worsley Building, University of Leeds.
Each sample will be stored in a micro-centrifuge tube with the investigator's name, date of collection and the same ID on it.

How will the tissue be disposed of?

[X] Clinical waste [ ] Returned to Tissue Bank

If disposed by clinical waste, provide details:
After the samples have been used in the experiment along with any remaining unwanted sectioned parts of tissue will be disposed in accordance with the Human Tissue Authority's Code of Practice

How is the proposed work to be funded?

Funded by the University of Leeds (Bench fee)

How was the work scientifically reviewed? (e.g. details should include name(s) of peer reviewer(s) and/or supervisor(s):

Discussion and decision on the methods have been done with my research supervisors, Dr Richard Balmer, Dr Al-Tale and Professor Drummond who is supervising three similar projects with different treatments.

How will the results of the work be disseminated?

[X] Peer reviewed scientific journals [X] Conference presentation
[X] D Raed Dent Thesis [ ] No plans to disseminate results
[ ] Other

Provide additional details here:

Please ensure you attach a Protocol of your research study which includes a brief description of the proposed work, highlighting the aims, objectives and methods that will be used.

I have enclosed the following documents where required (please tick which documents are being enclosed):

[x] Completed Tissue Bank application form

[x] Protocol

[ ] Tissue Transfer Agreement (if material is to be sent outside of the University of Leeds/Leeds Teaching Hospitals NHS Trust)

I confirm that the proposed work meets with the requirements of the Leeds Skeletal Tissue Bank Policy and that the tissue released under the remit of this SOP will be used for no other purpose.

Lead applicant (print name): ...Salama Humaid Khalfan Almahruqi.....

Signature: ....Salama Almahruqi.....

Date: .....15/12/2020.....

Supervisor approval:

Supervisor (print name): Dr Asmaa Al-Taie

Signature:

Date: 15/12/2020

Appendix 2: Approval email from Tissue Bank

From: Julie McDermott <J.K.McDermott@leeds.ac.uk>
Sent: 13 January 2021 10:15
To: Salama Almahruqi <dnshka@leeds.ac.uk>
Cc: David Wood <D.J.Wood@leeds.ac.uk>; Richard Balmer <R.C.Balmer@leeds.ac.uk>; Asmaa Al-Taie <A.Altaie@leeds.ac.uk>; Jianhua Wu <J.H.Wu@leeds.ac.uk>
Subject: DREC ref: 040121/SA/314

Dear Salama

DREC ref: 040121/SA/314
Study title: Comparative evaluation of Icon (resin infiltration) and micro-abrasion with CPP-ACP on colour, mineral content and surface roughness of MIH teeth in vitro

Thank you for submitting the above Tissue Bank application to the Dental Research Ethics Committee (DREC). Your application has been reviewed and I am pleased to confirm that is has been approved for 20 teeth (10 sound and 10 MIH teeth).

Please note: Due to the current Covid-19 pandemic, there are fewer teeth coming into the Bank. Please be aware that teeth may not be supplied to you all at once and will be made available to you as and when become available.

If you have any questions, please do not hesitate to contact me.
With best wishes for the success of your study.

For and on behalf of
Professor David Wood
DREC Chair

Appendix 3: Mean surface roughness (profilometry readings) in µm for the SE group at baseline, 7-days, 1 and 3 months after treatment

Sound enamel slabs	Baseline	7-days	1-month	3-months
S1	0.959	0.725	0.555	0.758
S2	0.457	0.487	0.426	0.583
S3	0.563	0.422	0.431	0.426
S4	0.516	0.506	0.656	0.712
S5	0.573	0.501	0.538	0.476
S6	0.483	0.433	0.384	0.309
S7	0.411	0.435	0.392	0.412
S8	0.749	0.436	0.517	0.771
S9	0.630	0.534	0.589	0.639
S10	0.816	0.516	0.397	0.770

Appendix 4: Mean surface roughness (profilometry readings) in µm for the white-creamy MIH lesions treated with IRF before treatment(baseline), 7-days, 1 and 3 months after treatment

White-creamy MIH slabs	Baseline	7-days	1-month	3-months
MIH21	0.725	0.849	0.864	0.594
MIH24	0.605	0.231	0.390	0.355
MIH25	0.604	0.709	1.575	0.770
MIH27	1.606	1.090	1.196	0.792
MIH30	0.675	0.693	0.937	0.895
MIH33	0.879	0.645	0.929	0.486
MIH36	1.108	0.570	0.785	0.796
MIH38	0.914	0.643	0.959	0.829
MIH39	0.859	0.685	0.982	1.278
MIH40	0.519	0.758	0.972	1.478

Appendix 5: Mean surface roughness (profilometry readings) in µm for white-creamy MIH lesions treated with MCP before treatment (baseline), 7-days, 1 and 3 months after treatment

White-creamy MIH slabs	Baseline	7-days	1-month	3-months
MIH22	0.684	1.013	1.373	1.365
MIH23	0.857	0.370	0.859	0.458
MIH26	0.837	0.379	1.080	0.381
MIH28	0.837	0.703	0.531	0.744
MIH29	0.675	0.480	0.547	0.552
MIH31	0.511	0.396	0.545	0.565
MIH32	0.527	0.549	0.554	0.486
MIH34	0.519	0.297	0.624	0.478
MIH35	0.398	0.457	0.670	0.726
MIH37	1.009	0.446	00.625	0.404

Appendix 6: Mean surface roughness (profilometry readings) in µm for the yellow-brown MIH lesions treated with IRF before treatment (baseline), 7-days, 1 and 3 months after treatment

Yellow-brown MIH slabs	Baseline	7-days	1-month	3-months
MIH2	1.113	1.075	2.592	2.084
MIH5	0.803	1.008	0.605	0.809
MIH8	0.632	0.753	0.785	0.561
MIH10	1.013	0.726	0.895	0.859
MIH12	0.612	1.171	0.642	0.763
MIH15	0.551	1.420	1.073	0.895
MIH16	0.557	1.054	0.898	0.816
MIH17	1.082	0.827	0.688	0.632
MIH19	0.438	0.916	1.137	0.723
MIH20	0.579	0.632	0.958	1.072

