Enhancing the Shear Bond Strength of Composite Restorations in Defective Enamel of Molar Incisor Hypomineralisation using Self-Assembling Peptide (SAPs) (P_{11-4}) in vitro.

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

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II. Abstract

Background: Molar Incisor Hypomineralisation (MIH) represents a prevalent enamel defect. While resin composites are often the treatment of choice, there is evidence indicating that resin composite has a weaker bond strength to MIH teeth when compared to sound enamel. Here, we investigate if pre-treatment of hypomineralised enamel of MIH teeth with Curodont™ Repair Self-Assembling Peptide (SAPs) P_{11-4} improves the bond strength of resin composite restorations to MIH teeth.

Method: Enamel specimens were collected from 25 sound and 50 MIH teeth. Baseline analysis consisted of Laser Fluorescence to assess the mineral content of sound teeth and hypomineralised enamel in MIH teeth. Followed by digital photography, and colour measurements which were used to divide the MIH specimens into two distinct severity groups (mild/moderate and severe). Enamel specimens were divided into three groups: Sound (SE), hypomineralised enamel (MIH), and SAPs-pre-treated enamel (MIH+T), (n=25 each). To produce a smooth enamel surface, crowns were examined and sectioned into 3x3 mm enamel specimens which were then placed in resin blocks. Specimens were initially etched with 37% wt phosphoric acid for 20 s, and then, only group (MIH+T) surfaces were pretreated with P_{11-4}. All the groups were followed by applying Optibond Solo (Kerr Corporation), then light-cured using Demi Plus LED Light Curing System for 40s (Kerr). Resin composite cylinder Filtek Supreme Universal (3M Oral Care ESPE) was then attached to each enamel specimen as per ISO 29022. Specimens were stored in phosphate-buffered saline (PBS) storage media for 24 hours in 37 °C incubator before testing. Each group underwent ISO 29022- notch-shear bond strength (SBS) testing using an Universal Testing Machine (UTM) at a crosshead speed of 1 mm/min until failure. Further analysis was conducted on 15 representative specimens from the three groups (SE), (MIH), (MIH+T) (n=5 each), using Micro CT to measure the mineral density, 9 representative specimens (n=3 each) for Scan Electron Microscope (SEM) and Energy-dispersive X-ray (EDX) to understand the type of the failure. SPSS software version 28 was used to analyse the data.

Results: The mean SBS of composite to sound teeth in group (SE) was 15.87 MPa +/-12.87 (SD), and for group (MIH) teeth, the mean value was 1.69 MPa +/-0.81 (SD), and for the group (MIH+T) it was 4.5 MPa +/- 1.45 (SD). There was a significant difference between all three groups (SE), (MIH) and (MIH +T) $P<0.001$, with a significant difference between (SE) and (MIH) with $P<0.001$, (MIH) and (MIH+T) $P<0.001$, and (SE) and (MIH+T) $P<0.001$.

Conclusion: $P_{11\text{-}4}$ enhanced the SBS of resin composites to MIH affected teeth in vitro when compared to MIH teeth without pre-treatment. However, this remained significantly lower than SBS to sound enamel.

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VI. List of Abbreviations

AI	Amelogenesis Imperfecta.
AMEL	Amelogenin.
APF	Acidulated Phosphate Fluoride.
Bis-GMA	Bisphenol A-glycidyl methacrylate.
COOH	carboxyl group.
CPP-ACP	Casein Phosphopeptide–Amorphous Calcium Phosphate.
DDE	Developmental Defects of Enamel.
DSLR	Digital Single Lens Reflex.
EDX	Energy Dispersive Xray.
FDI	World Dental Federation.
GIC	Glass Ionomer Cement.
HAP	Hydroxyapatite.
HEX	Colour code
HNSCs	Human Neural Stem Cells.
IQR	Interquartile range.
IR	Infrared.
ISO	International Organization for Standardization.
Kg	Kilograms.
Kg/cm2	Kilograms per square centimetre.
Lb.	Pounds.
lb./in2 or psi	Pounds per square inch.
LDI	Leeds Dental Institute.
LED	Light Emitting Diode.
LF	Laser Fluorescence.
Micro-CT	Micro-Computed Tomography.
MIH	Molar Incisor Hypomineralisation.
MPa	Megapascals.
N	Newtons.
NaF	Sodium Fluoride.
NH3	Trihydridonitrogen.

PBS	phosphate-buffered saline.
PCBs	Polychlorinated Biphenyl.
PEB	Post-Eruptive Breakdown.
PFM	Permeant First Molar.
PO_4^3	Phosphate ion.
QLF	Quantitative Light-induced Fluorescence.
RBC	Resin Based Composite.
RMGIC	Resin-modified glass ionomer cement.
ROI	Regions of interest.
SAPs	Self-Assembling Peptide.
SBS	Shear Bond Strength.
SD	Standard deviation.
SEM	Scanning Electron Microscope.
SSC	Stainless Steel Crowns.
TBS	Tensile Bond strength.
TEGDMA	Triethylene Glycol Dimethacrylate.
UK	United Kingdom.
μSBS	Micro Shear Bond Strength.
μTBS	Micro Tensile Bond Strength.
wt	Weight
v/wt	Volume / weight

CHAPTER 1: INTRODUCTION

Molar Incisor Hypomineralisation (MIH) is a condition that currently affects around 25% of the general population globally [7]. It is characterised by defects in the enamel of permanent dentition, mainly the first molars and, to a lesser extent, the incisors [8]. The condition impacts patients negatively, lowering their confidence and socialisation. [9], increasing the affected teeth sensitivity, and diminishing the effectiveness of applied anaesthesia [10]. The latter exaggerates the level of dental fear, especially during painful procedures, rendering their management more challenging [11]. Moreover, occlusal forces may cause the loss of the defective enamel, resulting in Post-Eruptive Breakdown (PEB), sharp edges [12], exposed dentine [13, 14] and increased liability for decay [15]. It is noteworthy that MIH may co-occur with one or more cases of hypoplastic dentition. The latter is dependent on factors such as the timing, duration, severity, and the individual's susceptibility to trauma [16].