Appendix 7: Mean surface roughness (profilometry readings) in µm for yellow-brown MIH lesions treated with MCP before treatment(baseline), 7-days, 1 and 3 months after treatment

Yellow-brown MIH slabs	Baseline	7-days	1-month	3-months
MIH1	0.935	0.642	0.657	0.583
MIH3	0.595	1.334	0.834	0.740
MIH4	1.906	0.866	0.999	0.790
MIH6	0.530	0.860	0.801	0.710
MIH7	0.597	1.014	0.760	0.561
MIH9	0.438	0.642	0.771	0.515
MIH11	0.567	0.824	0.884	0.789
MIH13	0.796	0.518	0.433	0.571
MIH14	0.552	0.923	0.960	0.738
MIH18	0.441	1.321	0.799	0.768

Appendix 8: Multiple Comparison within SE group on Profilometry reading (Ra values in µm) before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4)

Time(I)-Time(J)	Test statistics	Degree of freedom	Asymptotic Sig. (2-sided test)
Time1-Time2	0.756	3	0.056
Time2-Time3	0.756	3	0.056
Time3-Time4	0.756	3	0.056
Time1-Time4	0.756	3	0.056

Appendix 9: Multiple Comparisons within IRF group on Profilometry reading (Ra values in µm) before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of white-creamy MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	.162	.087	.564	-.129	.453
	3	-.110	.127	1.000	-.537	.318
	4	.022	.155	1.000	-.500	.544
2	1	-.162	.087	.564	-.453	.129
	3	-.272*	.072	.027	-.515	-.029
	4	-.140	.105	1.000	-.494	.214
3	1	.110	.127	1.000	-.318	.537
	2	.272*	.072	.027	.029	.515
	4	.132	.119	1.000	-.268	.531
4	1	-.022	.155	1.000	-.544	.500
	2	.140	.105	1.000	-.214	.494
	3	-.132	.119	1.000	-.531	.268

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 10: Multiple Comparison within MCP group on Profilometry reading (Ra values in µm) before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months(Time 4) after treatment of white-creamy MIH lesions

Time(I)-Time(J)	Test statistics	Std Error	Std.Test Statistics	Sig	Adj.Sig
Time1-Time2	1.100	0.577	1.905	0.057	0.340
Time2-Time3	-1.600	0.577	-2.771	0.006	0.034
Time3-Time4	0.700	0.577	1.212	0.225	1.000
Time1-Time4	0.200	0.577	0.346	0.729	1.000

Appendix 11: Multiple Comparisons within IRF group on Profilometry reading (Ra values in µm) before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of yellow-brown MIH lesions

Time(I)-Time(J)	Test statistics	Degree of freedom	Asymptotic Sig. (2-sided test)
Time1-Time2	4.320	3	0.229
Time2-Time3	4.320	3	0.229
Time3-Time4	4.320	3	0.229
Time1-Time4	4.320	3	0.229

Appendix 12: Multiple Comparisons within MCP on Profilometry reading (Ra values in µm) before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of yellow-brown MIH lesions

Time(I)-Time(J)	Test statistics	Std Error	Std.Test Statistics	Sig	Adj.Sig
Time1-Time2	-1.100	0.577	-1.905	0.057	0.340
Time2-Time3	0.000	0.577	0.000	1.000	1.000
Time3-Time4	1.300	0.577	2.252	0.024	0.146
Time1-Time4	0.200	0.577	0.346	0.729	1.000

Appendix 13:Comparison between SE group and IRF group (white-creamy MIH lesions) on Profilometry reading (Ra values in µm) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]

	Baseline	7-days	1-month	3-months
Mann-Whitney U	23.000	16.000	9.000	21.500
Wilcoxon W	78.000	71.000	64.000	76.500
Z	-2.041	-2.570	-3.099	-2.155
Asymp. Sig. (2-tailed)	.041	.010	.002	.031
Exact Sig. [2*(1-tailed Sig.)]	.043[b]	.009[b]	.001[b]	.029[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 14: Comparison between SE group and IRF group (yellow-brown MIH lesions) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	34.000	1.000	2.000	16.000
Wilcoxon W	89.000	56.000	57.000	71.000
Z	-1.209	-3.704	-3.628	-2.570
Asymp. Sig. (2-tailed)	.226	.000	.000	.010
Exact Sig. [2*(1-tailed Sig.)]	.247[b]	.000[b]	.000[b]	.009[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 15: Comparison between SE group and MCP group (white-creamy MIH lesions) on Profilometry reading (Ra values in µm) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	38.000	40.000	15.000	45.000
Wilcoxon W	93.000	95.000	70.000	100.000
Z	-.907	-.756	-2.646	-.378
Asymp. Sig. (2-tailed)	.364	.450	.008	.705
Exact Sig. [2*(1-tailed Sig.)]	.393[b]	.481[b]	.007[b]	.739[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 16: Comparison between SE group and MCP group (yellow-brown MIH lesions) on Profilometry reading (Ra values in µm) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	46.000	4.000	5.000	35.500
Wilcoxon W	101.000	59.000	60.000	90.500
Z	-.302	-3.479	-3.402	-1.097
Asymp. Sig. (2-tailed)	.762	.001	.001	.273
Exact Sig. [2*(1-tailed Sig.)]	.796[b]	.000[b]	.000[b]	.280[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 17: Comparison between IRF group and MCP group on Profilometry reading (Ra values in µm) at baseline, 7-days, 1 and 3 months after treatment of white-creamy MIH lesions

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	32.000	23.000	26.000	24.500
Wilcoxon W	87.000	78.000	81.000	79.500
Z	-1.362	-2.041	-1.814	-1.928
Asymp. Sig. (2-tailed)	.173	.041	.070	.054
Exact Sig. [2*(1-tailed Sig.)]	.190[b]	.043[b]	.075[b]	.052[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 18: Comparison between IRF group and MCP group on Profilometry reading (Ra values in μm) at baseline, 7-days, 1 and 3 months after treatment of yellow-brown MIH lesions

Test Statistics[a]