Treatment options are various and range from simple restorative procedures to extractions [17, 18]. The treatment choice depends on several factors, including the case’s severity [13], the dental developmental stage, the presence of sensitivity, the general oral hygiene, the assessment of the risk of developing dental decay, the patient’s cooperation, and the convenience of the restoration. The latter must possess the capability to form strong and durable bonds with dental tissue [19]. However, more extensive scenarios may necessitate the application of stainless-steel crowns (SSCs), which can neglect the aesthetical considerations. Therefore, alternatives were opted for when possible, such as resin-based composites (RBCs), glass ionomer cement (GICs), and Resin-modified glass ionomer cement (RMGIC) [18]. Additionally, the application of indirect alloys was proposed and investigated in particular instances [20]All these restorative materials require enhancing their adherence to MIH-affected enamel to extend teeth's lifespan, highlighting the ongoing challenges in MIH treatment.

The altered composition of the enamel in MIH teeth, characterised by reduced mineral and increased protein content, poses a challenge to the bonding process as the diffusion of the acidic etchant into the crystals is limited [8, 21, 22]. RBCs are commonly used as restorative materials for MIH treatment [23, 24]; they are

highly aesthetic and resistant to wear. However, prior to their application, its recommended to remove all porous enamel [25],to eliminate the cracks. This ensures a successful bonding through the formation of a 'uniform hybrid layer' at the enamel/adhesive interface, hence preventing micro-leakage [14, 26]. While the decrease in the bonding strength between RBCs and hypomineralised enamel has been reported in several studies [24, 27], RBCs are still recommended for MIH treatment whenever it's clinically required [27]. Therefore, it's crucial to understand the alterations between the dental tissue and the materials when considering using bonded materials for intervention or restorative treatments.

Attempts were made to ameliorate the base material (MIH defective enamel) to increase their bond strength and bond longevity to restorations [19]. Self-Assembling Peptides (SAPs), especially P_{11-4}, have recently emerged as a technique for repairing enamel defects and infiltrating demineralised lesions [28]. The ability of these peptides to self-assemble allows them to form supra-molecular structures that are capable of sequestering calcium and, subsequently, phosphate, which leads to the deposition of minerals within the subsurface lesion. This enamel remineralisation was shown in vitro and was proven clinically [29, 30]. However, to our knowledge, no studies have been conducted to investigate whether applying P_{11-4} to the defective enamel lesion of MIH teeth improved their shear bond strength to composite restorations. Hence, this was the purpose of performing this study.

CHAPTER 2: LITERATURE REVIEW

2.1 Dental Enamel

Dental enamel is a hard tissue structure that contains high mineral concentration and molecular organisation, making it the toughest tissue in the human body [31]. The enamel's composition consists predominantly of inorganic crystallites, making up roughly 87% of its volume and 95% of its weight, arranged in an ordered fashion and displaying a high density. However, the amount of organic substance is less than 1%. A characteristic feature of enamel tissue is that, once tooth eruption is complete, it has a reduced capacity for regeneration or self-healing [32] as the layer of cells that forms it is lost post-tooth eruption [33]. This is particularly important in hypomineralised teeth, where the teeth are incapable of self-healing and, therefore, warrant external intervention.

2.1.1 Enamel Structure

Enamel development and mineralisation is a complex process that is regulated by ameloblast cells [34]. The enamel matrix secretes structural proteins (ENAM) such as Amelogenin (AMELx), Ameloblastin (AMBN), and Enamelin, which are an important constituent of the matrix, making up almost 90% of it [35]. The Amelogenin protein is involved in the process of mineralisation and morphological variations that occur in enamel. Amelogenin's structure is made up of two self-assembled parts: the amino terminal, which is hydrophobic, and the carboxy terminal, which is hydrophilic. The former contains a concentrated amount of histidine, which is capable of absorbing hydrogen ions and buffering the enamel fluid [36]. Meanwhile, the latter shows a remarkable ability to link to hydroxyapatite crystals as it is vigorously attracted to them. Additionally, at the protein center, there is a third terminal which forms a tightly packed core within the nanospheres. This core is encircled by elongated tails that extend from both ends of the molecule [37]. It undergoes proteolytic cleavage post-protein secretion that controls the formation of robust linkages with the enamel crystals. On the other hand, the ability of these cleaved products to attract enamel crystals is limited. As the enamel development and mineralisation proceed, eradication of the majority of the ENAM occurs, especially AMELx, which disappears

completely. This eradication occurs through their continuous degradation by specific proteases. Gradually, the mineral ions - calcium and phosphorus- replace the original enamel components, leading to the complete mineralisation of the hard, mature enamel [36] (shown in Figure 1).

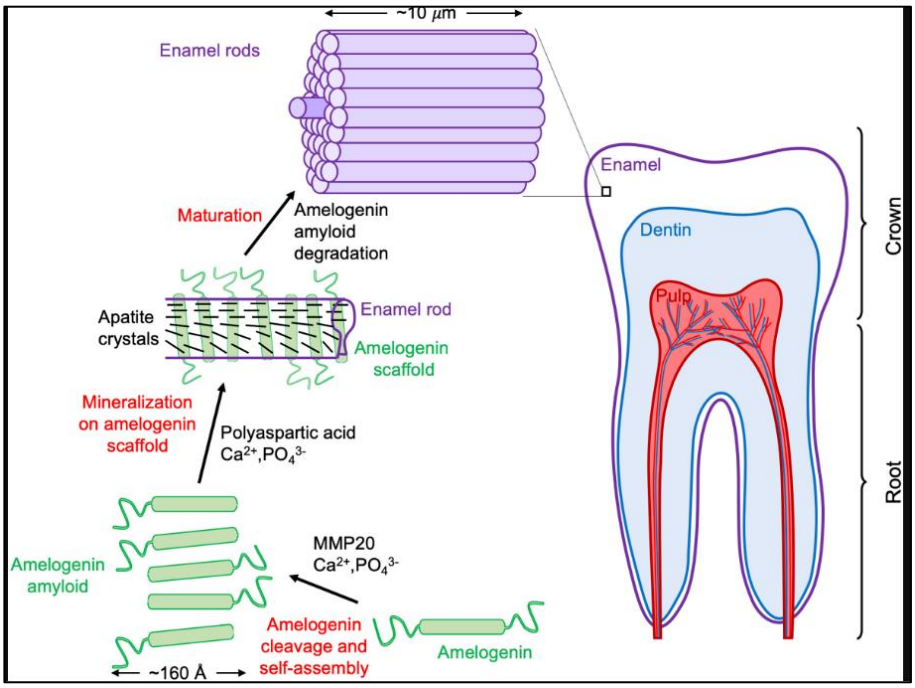

Figure 1: Oriented growth of apatite crystals in enamel tissues. MMP20 self-assembles into amyloid-like structures that guide the growth of apatite minerals along one direction. The packing of the resulting rods gives enamel its unique microstructure and therefore its mechanical properties. This Figure is sourced from an open-access repository, no permission from the publisher was required [2].