	Baseline	7-days	1-month	3-months
Mann-Whitney U	38.500	42.000	38.000	22.500
Wilcoxon W	93.500	97.000	93.000	77.500
Z	-.870	-.605	-.907	-2.080
Asymp. Sig. (2-tailed)	.384	.545	.364	.038
Exact Sig. [2*(1-tailed Sig.)]	.393[b]	.579[b]	.393[b]	.035[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 19: Mean DIAGNOdent readings for SE group before at baseline, 7-days, 1 and 3 months

Sound enamel slabs	Baseline	7-days	1-month	3-months
S1	5.400	5.200	5.400	5.400
S2	7.800	7.000	6.600	6.200
S3	4.600	4.000	4.200	4.400
S4	6.600	6.000	6.000	5.800
S5	6.600	6.000	6.000	6.600
S6	8.600	8.400	9.800	9.600
S7	7.000	6.400	7.000	6.600
S8	5.800	5.800	6.400	6.000
S9	5.200	5.000	5.000	5.200
S10	5.400	4.800	5.000	5.200

Appendix 20: Mean DIAGNOdent readings for the white-creamy MIH lesions treated with IRF before treatment (baseline), 7-days, 1 and 3 months after treatment

White-creamy MIH slabs	Baseline	7-days	1-month	3-months
MIH21	24.000	15.000	13.600	25.000
MIH24	12.200	10.600	10.400	10.000
MIH25	19.000	18.000	17.000	13.600
MIH27	12.000	11.200	11.000	11.400
MIH30	17.600	18.000	17.200	16.600
MIH33	13.000	8.600	8.200	8.200
MIH36	19.400	18.600	18.200	18.800
MIH38	8.600	8.200	8.200	8.800
MIH39	15.800	13.000	13.600	15.600
MIH40	8.000	9.000	9.400	7.600

Appendix 21: Mean DIAGNOdent readings for the white-creamy MIH lesions treated with MCP before treatment (baseline), 7-days, 1 and 3 months after treatment

White-creamy MIH slabs	Baseline	7-days	1-month	3-months
MIH22	14.800	16.200	17.200	24.800
MIH23	27.600	17.200	17.600	21.000
MIH26	8.800	27.200	25.600	20.600
MIH28	6.600	10.600	10.800	11.800
MIH29	10.800	17.800	17.400	20.400
MIH31	21.600	7.800	8.800	8.400
MIH32	14.600	7.200	8.200	7.200
MIH34	8.000	6.800	6.600	6.400
MIH35	19.200	10.800	11.600	11.400
MIH37	13.600	13.400	12.800	14.400

Appendix 22: Mean DIAGNOdent readings for the yellow-brown MIH lesions treated with IRF before treatment (baseline), 7-days, 1 and 3 months after treatment

Yellow-brown MIH slabs	Baseline	7-days	1-month	3-months
MIH2	67.400	74.400	67.200	67.400
MIH5	91.400	67.600	67.800	63.800
MIH8	53.600	55.000	58.800	63.200
MIH10	82.400	74.000	77.000	80.600
MIH12	33.000	20.000	23.400	32.400
MIH15	33.000	25.400	30.600	27.600
MIH16	26.400	24.000	22.800	26.400
MIH17	16.000	59.800	52.600	52.600
MIH19	31.000	18.200	20.000	22.400
MIH20	33.000	46.000	31.000	30.200

Appendix 23: Mean DIAGNOdent readings for the yellow-brown MIH lesions treated with MCP before treatment (baseline), 7-days, 1 and 3 months after treatment

Yellow-brown MIH slabs	Baseline	7-days	1-month	3-months
MIH1	84.800	68.800	60.000	65.400
MIH3	66.400	73.200	54.800	65.400
MIH4	71.200	38.800	45.600	44.600
MIH6	69.000	76.400	73.600	71.200
MIH7	56.800	91.600	89.600	88.000
MIH9	92.000	75.200	73.600	72.200
MIH11	22.800	42.200	77.800	58.000
MIH13	22.000	29.600	34.800	35.000
MIH14	42.000	54.000	58.000	60.200
MIH18	47.600	25.000	29.000	28.000

Appendix 24: Multiple Comparison within SE group on DIAGNOdent reading before treatment(Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4)

Time(I)-Time(J)	Test statistics	Std Error	Std.Test Statistics	Sig	Adj.Sig
Time1-Time2	1.800	0.577	3.118	0.002	0.011
Time2-Time3	-1.100	0.577	-1.905	0.057	0.340
Time3-Time4	0.000	0.577	0.000	1.000	1.000
Time1-Time4	0.700	0.577	1.212	0.225	1.000

Asymptomatic significance are displayed

The significance level is 0.05

Appendix 25: Multiple Comparison within IRF group on DIAGNOdent reading before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of white-creamy MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	1.940	.923	.390	-1.166	5.046
	3	2.280	1.033	.328	-1.195	5.755
	4	1.400	.670	.397	-.854	3.654
2	1	-1.940	.923	.390	-5.046	1.166
	3	.340	.193	.675	-.310	.990
	4	-.540	1.193	1.000	-4.553	3.473
3	1	-2.280	1.033	.328	-5.755	1.195
	2	-.340	.193	.675	-.990	.310
	4	-.880	1.258	1.000	-5.112	3.352
4	1	-1.400	.670	.397	-3.654	.854
	2	.540	1.193	1.000	-3.473	4.553
	3	.880	1.258	1.000	-3.352	5.112

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Appendix 26: Multiple Comparison within MCP group on DIAGNOdent reading before treatment (Time 1), 7-days(Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of white-creamy MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	1.060	3.018	1.000	-9.093	11.213
	3	.900	2.801	1.000	-8.524	10.324
	4	-.080	2.779	1.000	-9.429	9.269
2	1	-1.060	3.018	1.000	-11.213	9.093
	3	-.160	.273	1.000	-1.078	.758
	4	-1.140	1.197	1.000	-5.168	2.888
3	1	-.900	2.801	1.000	-10.324	8.524
	2	.160	.273	1.000	-.758	1.078
	4	-.980	1.046	1.000	-4.500	2.540
4	1	.080	2.779	1.000	-9.269	9.429
	2	1.140	1.197	1.000	-2.888	5.168
	3	.980	1.046	1.000	-2.540	4.500