2.1.2 Developmental Defects of Enamel (DDE)

Tooth development and mineralisation can be influenced by genetic and environmental factors. Interruption of the ameloblasts may disturb the quantity and/or quality of the enamel [38], resulting in Developmental Defects of the Enamel (DDE) [39]. DDEs can be categorised as quantitative defects, where the enamel thickness is reduced and hence insufficient organic matrix formation (hypoplasia) [40] (illustrated in Figure 2 (f)), or qualitative, where the defect is due to the increased enamel porosity that causes changes to the translucency, presenting as opacities (hypomineralisation) [41]. One of the qualitative enamel

defects is Molar Incisor Hypomineralisation (MIH), which is characterised by hypomineralised lesions that are well-demarcated (shown in Figure 2 (b)) and asymmetrical. The location of these lesions is usually the incisal or cuspal third of the crown, while the cervical third is spared. In some cases, more than one type of defect may present at the same time.[38].

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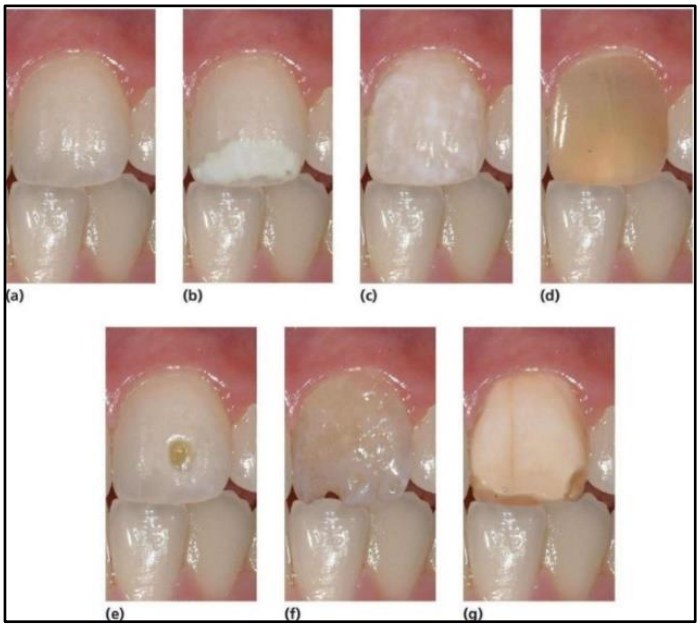


Figure 2 illustrates the classification and instances of enamel defects, including (a) sound tooth enamel, (b) distinct opaque white lesions, (c) diffused opaque white lesions, (d) enamel discolouration, (e) solitary hypoplastic enamel, (f) multiple hypoplastic defects, and (g) post-eruptive harm caused by enamel hypomineralisation. This Figure is sourced from an open-access repository, no permission from the publisher was required [3]

Differentiating between MIH and other lesions is mandatory to apply the most suitable treatment scenario. (Shown in Figure 3 and Figure 4). This can be challenging as the lesions give a similar presentation opacity. Lesions can be inherited, developed later or caused by trauma. Among the possible inherited conditions is Amelogenesis Imperfecta (AI) (presented in Figure 4). Usually, the condition runs in the family, affecting both primary and permanent dentitions [17]. Additionally, it may manifest as a combination of hypoplastic (quantitative) and hypomaturation (qualitative) defects with a high level of residual Amelogenins. In contrast, MIH is a hypocalcified (qualitative) defect with a normal level of residual Amelogenins [42]. Meanwhile, fluorosis lesions (depicted in Figure 3 (B)) are

usually bilateral and symmetrical, as opposed to MIH's well-demarcated and asymmetrical lesions (demonstrated in Figure 2 (C) and Figure 3 (D)); the lesions can be diffuse, linear, or sometimes patchy.

Another type of lesion would be hypoplastic and hypomineralised enamel. The lesion's borders are smooth and regular, as opposed to MIH. Amongst the non-inherited conditions that develop later and may present as opaque lesions is the development of early enamel carious lesions [17] which is represented by whitish opaque lesions and usually appear at the teeth' cervical margin, where plaque accumulates (indicated in Figure 3 (A)). In contrast, MIH lesions are located at the incisal or cuspal third of the crown and usually spare the cervical third [43]. Finally, trauma may cause Turner's hypoplasia. The lesion is confined to the traumatised tooth, though its location, border, form, and even colour may differ (shown in Figure 3(C)) [17].