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Appendix 27: Multiple Comparison within IRF group on DIAGNOdent reading before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of yellow-brown MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	.280	5.936	1.000	-19.689	20.249
	3	1.600	4.902	1.000	-14.893	18.093
	4	.060	5.064	1.000	-16.976	17.096
2	1	-.280	5.936	1.000	-20.249	19.689
	3	1.320	2.049	1.000	-5.575	8.215
	4	-.220	2.702	1.000	-9.311	8.871
3	1	-1.600	4.902	1.000	-18.093	14.893
	2	-1.320	2.049	1.000	-8.215	5.575
	4	-1.540	1.220	1.000	-5.645	2.565
4	1	-.060	5.064	1.000	-17.096	16.976
	2	.220	2.702	1.000	-8.871	9.311
	3	1.540	1.220	1.000	-2.565	5.645

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Appendix 28: Multiple Comparisons within MCP group on DIAGNOdent reading before treatment (Time 1), 7-days (Time 2), 1month (Time 3) and 3 months (Time 4) after treatment of yellow-brown MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-.020	6.657	1.000	-22.417	22.377
	3	-2.220	8.552	1.000	-30.990	26.550
	4	-1.340	7.119	1.000	-25.288	22.608
2	1	.020	6.657	1.000	-22.377	22.417
	3	-2.200	4.415	1.000	-17.054	12.654
	4	-1.320	2.270	1.000	-8.958	6.318
3	1	2.220	8.552	1.000	-26.550	30.990
	2	2.200	4.415	1.000	-12.654	17.054
	4	.880	2.456	1.000	-7.383	9.143
4	1	1.340	7.119	1.000	-22.608	25.288
	2	1.320	2.270	1.000	-6.318	8.958
	3	-.880	2.456	1.000	-9.143	7.383

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Appendix 29: Comparison between SE group and IRF group (white-creamy MIH lesions) on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]

	Baseline	7-days	1-month	3-months
Mann-Whitney U	1.500	1.000	3.000	3.000
Wilcoxon W	56.500	56.000	58.000	58.000
Z	-3.670	-3.707	-3.558	-3.556
Asymp. Sig. (2-tailed)	.000	.000	.000	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]	.000[b]	.000[b]	.000[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 30: Comparison between SE group and IRF group (yellow-brown MIH lesions) on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	55.000	55.000	55.000	55.000
Z	-3.788	-3.781	-3.782	-3.782
Asymp. Sig. (2-tailed)	.000	.000	.000	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]	.000[b]	.000[b]	.000[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 31: Comparison between SE group and MCP group (white-creamy MIH lesions) on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	5.000	4.000	4.500	5.000
Wilcoxon W	60.000	59.000	59.500	60.000
Z	-3.408	-3.479	-3.443	-3.404
Asymp. Sig. (2-tailed)	.001	.001	.001	.001
Exact Sig. [2*(1-tailed Sig.)]	.000[b]	.000[b]	.000[b]	.000[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 32:Comparison between SE and MCP group (yellow-brown MIH lesions) on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	.000	.000	.000	.000
Wilcoxon W	55.000	55.000	55.000	55.000
Z	-3.782	-3.781	-3.784	-3.784
Asymp. Sig. (2-tailed)	.000	.000	.000	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]	.000[b]	.000[b]	.000[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 33: Comparison between IRF group and MCP group on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of white-creamy MIH lesions

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.282	.602	.151	18	.882	.400000	2.646524	-5.160140	5.960140
	Equal variances not assumed			.151	16.894	.882	.400000	2.646524	-5.186344	5.986344
7-days	Equal variances assumed	1.123	.303	-.201	18	.843	-.480000	2.386760	-5.494396	4.534396
	Equal variances not assumed			-.201	15.473	.843	-.480000	2.386760	-5.553741	4.593741
1-month	Equal variances assumed	1.561	.227	-.448	18	.660	-.980000	2.188911	-5.578732	3.618732
	Equal variances not assumed			-.448	15.557	.661	-.980000	2.188911	-5.631038	3.671038
3-months	Equal variances assumed	.933	.347	-.396	18	.697	-1.080000	2.726756	-6.808702	4.648702
	Equal variances not assumed			-.396	17.464	.697	-1.080000	2.726756	-6.821325	4.661325

Appendix 34: Comparison between IRF group and MCP group on DIAGNOdent reading at baseline, 7-days, 1 and 3 months after treatment of yellow-brown MIH lesions

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.186	.671	-.969	18	.345	-10.740000	11.080804	-34.019906	12.539906
	Equal variances not assumed			-.969	17.912	.345	-10.740000	11.080804	-34.028117	12.548117
7-days	Equal variances assumed	.001	.970	-1.085	18	.292	-11.040000	10.177317	-32.421749	10.341749
	Equal variances not assumed			-1.085	18.000	.292	-11.040000	10.177317	-32.421786	10.341786
1-month	Equal variances assumed	1.105	.307	-1.577	18	.132	-14.560000	9.230885	-33.953370	4.833370
	Equal variances not assumed			-1.577	17.768	.132	-14.560000	9.230885	-33.971531	4.851531
3-months	Equal variances assumed	1.446	.245	-1.375	18	.186	-12.140000	8.827787	-30.686492	6.406492
	Equal variances not assumed			-1.375	17.621	.186	-12.140000	8.827787	-30.715142	6.435142

Appendix 35: Surface lightness (L* values) for SE group before at baseline, 7-days, 1 and 3 months

Sound enamel slabs	Baseline	7-days	1-month	3-months
S1	56.318	47.289	46.255	38.077
S2	52.088	34.725	33.395	31.452
S3	63.230	47.456	47.757	43.202
S4	53.819	39.736	44.018	43.523
S5	43.298	34.810	38.406	35.086
S6	54.055	36.217	44.008	39.107
S7	53.798	40.698	42.613	38.730
S8	52.384	39.435	45.449	42.900
S9	51.669	37.270	42.755	41.999
S10	59.712	43.471	48.839	42.017