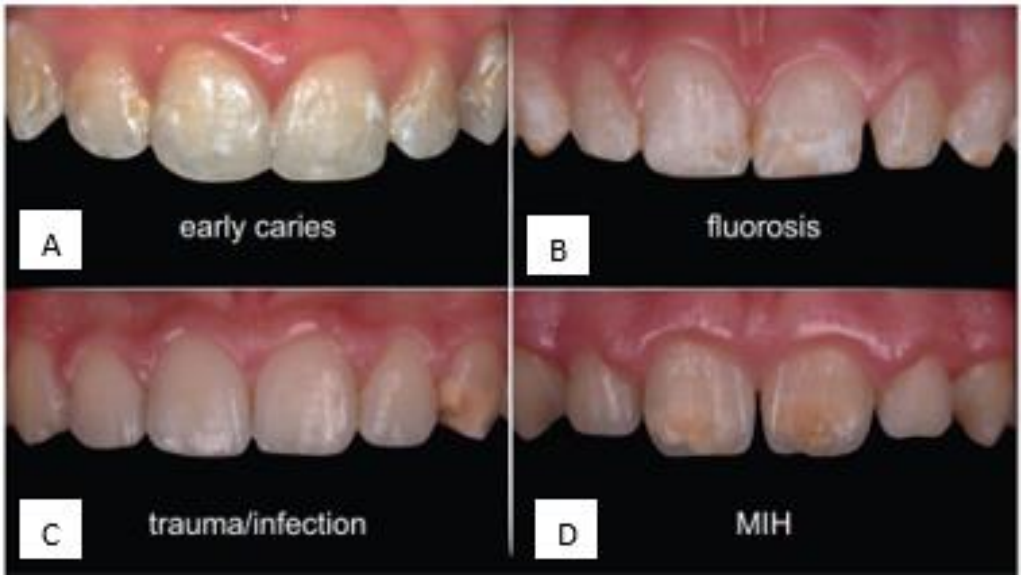


Figure 3 illustrates the clinical presentation of different enamel defects (A) early caries on facial, cervical and proximal surfaces of the teeth. (B) Fluorosis which shows generalised diffused discoloration on enamel surface. (C) represent Trauma/ infection affected tooth which is localised. (D) MIH with well demarcated discoloration of the facial surface of the upper central incisors. This Figure is sourced from an open-access repository, no permission from the publisher was required From [4]

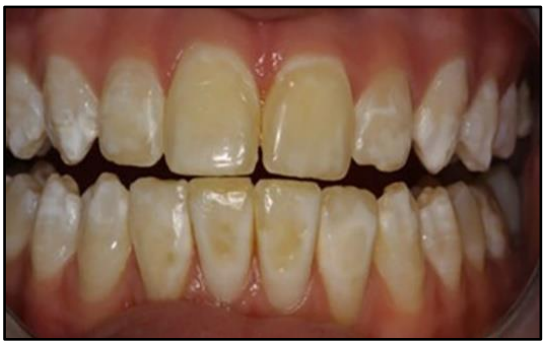


Figure 4 Amelogenesis imperfecta which represent generalised discoloration of the teeth. This Figure is sourced from an open-access repository, no permission from the publisher was required from [1]

The status of DDEs as a public health concern is debatable [44-46]. Usually, a disease becomes a public health concern due to its widespread occurrence or severe impact, especially if it's rare. This severity encompasses adverse effects on affected individuals' functionality, psychological well-being, and social interactions [47, 48]. DDEs, which may not have a high prevalence, may influence tooth esthetics and impact a person’s daily activities [47], may affect the mastication and tend to increase tooth sensitivity [49-51]. Furthermore, when contemplating restorative measures for tooth treatment, difficulties are encountered with tooth (restoration-enamel) bonding and anaesthesia [52]. Therefore, a primary approach for DDE should be prevention [53, 54] and avoiding extensive treatment. Studies on MIH, for example, have also proven that teeth with such lesions often require more invasive, complex and repetitive treatment compared to unaffected patients [55], and, as such, patients are more likely to develop dental anxiety [47].

2.2 Molar Incisor Hypomineralisation (MIH)

2.2.1 Definition and Prevalence

Molar Incisor Hypomineralisation (MIH) is a dental condition characterised by distinct opacities in the structure of the enamel. Most of the time, it affects the one

to four first permanent molars as well as the incisors. [56]. MIH is commonly seen in the UK and worldwide [57]. The prevalence of MIH reported in the literature ranged between 5-25 % worldwide, while 14.6% in the UK [7]. It is crucial to note that the prevalence of MIH often varies. This could be because there aren't any diagnostic standards or guidelines. Consequently, this renders the diagnosis rather subjective, primarily depending on the clinician's interpretations, leading to faulty classification or even missed cases [58], which may be due to a lack of a standardised method for recording the number of cases diagnosed [56].

2.2.2 MIH: Organic and Inorganic Content

The inorganic and organic materials of the MIH's enamel may present with developed anomalies [59]. The amount of mineral content in the enamel of MIH teeth is significantly reduced to 58.8%, compared to that of sound teeth, at 96%. MIH-affected teeth also have reduced concentrations of phosphate and calcium - which composes a 5-20% reduction of inorganic material [60], and a disorganised crystalline structure with thin rods [19] - diminishing the hardness and elastic modulus [19, 61]. Farah et al. studied whether the visual assessment of the defect discolouration's intensity is a clinical indicator of its severity. The mineral density was assessed and was found to be lower in intensively discoloured lesions (brownish) compared to lightly discovered ones (whitish or creamy). Then, the protein content was assessed and compared to that of sound enamel. It was found to be 8-21-fold higher in yellow and brown enamel, respectively, compared to sound enamel. Additionally, the protein content in whitish lesions was higher than that of sound enamel; however, it was lower compared to yellowish and brownish lesions. This signifies that the protein content increased as the discolouration increased. Amongst the increased proteins were serum albumin (which is hypothesised to cause the porous hypomineralisation of the enamel [62]), alpha-1 antitrypsin, and antithrombin III than white/opaque and sound enamel [59]. These variations of content properties have resulted in the hypomineralisation of the enamel and reduced binding strengths [24, 61, 63, 64]. In addition, according to the findings of the systematic review on bonding to hypomineralised enamel, the bond strength of resin dental adhesives to hypomineralised enamel is lower than sound enamel. The majority

of the studies incorporated in the review revealed that resin composites exhibited a significant lower bond strength to hypomineralised enamel. The altered mineralisation and structure of hypomineralised enamel may pose difficulties in employing resin adhesives to form durable and long-lasting bonds [65].

2.2.3 Etiology

The exact cause of MIH is still undetermined [66, 67], although several causes were identified in the pathogenesis and were presumed to be a result of multifactorial mechanisms [66-68]. Various reported etiological factors contribute to MIH, such as pyrexia, gestational diabetes, prolonged delivery, and caesarean section birth, to illnesses like upper respiratory infections, otitis media, and high fever [69]. Moreover, Jan et al. found a correlation between DDEs and serum concentration of Polychlorinated Biphenyls (PCB), suggesting exposure to toxins such as (PCBs) could be a potential risk factor for developing MIH-like enamel abnormalities [70]. Although pre-, peri- and neonatal issues are associated with an increased frequency of DDEs in general, especially with primary dentition, additional studies need to be undertaken to provide evidence for MIH. The same goes for the evidence that early childhood malnutrition is correlated with increased incidence of DDEs, and further studies need to investigate their direct connection [71]. It should be noted that DDEs are more common in medically challenged populations, especially those who are diagnosed during their first 2-4 years (like developing respiratory illness or seizures) when compared to those without MIH [69].

2.2.4 MIH Management.

The nature of the anomalies of MIH teeth may pose several clinical challenges to its treatment; these challenges may include those affecting the patient themselves, such as tooth sensitivity [72] and esthetic presentation, and those faced by the dentist, such as restorative challenges – due to the reduced the bonding strength. Patients with MIH teeth often experience heightened sensitivity, particularly to cold temperatures and during teeth brushing, which can have a negative impact on their daily lives [11]. This heightened sensitivity may also contribute to an increased risk of caries or plaque accumulation due to the

porous enamel, allowing bacteria to penetrate through dentinal tubules and elicit chronic inflammation in the pulp [17, 72]. As a result, it becomes challenging to achieve adequate local analgesia for clinical management, thus placing patients at a higher need for behaviour management issues and dental fear and anxiety [11, 73]. Furthermore, the challenge of bonding adhesive dental materials to MIH teeth arises from the enamel's high protein content, often requiring multiple repetitive treatments [74], which may also increase dental fear and anxiety [75].

The available treatment options include preventive measures (mineralisation), restorative procedures, and extraction [76]. Recent research is investigating therapeutic solutions using materials for teeth affected by MIH, especially in cases where restoration is not viable and tooth loss is possible. The need for restorative materials can be postponed or eliminated by implementing comprehensive individualised prevention plans and strategies. For example, fissure sealants can be used to prevent cavities and provide coronal protection on molar teeth [77]. Furthermore, teeth that are hypersensitive or only partially erupted can be temporarily sealed using glass-ionomer materials. Nevertheless, it's essential to consider potential clinical issues, such as the retention of resin sealants. A study revealed a decrease in the retention of fissure sealants in teeth affected by MIH, indicating a need for careful monitoring and management [78].

The treatment options for MIH-affected teeth vary depending on the severity. In mild to moderate cases, composite restorations are commonly applied, often preceded by using modern adhesives. In more severe cases, the treatment is focused on addressing functional and aesthetic concerns. The timing of treatment intervention is typically before puberty to deliver a permanent prosthetic solution, such as providing veneers for the incisors and crowns for the molars [79]. The composite resin restorations of MIH-affected teeth were longer-lasting than those of alternative restorative materials. Hence, they are more commonly used [57]. However, the retention of composite restorations continues to be lower in MIH patients (median survival rate: 5.2 years +3.29 [23, 80, 81] and those applied in posterior teeth had a median survival rate of 92% after ten years [82]), meaning that more repetitive dental treatments were required.

2.3 Resin composites materials.

2.3.1 Composition of Resin Composites.

Composites are resin-based materials. They are composed of specific basic components such as an organic resin matrix, filler particles (inorganic fillers or dispersed phases of non-organic fillers) and a silane bonding agent (organic-nonorganic) [83-85].

The organic resin matrix is made of monomers, amongst which the most commonly used is Bisphenol A-glycidyl Methacrylate (Bis-GMA). As viscous, Bis-GMA is typically diluted with Triethylene Glycol Dimethacrylate (TEGDMA) to ease its handling [86]. When the organic matrix undergoes polymerisation, the polymers form a three-dimensional network. Unfortunately, the formed network suffers from some drawbacks, such as low physical, mechanical (tensile strength and compressive strength), and surface (wear resistance) properties [87]. It also shows high polymerisation shrinkage and water absorption [88].

Fillers were added to overcome these drawbacks, where the drawbacks decreased as their amount increased [83]. Fillers are usually inorganic part, with glass/quartz or fused glass particle fillers being the most commonly used ones [89]. The addition of fillers necessitates the presence of an agent capable of bonding the organic matrix to the inorganic fillers. Silane was hence added. It is an organic-inorganic molecule, where its inorganic part bonds to the fillers' surface while its organic part bonds to the resin matrix. Other minute components of composite include polymerisation initiators, inhibitors, additives, stabilisers, and pigments [87].

2.3.2 Composite Restoration Classification

Composite properties are influenced by the characteristics of the inorganic filler applied in terms of size, content, geometry, and composition. Hence, composites are usually classified according to their fillers [90]. The most famous classification

is the fillers size; it ranges from macro sized particles (> 10 μm) to Nano sized particles (5-100nm). The large sized particles show low attrition rate while the tiny sized ones deliver superior aesthetics. Hybrid complexes (10-50μm & 40nm) were introduced to combine the advantages of different sizes [89].

Another well-known classification relies on the fillers size distribution or rather the fillers content [91]. Fillers contents are inversely correlated to solvent diffusion into the material which leads to a gradual expansion and increase in the material's volume. The latter phenomenon is known as solvent sorption [92]. Moreover, there is another inverse correlation between the fillers content and the material deformation which is assessed through the measurement of the flexural modulus. Increased levels of sorption lead to strength reductions, which stresses the significance of effectually managing solvent absorption [91].

It is noteworthy that an additional classification method was developed relating to the filler's content. It had two distinct levels of filler content, 50% and 74% volume. Several terminologies, "ultra-low fill," "low fill," and "compact resin composites," are used synonymously to describe substances characterised by filler quantities ranging from less than 50% to more than 74%, respectively [90].

2.3.3 Advantages of Composite Restoration

A restorative material should fulfil the biological, mechanical, and aesthetic needs when used. Composites are less intrusive choice when combined with resin bonding adhesives than other restorative materials used in dentistry. It is retained in a micro-mechanical manner similar to the enamel and dentine [93]. As a result, less dental tissue is lost during cavity preparation [94]. Furthermore, they show an increased compressive strength compared to most restorative materials [95] and a reduced rate of cusp fractures – at a rate of 2.29 %[96]. It can also sustain wear readily; the latest developments in composites technology have resulted in wear rates of 110–149 micrometres after three years, compared to human enamel -122 micrometres after three years [97, 98]. Finally, composites can provide a broad range of shades of colour that mimic those of natural shades of enamel and dentine, which allows for the restoration to be undetectable, and

hence more aesthetically acceptable. To sum up, composites are the optimum restorative choice that respects the biological, mechanical, and esthetical principles when treating DDE.