Appendix 36: Surface lightness (L* values) for the white-creamy MIH lesions treated with IRF before treatment (baseline), 7-days, 1 and 3 months after treatment

White/creamy MIH slabs	Baseline	7-days	1-month	3-months
MHI21	29.757	57.751	39.991	36.371
MIH24	34.028	44.157	40.330	37.457
MIH25	43.019	54.400	50.042	37.372
MIH27	37.485	48.725	40.972	35.354
MIH30	41.725	59.035	51.182	46.391
MIH33	30.189	39.552	34.471	30.769
MIH36	42.787	61.478	53.825	45.557
MIH38	31.302	48.377	41.901	33.976
MIH39	35.638	49.229	40.513	32.305
MIH40	28.586	44.774	39.760	39.697

Appendix 37: Surface lightness (L* values) for the white-creamy MIH lesions treated with MCP before treatment (baseline), 7-days, 1 and 3 months after treatment

White/creamy MIH slabs	Baseline	7-days	1-month	3-months
MIH22	46.624	67.661	57.045	48.374
MIH23	35.366	60.896	55.009	37.520
MIH26	39.466	65.797	53.664	44.646
MIH28	33.691	51.872	43.495	34.659
MIH29	32.147	55.686	47.501	37.778
MIH31	22.811	42.486	28.546	23.429
MIH32	32.633	51.676	41.410	39.585
MIH34	30.545	46.575	37.139	30.026
MIH35	34.762	59.901	37.139	34.239
MIH37	28.632	51.111	43.042	34.363

Appendix 38: Surface lightness (L* values) for the yellow-brown MIH lesions treated with IRF before treatment (baseline), 7-days, 1 and 3 months after treatment

Yellow/brown MIH slabs	Baseline	7-days	1-month	3-months
MIH2	61.344	51.394	58.727	48.096
MIH5	58.612	40.063	46.075	35.618
MIH8	66.822	54.583	57.563	45.121
MIH10	63.194	45.494	52.133	40.344
MIH12	70.162	51.070	60.672	50.577
MIH15	61.056	46.537	51.438	40.979
MIH16	57.508	41.553	48.977	37.287
MIH17	65.495	48.615	57.327	39.253
MIH19	54.774	39.067	46.345	38.007
MIH20	58.879	39.699	46.534	40.167

Appendix 39: Surface lightness (L* values) for the yellow-brown MIH lesions treated with MCP before treatment (baseline), 7-days, 1 and 3 months after treatment

Yellow/brown MIH slabs	Baseline	7-days	1-month	3-months
MIH1	61.323	48.384	52.756	40.244
MIH3	55.744	47.493	54.333	40.735
MIH4	64.526	60.449	64.047	53.542
MIH6	51.818	54.359	60.766	48.129
MIH7	67.699	45.100	48.718	39.253
MIH9	60.516	57.859	65.209	48.713
MIH11	67.898	50.112	51.849	40.802
MIH13	68.913	57.091	66.017	53.054
MIH14	65.855	60.745	66.230	49.774
MIH18	66.743	55.359	65.689	50.927

Appendix 40: Multiple Comparison within SE group on surface lightness (L* values) before treatment (Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4)

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	13.926*	1.007	.000	10.537	17.315
	3	10.688*	1.246	.000	6.497	14.879
	4	14.428*	1.485	.000	9.431	19.425
2	1	-13.926*	1.007	.000	-17.315	-10.537
	3	-3.239	.993	.059	-6.579	.102
	4	.501	1.392	1.000	-4.180	5.183
3	1	-10.688*	1.246	.000	-14.879	-6.497
	2	3.239	.993	.059	-.102	6.579
	4	3.740*	.785	.006	1.099	6.382
4	1	-14.428*	1.485	.000	-19.425	-9.431
	2	-.501	1.392	1.000	-5.183	4.180
	3	-3.740*	.785	.006	-6.382	-1.099

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 41: Multiple Comparison within IRF group on surface lightness (L* values) before treatment(Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4) after treatment of white-creamy MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-15.296*	1.756	.000	-21.202	-9.390
	3	-7.847*	.947	.000	-11.033	-4.661
	4	-2.073	1.565	1.000	-7.338	3.192
2	1	15.296*	1.756	.000	9.390	21.202
	3	7.449*	1.262	.001	3.203	11.695
	4	13.223*	1.607	.000	7.817	18.628
3	1	7.847*	.947	.000	4.661	11.033
	2	-7.449*	1.262	.001	-11.695	-3.203
	4	5.774*	1.132	.004	1.966	9.581
4	1	2.073	1.565	1.000	-3.192	7.338
	2	-13.223*	1.607	.000	-18.628	-7.817
	3	-5.774*	1.132	.004	-9.581	-1.966

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 42:Multiple Comparison within MCP group on surface lightness (L* values) before treatment(Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4) after treatment of white-creamy MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-21.698*	1.096	.000	-25.385	-18.012
	3	-10.731*	1.643	.001	-16.259	-5.203
	4	-2.794	.889	.071	-5.787	.198
2	1	21.698*	1.096	.000	18.012	25.385
	3	10.967*	1.494	.000	5.940	15.994
	4	18.904*	1.208	.000	14.840	22.968
3	1	10.731*	1.643	.001	5.203	16.259
	2	-10.967*	1.494	.000	-15.994	-5.940
	4	7.937*	1.370	.002	3.329	12.545
4	1	2.794	.889	.071	-.198	5.787
	2	-18.904*	1.208	.000	-22.968	-14.840
	3	-7.937*	1.370	.002	-12.545	-3.329

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 43: Multiple Comparison within IRF group on surface lightness (L* values) before treatment(Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4) after treatment of yellow-brown MIH lesions

Pairwise Comparisons

Measure MEASURE_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	15.977*	.960	.000	12.749	19.205
	3	9.206*	.883	.000	6.234	12.177
	4	20.240*	1.137	.000	16.415	24.064
2	1	-15.977*	.960	.000	-19.205	-12.749
	3	-6.772*	.589	.000	-8.754	-4.789
	4	4.263*	1.071	.019	.658	7.867
3	1	-9.206*	.883	.000	-12.177	-6.234
	2	6.772*	.589	.000	4.789	8.754
	4	11.034*	.962	.000	7.798	14.271
4	1	-20.240*	1.137	.000	-24.064	-16.415
	2	-4.263*	1.071	.019	-7.867	-.658
	3	-11.034*	.962	.000	-14.271	-7.798