2.3.4 Limitations

Composite restoration application is not without limitations. Such limitations may include the patient's increased sensitivity to the procedure. This could be related to the type of enamel, as it is defective, or to the material itself, as BIS-GMA biocompatibility shows concerns [99]. In addition to the potential impact of polymerization shrinkage of composite resins on the bond strength results. Polymerization shrinkage occurs as the hydrogen bonds are substituted with shorter- stronger bonds (covalent);hence, the material volume shrinks, resulting in stresses that may affect the restoration's integrity [100]. In addition, if not properly applied, micro-leakage may occur at the margins, putting the patients at higher risk of secondary caries [99]. Finally, in composite restoration the main disadvantage is the difficulty in determining the extent of cavity preparation due to the poor bonding properties of affected enamel which lead to removal of large amount of enamel [13].

2.4 Evolution of Restorative Dentistry (bonding to enamel and dentine)

The Introduction of adhesive materials was revolutionary within the field of preventative and restorative dentistry [101]. Dental adhesives are resin monomer solutions applied at the interface between the tooth and the restoration [102]. They eliminated the need of mechanical retentive features during cavity preparation [101], Hence tooth structure was rather preserved [102].

Originally, dental adhesives are composed of monomers that display both hydrophobic and hydrophilic features, inorganic fillers, solvents, inhibitors, curing initiators, and stabilizers [103]. Adhesive success mainly depends on the microstructure and composition of the dental tissue, enamel, or dentine. Enamel contains hard solid crystals, such as hydroxyapatite (HAP), which are securely bonded to each other at the molecular level with high energy at the surface. On

the other hand, dentinal crystals are less with a more collagenous network, approximately 25% of the total tissue. Hence, the intermolecular bonding forces, hardness and surface energy are all less than that of the enamel. Additionally, dentine contains tubules that hold smear, organic substances, and fluid. These tubules width and density show variation according to dentineal depth; their width is larger at the surface while their density is lower. Therefore, adhesion of the superficial dentine is attained through inter-tubular resin absorption, while it is attained through intratubular permeability of resins in the deep parts [104]. Moreover, dentinal tissue undergoes alterations over time; its thickness and permeability are reduced as its mineral content increases. This has significant implications for restorative dentistry, as bonding to dentine is provocative [105]. Finally, as optimum adhesion is questionable, adhesive materials suffer from two major limitations: microleakage and, subsequently, secondary caries. Contemporary advancements in adhesive technology helped decline these limitations [103].

2.4.1 Etching

During the etching process, the etchant used is 35- 37% phosphoric acid [106]. Enamel requires an application of 30 seconds to demineralise. This occurs by removing calcium in the outer enamel layer, subsequently creating pores and enhancing the surface reactivity, allowing enamel tags to be formed. These tags provide a micromechanical attachment of the resin to the tooth tissue, resulting in a strong bond. It prepares dental tissues for primer application by producing micro porosities, which allow the resin to penetrate and form tags, ensuring micro-mechanical bonding. It is essential to stress that this specific step increases the enamel's surface reactivity [107]. However, the self-etch adhesives have an acidic monomer acting as an etchant and primer [106].

2.4.2 Primer and Bonding agent

A primer is made of monomers that are hydrophilic and can dissolve in water, acetone, or ethanol. In some circumstances, a primer is necessary after the etching process and right before adding the adhesive material to the teeth, as it

permits sufficient flow and penetration into the hydrophilic dentine, creating a bridge between the hydrophilic and hydrophobic components [106].

The application of a bonding agent, which is a thin coating of resin between the restoration and the prepared dental tissue, assists in their adhesion, with the primer attached to the hydrophobic resin composite. Furthermore, ensuring the appropriate curing methods is essential to achieve optimal sealing and retention [106].

2.4.3 Generation Development of Dental Adhesive

Adhesive dentistry has advanced over time from total- to self-etch systems [108, 109]. The total-etch system comprises the 4^{th} and 5^{th} generations. The 4th generation consists of three basic parts: etch, primer, and bonding agent, where each constituent is packed individually and applied in a respective successive order. Unfortunately, the primer and bonding agent may penetrate the collagen network at different levels, causing the collapse of the demineralised collagen network. The 5th Generation (one bottle etch-and-rinse) minimised the risk of the collagen network breakdown by merging the primer and adhesive into a single solution [110-112]. Equally, in the 4th and 5th Generations, dentine and enamel are simultaneously etched for 15-20 seconds using phosphoric acid [113, 114]. This rendered the process to be technique sensitive as the conservation of the dehydrated demineralised dentine from collapse is a little burdensome.

Self-etch systems eliminated the separate etching step as they focused on removing or chemically integrating the etching into the other steps. They include the 6th and 7th generations. The 6th generation incorporated the etchant with the primer in a single bottle solution (also recognised as "self-etching primers") [115]. The process minimised the technique sensitivity of conserving hydrated dentine in total-etch systems [114]. Meanwhile, the 7th generation additionally simplified the process by merging all ingredients in a single bottle [114, 116]. However, the system still has limitations; its acidity makes it more susceptible to hydrolysis and chemical degradation [116, 117], and its produced pores are fewer than the other systems [118]. The protocol of the different generations of dental adhesives are summarised in table (1).

Generation	Protocol	Steps	Etch	Primer	Adhesive
4th	Etch and rinse	3	✓	✓	✓
5th		2	✓	One bottle	
6th	Self-etch	2	One bottle (acidic primer)		✓
7th		1	All in One bottle		

Table 1 Summary of the protocol of the different generations of dental adhesives.

2.5 In Vitro Bond Strength Testing

Testing the efficacy of new materials before their clinical implementation is mandatory. New adhesives are mainly evaluated through their produced bond with the dental tissue. This evaluation is accomplished in vitro as attempts of debonding are performed and analysed. The analysis primarily aims to quantify the debonding force in relation to the bonded surface area while secondarily aiming to localise the fracture. The concept of bond strength encompasses the quantitative measure of the force necessary to initiate the debonding process. The expression of the provided values could be in the metric unit of Megapascals (MPa) or kilogrammes per square centimetre (kg/cm^2). Quantifying the debonding force in any unit necessitates a careful adjustment in proportion to the nominal bonding area [119].

The surface area of the bonded surface to be treated influences the produced bond strength values. The diameters of bonded specimens range from 1 to 4.5 mm, with areas ranging from 0.8 to 15.9 mm^2. Smaller bonded areas may give higher bond strength values. It is noteworthy that the bond strength could be evaluated either through macro-test or micro-test configuration. This is dependent on the size of the bonded area, with macro-test for areas of 2-3 mm^2, whereas micro-test strength is used for areas < $1mm^2$. The test could be performed in multiple ways; ‘shear’, ‘tensile’, or a ‘push-out’ bonding.

2.5.1 Shear Bond Strength (SBS) Test

The Shear Bond Strength (SBS) test is commonly utilised to assess the adhesive properties of materials, providing a simple and fast method that does not require further post-bonding specimen processing [120, 121]. However, the 'Micro' shear bond strength (μSBS) test, which allows for the assessment of multiple specimens per tooth, is considered a sensitive technique with numerous limitations. The diameters of composite cylinders required for the test typically range from 0.7-0.8 mm. Furthermore, a thin adhesive layer is required to avoid significant deformation and non-uniform loading. Compared to macro-SBS, its variable stress dispersal will likely be more evident. Therefore, using the μSBS test is uncommon, as only a limited number of bond-strength studies have employed it [122, 123].

Numerous in vitro experimental trials focused on measuring the adhesive strength between two materials [124, 125], where they recommended the use of conventional standards for measuring the bond strengths to the tooth structure to avoid potential variations in testing parameters. Presently, the recommended standardised tests are the ISO/TS 11405 (2015) for tensile bond strength testing, DIN 13990 (2017) using a straight blade recommended for SBS test on orthodontic attachments, and ISO 29022 (2013), where a notched-edge blade is used for testing SBS of dental adhesives and direct restorative materials, as it concentrates stress closer to the contact and interface more than the knife-edge [120]. Exclusive jigs, such as the Ultradent jig and the SDI jig, have been formulated to standardise the test process [120, 121]. Following these standards, a Universal Testing Machine equipped with an appropriate assembly has been commonly used to assess bond strength in dentistry. In a compression state, measurements are made with a blade moving at a crosshead speed of 1 mm/minute parallel to the adhesive-enamel interface. The results are calculated by dividing the surface area by the peak load at failure in Megapascal (MPa) [126]. Despite these experiments for standardisation, a meta-analysis of the elements affecting SBS testing conveyed that there is a substantial effect of several parameters, including those associated with the dentine substrate or tooth nature, the composite and bonding area or composite stiffness, the storage conditions of the bond assemblies or thermocycling, and the test design or

crosshead speed, but this is true for all bond strength tests [120, 121]. Nonetheless, the SBS testing is likely to remain commonly utilised to calculate the bonding efficacy of new adhesive inventions.

2.5.2 Tensile Bond Strength Test

Tensile bond strength testing is a widely used technique for assessing adhesive strength between different materials. The utilisation of macro-tensile bond strength in assessing bond strength for dental materials, including ceramics and metal alloys, is uncommon [120, 121]. With microtensile bond strength testing (μTBS) was developed in 1994 by Sano et al. [127], the bond area tested is far smaller than the bond area evaluated in macro tests. One of the advantages of μTBS is being more economical, with multiple micro-specimens originating from a single tooth. This enhances the test's adaptability since obtaining several specimens enables more creative study settings. Another advantage of μTBS is the more precise control of substrate parameters [120], of that of regional differences (e.g. peripheral versus central dentine), and a more uniform stress distribution at the actual interface (avoiding cohesive failure in the tooth substrate/ composite) [127]. Additionally, the micro-tensile protocol appears to be more effective than the SBS approach at discriminating adhesives based on their bonding performance [120, 128, 129]. However, further specimen processing or preparation of the micro-specimens is required after the bonding approach, extending the test and making it technique-dependent [127].

2.6 Failure mode analysis

Bond quality evaluation should include a detailed investigation of deboned surfaces to understand material performance under actual stresses, not only nominal bond strength [130]. It is essential to evaluate bond strength and failure mode to establish the adhesion quality for a certain bonding agent and enhance adhesion techniques or testing processes by resolving shortcomings [131]. A higher average bond strength is related to a higher occurrence of cohesive failure. Cohesive failures increase with adhesive system advancement, limiting testing [129]. Traditional bond strength tests (shear/micro-shear and

tensile/micro-tensile) should consider adhesive failures, mixed failures with minimal composite (<10%), and/or the involvement of the dentine. This requires microscopic examination of fractured surfaces using Stereo and Scanning Electron Microscopy (SEM) [132].

2.7 Biomimetic Material

2.7.1 Self-Assembling Peptides (SAPs) Structure and Formation

2.7.1.1 Proteins

A protein is a polypeptide with fifty or more amino acids [133]. The structure of a protein can be categorised as primary, secondary, tertiary, or quaternary [134]. A primary structure has a linear amino acid sequence, thus the hydrogen bond between the polypeptide backbone, oxygen, and nitrogen atoms constitutes its secondary structure; the polypeptide chains are coiled together, forming the protein's structure; these coils are known as α- helix and β-sheet [135], which are the two most common secondary structures. The β-sheet (Figure 5 D, E) encompasses two distinct parts of a polypeptide chain joined by a hydrogen. These sheets are either parallel if they are less twisted and always buried (Figure 5E) or antiparallel [136], surviving greater twisting and solvent exposure. The increased stability of antiparallel sheets can be credited to the hydrogen bond geometry and the infrequency of small parallel sheets [136, 137].

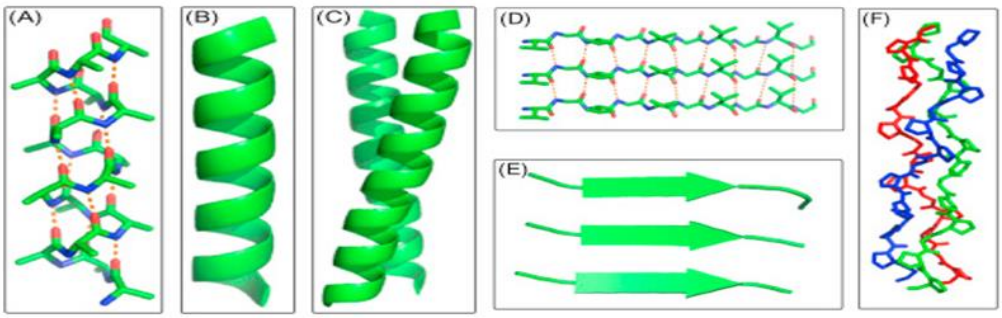


Figure 5 illustrates peptide structure and folding (A) Molecular view of an alpha -helix with intramolecular hydrogen bonds in orange ;(B) alpha-helix ;(C) dimeric coiled coil; (D) molecular representation of three Beta -strands forming alpha Beta- sheet, intermolecular hydrogen bonds are orange; (E) three parallel Beta-strands (F) collagen triple helix with strands coloured differently. This Figure reproduced with a permission of the rights holder From:[138]

2.7.1.2 Synthetic Peptides

Self-assembling peptides (SAPs) are synthetic peptides that are designed beta-sheet-forming peptides, naturally forming three-dimensional fibrillar scaffolds because of environmental triggers, such as ionic strength and pH. SAPs have the potential to be used in skeletal tissue engineering [139] and the prevention and treatment of dental decay [139, 140].