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 44: Multiple Comparison within MCP group on surface lightness (L* values) before treatment(Time 1), 7-days(Time 2), 1month (Time 3) and 3 months(Time 4) after treatment of yellow-brown MIH lesions

Time(I)-Time(J)	Test statistics	Std Error	Std.Test Statistics	Sig	Adj.Sig
Time1-Time2	1.500	0.577	2.598	0.009	0.056
Time2-Time3	-1.200	0.577	-2.078	0.038	0.226
Time3-Time4	2.300	0.577	3.984	0.000	0.000
Time1-Time4	2.600	0.577	4.503	0.000	0.000

Appendix 45: Comparison between SE group (Sound enamel) and IRF group (white-creamy MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.721	.407	7.654	18	.000	18.585500	2.428062	13.484331	23.686669
	Equal variances not assumed			7.654	17.936	.000	18.585500	2.428062	13.483032	23.687968
7-days	Equal variances assumed	2.625	.123	-3.926	18	.001	-10.63710	2.70952	-16.32960	-4.94460
	Equal variances not assumed			-3.926	15.512	.001	-10.63710	2.70952	-16.39577	-4.87843
1-month	Equal variances assumed	1.657	.214	.021	18	.984	.05080	2.43007	-5.05458	5.15618
	Equal variances not assumed			.021	16.572	.984	.05080	2.43007	-5.08631	5.18791
3-months	Equal variances assumed	.316	.581	1.014	18	.324	2.08440	2.05570	-2.23448	6.40328
	Equal variances not assumed			1.014	16.869	.325	2.08440	2.05570	-2.25532	6.42412

Appendix 46: Comparison between SE group (Sound enamel) and MCP group (white-creamy MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.227	.639	7.815	18	.000	20.369400	2.606441	14.893470	25.845330
	Equal variances not assumed			7.815	17.411	.000	20.369400	2.606441	14.880166	25.858634
7-days	Equal variances assumed	3.485	.078	-5.128	18	.000	-15.25540	2.97466	-21.50494	-9.00586
	Equal variances not assumed			-5.128	14.370	.000	-15.25540	2.97466	-21.62005	-8.89075
1-month	Equal variances assumed	4.353	.051	-.328	18	.747	-1.04950	3.20416	-7.78119	5.68219
	Equal variances not assumed			-.328	13.309	.748	-1.04950	3.20416	-7.95537	5.85637
3-months	Equal variances assumed	5.303	.033	-1.534	18	.142	-4.78970	3.12200	-11.34877	1.76937
	Equal variances not assumed			-1.534	12.324	.150	-4.78970	3.12200	-11.57218	1.99278

Appendix 47: Comparison between SE group (Sound enamel) and IRF group (yellow-brown MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.035	.854	-3.475	18	.003	-7.747500	2.229773	-12.432079	-3.062921
	Equal variances not assumed			-3.475	17.765	.003	-7.747500	2.229773	-12.436532	-3.058468
7-days	Equal variances assumed	.632	.437	-2.476	18	.023	-5.696800	2.300535	-10.530045	-.863555
	Equal variances not assumed			-2.476	17.503	.024	-5.696800	2.300535	-10.539891	-.853709
1-month	Equal variances assumed	1.595	.223	-4.036	18	.001	-9.229600	2.286622	-14.033614	-4.425586
	Equal variances not assumed			-4.036	17.293	.001	-9.229600	2.286622	-14.047729	-4.411471
3-months	Equal variances assumed	.420	.525	-.977	18	.341	-1.935600	1.980204	-6.095855	2.224655
	Equal variances not assumed			-.977	17.299	.342	-1.935600	1.980204	-6.107970	2.236770

Appendix 48: Comparison between SE group (Sound enamel) and MCP group (yellow-brown MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]				
	Baseline	7-days	1-month	3-months
Mann-Whitney U	13.000	2.000	1.000	20.000
Wilcoxon W	68.000	57.000	56.000	75.000
Z	-2.797	-3.628	-3.704	-2.268
Asymp. Sig. (2-tailed)	.005	.000	.000	.023
Exact Sig. [2*(1-tailed Sig.)]	.004[b]	.000[b]	.000[b]	.023[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 49: Comparison between IRF and MCP group (white-creamy MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline	Equal variances assumed	.050	.825	.667	18	.513	1.783900	2.672978	-3.831819	7.399619
	Equal variances not assumed			.667	17.720	.513	1.783900	2.672978	-3.838185	7.405985
7-days	Equal variances assumed	.157	.696	-1.345	18	.195	-4.61830	3.43352	-11.83186	2.59526
	Equal variances not assumed			-1.345	17.711	.196	-4.61830	3.43352	-11.84032	2.60372
1-month	Equal variances assumed	1.236	.281	-.318	18	.754	-1.10030	3.46416	-8.37823	6.17763
	Equal variances not assumed			-.318	15.900	.755	-1.10030	3.46416	-8.44775	6.24715
3-months	Equal variances assumed	3.057	.097	-2.088	18	.051	-6.87410	3.29255	-13.79149	.04329
	Equal variances not assumed			-2.088	14.293	.055	-6.87410	3.29255	-13.92236	.17416

Appendix 50: Comparison between IRF and MCP group (yellow-brown MIH lesions) on surface lightness (L* values) at baseline, 7-days, 1 and 3 months after treatment of MIH lesion

Test Statistics[a]

	Baseline	7-days	1-month	3-months
Mann-Whitney U	40.000	18.000	20.000	22.500
Wilcoxon W	95.000	73.000	75.000	77.500
Z	-.756	-2.419	-2.268	-2.080
Asymp. Sig. (2-tailed)	.450	.016	.023	.038
Exact Sig. [2*(1-tailed Sig.)]	.481[b]	.015[b]	.023[b]	.035[b]

a. Grouping Variable: Groups

b. Not corrected for ties.

Appendix 51: Colour change (ΔE) of SE from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Sound enamel slabs	ΔE_1(T1-T2)	ΔE_2(T2-T3)	ΔE_3(T1-T4)
S1	15.702	12.911	18.962
S2	21.385	21.360	23.988
S3	16.781	17.005	20.560
S4	16.238	10.636	14.602
S5	13.621	7.741	13.022
S6	20.037	12.688	21.551
S7	16.943	12.256	16.124
S8	16.000	8.574	14.478
S9	16.416	10.617	13.754
S10	17.148	13.116	18.596

Appendix 52: Colour change (ΔE) of white-creamy MIH lesions treated with IRF from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

White/creamy MIH slabs	ΔE_1(T1-T2)	ΔE_2(T2-T3)	ΔE_3(T1-T4)
MHI21	28.160	11.633	7.954
MIH24	12.129	9.604	6.593
MIH25	11.978	10.226	9.073
MIH27	14.563	9.506	8.844
MIH30	18.762	11.833	8.000
MIH33	19.344	7.715	5.709
MIH36	19.275	11.864	5.593
MIH38	18.049	12.131	5.142
MIH39	16.873	11.734	7.869
MIH40	17.975	12.866	12.247

Appendix 53: Colour change (ΔE) of white-creamy MIH lesions treated with MCP from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

White/creamy MIH slabs	ΔE_1(T1-T2)	ΔE_2(T2-T3)	ΔE_3(T1-T4)
MIH22	21.357	11.596	4.565
MIH23	25.643	19.874	4.713
MIH26	27.807	15.850	9.874
MIH28	18.776	10.494	3.309
MIH29	24.475	16.482	7.841
MIH31	21.119	10.033	6.413
MIH32	19.344	8.803	7.063
MIH34	16.558	7.212	2.832
MIH35	25.145	3.563	3.000
MIH37	23.059	14.473	6.457

Appendix 54: Colour change (ΔE) of yellow-brown MIH lesions treated with IRF from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Yellow/brown MIH slabs	ΔE_1(T1-T2)	ΔE_2(T2-T3)	ΔE_3(T1-T4)
MIH2	17.249	10.296	13.617
MIH5	21.828	16.616	24.107
MIH8	15.891	12.645	22.461
MIH10	20.834	14.019	23.534
MIH12	20.732	10.739	19.791
MIH15	16.752	11.324	20.921
MIH16	18.961	9.663	20.885
MIH17	20.063	12.605	26.780
MIH19	22.268	17.183	23.157
MIH20	21.838	13.175	20.802

Appendix 55:Colour change (ΔE) of yellow-brown MIH lesions treated with MCP from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Yellow/brown MIH slabs	ΔE_1(T1-T2)	ΔE_2(T2-T3)	ΔE_3(T1-T4)
MIH1	23.850	17.952	24.412
MIH3	17.293	14.511	16.736
MIH4	25.974	24.545	21.779
MIH6	26.152	27.011	15.317
MIH7	26.718	24.637	30.183
MIH9	22.253	23.261	19.525
MIH11	20.810	17.577	28.927
MIH13	21.826	16.409	18.286
MIH14	20.096	18.051	22.632
MIH18	23.247	19.834	19.891

Appendix 56: Multiple Comparison within SE group on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Pairwise Comparisons

Measure MEASURE_1

(I) ΔE	(J) ΔE	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	4.337*	.861	.002	1.811	6.863
	3	-.537	.717	1.000	-2.641	1.567
2	1	-4.337*	.861	.002	-6.863	-1.811
	3	-4.873*	.582	.000	-6.582	-3.165
3	1	.537	.717	1.000	-1.567	2.641
	2	4.873*	.582	.000	3.165	6.582

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 57: Multiple Comparisons within IRF group on Colour change (ΔE) for white-creamy MIH lesion from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Pairwise Comparisons

Measure MEASURE_1

(I) ΔE	(J) ΔE	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	6.800*	1.380	.002	2.750	10.849
	3	10.008*	1.659	.001	5.141	14.876
2	1	-6.800*	1.380	.002	-10.849	-2.750
	3	3.209*	.696	.004	1.167	5.251
3	1	-10.008*	1.659	.001	-14.876	-5.141
	2	-3.209*	.696	.004	-5.251	-1.167

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 58: Multiple Comparisons within MCP group on Colour change (ΔE) for white-creamy MIH lesion from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Pairwise Comparisons

Measure MEASURE_1

(I) ΔE	(J) ΔE	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	10.490*	1.352	.000	6.526	14.455
	3	16.722*	.960	.000	13.906	19.538
2	1	-10.490*	1.352	.000	-14.455	-6.526
	3	6.231*	1.303	.003	2.408	10.055
3	1	-16.722*	.960	.000	-19.538	-13.906
	2	-6.231*	1.303	.003	-10.055	-2.408

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 59: Multiple Comparisons within IRF group on Colour change (ΔE) for yellow-brown MIH lesion from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Pairwise Comparisons

Measure MEASURE_1

(I) ΔE	(J) ΔE	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	6.610*	.712	.000	4.461	8.758
	3	-2.297	1.113	.219	-5.654	1.060
2	1	-6.610*	.712	.000	-8.758	-4.461
	3	-8.907*	1.032	.000	-12.018	-5.796
3	1	2.297	1.113	.219	-1.060	5.654
	2	8.907*	1.032	.000	5.796	12.018

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 60: Multiple Comparisons within MCP group on Colour change (ΔE) for yellow-brown MIH lesion from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Pairwise Comparisons

Measure MEASURE_1

(I) ΔE	(J) ΔE	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	2.443*	.720	.024	.332	4.554
	3	1.053	1.633	1.000	-3.738	5.844
2	1	-2.443*	.720	.024	-4.554	-.332
	3	-1.390	2.027	1.000	-7.336	4.556
3	1	-1.053	1.633	1.000	-5.844	3.738
	2	1.390	2.027	1.000	-4.556	7.336

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 61: Comparison between SE group and IRF group (white-creamy MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	1.867	.189	-.424	18	.676	-.683705	1.611618	-4.069589	2.702179
	Equal variances not assumed			-.424	12.915	.678	-.683705	1.611618	-4.167733	2.800323
ΔE_2(T1-T3)	Equal variances assumed	2.374	.141	1.307	18	.208	1.779200	1.361332	-1.080853	4.639253
	Equal variances not assumed			1.307	11.758	.216	1.779200	1.361332	-1.193671	4.752071
ΔE_3(T1-T4)	Equal variances assumed	5.799	.027	7.285	18	.000	9.861300	1.353597	7.017499	12.705101
	Equal variances not assumed			7.285	14.260	.000	9.861300	1.353597	6.963080	12.759520

Appendix 62: Comparison between SE group and MCP group (white-creamy MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	3.639	.073	-4.042	18	.001	-5.301185	1.311674	-8.056909	-2.545460
	Equal variances not assumed			-4.042	15.106	.001	-5.301185	1.311674	-8.095238	-2.507132
ΔE_2(T1-T3)	Equal variances assumed	.873	.362	.428	18	.674	.852400	1.993183	-3.335123	5.039923
	Equal variances not assumed			.428	17.349	.674	.852400	1.993183	-3.346406	5.051206
ΔE_3(T1-T4)	Equal variances assumed	3.905	.064	8.624	18	.000	11.957000	1.386557	9.043951	14.870049
	Equal variances not assumed			8.624	15.068	.000	11.957000	1.386557	9.002788	14.911212

Appendix 63: Comparison between SE group and IRF group (yellow-brown MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	.570	.460	-2.594	18	.018	-2.614634	1.007906	-4.732167	-.497101
	Equal variances not assumed			-2.594	17.959	.018	-2.614634	1.007906	-4.732511	-.496757
ΔE_2(T1-T3)	Equal variances assumed	.620	.441	-.091	18	.929	-.136100	1.498697	-3.284746	3.012546
	Equal variances not assumed			-.091	15.232	.929	-.136100	1.498697	-3.326257	3.054057
ΔE_3(T1-T4)	Equal variances assumed	.701	.414	-2.510	18	.022	-4.041800	1.610068	-7.424427	-.659173
	Equal variances not assumed			-2.510	17.915	.022	-4.041800	1.610068	-7.425573	-.658027

Appendix 64: Comparison between SE group and MCP group (yellow-brown MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	1.478	.240	-4.930	18	.000	-5.794840	1.175349	-8.264156	-3.325524
	Equal variances not assumed			-4.930	16.517	.000	-5.794840	1.175349	-8.280146	-3.309535
ΔE_2(T1-T3)	Equal variances assumed	.678	.421	-4.200	18	.001	-7.688400	1.830559	-11.534261	-3.842539
	Equal variances not assumed			-4.200	17.965	.001	-7.688400	1.830559	-11.534795	-3.842005
ΔE_3(T1-T4)	Equal variances assumed	.396	.537	-2.157	18	.045	-4.205100	1.949620	-8.301099	-.109101
	Equal variances not assumed			-2.157	16.768	.046	-4.205100	1.949620	-8.322783	-.087417

Appendix 65: Comparison between IRF and MCP group (white-creamy MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	.019	.891	-2.523	18	.021	-4.617480	1.830385	-8.462977	-.771983
	Equal variances not assumed			-2.523	16.846	.022	-4.617480	1.830385	-8.481944	-.753016
ΔE_2(T1-T3)	Equal variances assumed	8.432	.009	-.572	18	.574	-.926800	1.619418	-4.329071	2.475471
	Equal variances not assumed			-.572	10.888	.579	-.926800	1.619418	-4.495597	2.641997
ΔE_3(T1-T4)	Equal variances assumed	.445	.513	2.112	18	.049	2.095700	.992065	.011448	4.179952
	Equal variances not assumed			2.112	17.850	.049	2.095700	.992065	.010189	4.181211

Appendix 66: Comparison between IRF and MCP group (yellow-brown MIH lesions) on Colour change (ΔE) from baseline-7 days (T1-T2), baseline-1month (T2-T3) and baseline-3months (T1-T4)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ΔE_1(T1-T2)	Equal variances assumed	.462	.505	-2.660	18	.016	-3.180206	1.195755	-5.692394	-.668018
	Equal variances not assumed			-2.660	16.896	.017	-3.180206	1.195755	-5.704217	-.656195
ΔE_2(T1-T3)	Equal variances assumed	4.986	.038	-4.882	18	.000	-7.552300	1.547109	-10.802655	-4.301945
	Equal variances not assumed			-4.882	14.838	.000	-7.552300	1.547109	-10.853016	-4.251584
ΔE_3(T1-T4)	Equal variances assumed	1.471	.241	-.086	18	.933	-.163300	1.903356	-4.162102	3.835502
	Equal variances not assumed			-.086	16.197	.933	-.163300	1.903356	-4.194249	3.867649

Appendix 67: Intra-examiner reliability for DIAGNOdent(DD) reading

Correlations

			DIAG1	DIAG2
Spearman's rho	DIAG1	Correlation Coefficient	1.000	.973**
		Sig. (2-tailed)	.	.000
		N	10	10
	DIAG2	Correlation Coefficient	.973**	1.000
		Sig. (2-tailed)	.000	.
		N	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 68: Intra-examiner reliability for Surface roughness in (Profilometry reading-Ra)

Correlations

			Prof1	Prof2
Spearman's rho	Prof1	Correlation Coefficient	1.000	.964**
		Sig. (2-tailed)	.	.000
		N	10	10
	Prof2	Correlation Coefficient	.964**	1.000
		Sig. (2-tailed)	.000	.
		N	10	10

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 69: Intra-examiner reliability for colour (surface lightness-L* values)

Correlations

			Colour1	Colour2
Spearman's rho	Colour1	Correlation Coefficient	1.000	.867**
		Sig. (2-tailed)	.	.001
		N	10	10
	Colour2	Correlation Coefficient	.867**	1.000
		Sig. (2-tailed)	.001	.
		N	10	10

**. Correlation is significant at the 0.01 level (2-tailed).