Generally, different types of structures of peptides are targeted to produce the synthetic. For example at University of Leeds, Synthetic peptides use the β-sheet secondary structures, as they can produce twisted elongated nanostructures [141]. This can be attained by strategically designing the primary structure of the peptides, permitting self-assembly into more stable three-dimensional structures that are linked by noncovalent bonds [142]. Peptides mostly exist in a monomeric and disordered form in very diluted solutions, inside the framework of surfactants, which are molecules capable of modifying the surface tension of a solution. The previous condition persists until surfactants, via the reduction of surface tension, provide an external force that has the potential to impact the structure and behaviour of the peptides. The self-assembly process begins at a particular concentration, when the change in free energy per molecule is greater than the change in free energy due to bonding interactions. Tapes, the simplest of hierarchical structures, form ribbons when stacked by the interaction of side chain-side chain interactions. The elastic energy (which is energy stored in an object) stops two tapes from joining to form a ribbon; if the free energy cost of fibril creation is bigger than the elastic penalty, then fibrils will self-assemble. The number of ribbons in a fibril is dictated by the equilibrium between the elastic penalty associated with untwisting and the increase in attraction energy resulting from ribbon accumulation. A minor angle of twist produces a long ribbon, while a large angle produces an infinite stack or crystal. When a stable fibril has formed, two fibrils can interweave to make a fibre [5]. The property of self-assembly can be assisted by hydrogen and metal-ligand bonding, electrostatics, charge transfer, van der Waals attractions, hydrophobic interactions, and π-stacking [143].

2.7.2 SAPs (P_{11-4})

A group of SAPs developed in Leeds use these phenomena in dentistry through the use of the specific peptide P_{11-4} (primary sequence $CH_3COQQRFEWEFEQQNH_2$). This particular peptide is composed of a total of 11 amino acids and is a form of SAPs that is pH-controlled [29, 144]. pH is a generally valuable trigger tool in relation to use in the oral environment: an acidic pH changes the shape of one specific peptide into biomimetic fibrillar scaffolds [28, 145]. pH disrupts the side chains of amino acids and ionic links between negatively and positively charged amino acids [146]. At variable pH values, the side chains of amino acids (terminated with -COOH or $-NH_3$ groups) can be protonated or deprotonated. These side chains exercise control over the electrostatic links between neighbouring peptides and efficiently dominate the free energy of association per peptide-peptide bond and overall net charge of -2 at physiological pH.

P_{11-4} also reacts to physio-chemical environmental triggers by producing hydrogels at peptide concentrations ranging from 10 to 30 mg/mL [147]. P_{11-4} self-assembles at or below a pH of 7.4 to produce a self-supporting hydrogel in a concentration-dependent fashion [147].Acid causes the activation of assembly, and then the disassembly is activated by a base [148-150] (illustrated in Figure 6). Moreover, P_{11-4} mimics the enamel matrix proteins: it forms a three-dimensional matrix inside the subsurface body of an initial enamel lesion [139]. The P_{11-4} matrix has a great affinity for calcium ions that is important to produce a de novo hydroxyapatite [139].

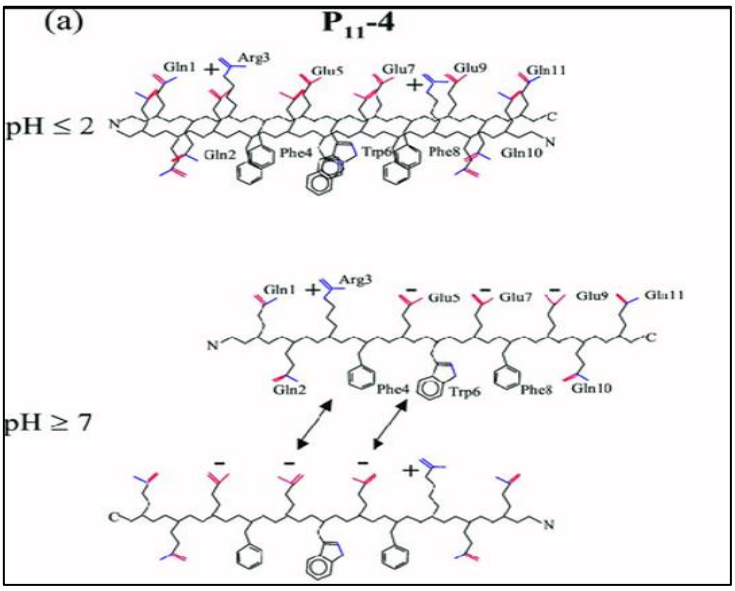


Figure 6 P_{11-4} a) Low and b) high Ph Levels demonstrating the colocalization of peptides in dimeric tape-like substructures and their dissociated monomeric forms. This Figure reproduced with a permission of the rights holder, From:[5]

2.7.3 Uses of SAPs P_{11-4}

The use of P_{11-4} varies in different specialities, such as dentistry and medicine. P_{11-4} can amend specific parameters, such as mechanical stiffness, cell adhesion and biodegradation. It has a specific physical advantage over other scaffolds in that it will return to its original gel form without phase separation when shear softened, making it a feasible encapsulating delivery and biomimetic scaffold [150]. These characteristics are appropriate for tissue repair, where they can be used as scaffolds in regenerative medicine [151]. In dentistry, enamel matrix proteins, which form self-assembling supramolecular structures, can control the organisation of hydroxyapatite crystals [28]. SAPs work in a comparable way to control the deposition of minerals. Furthermore, P_{11-4} has the capacity to nucleate hydroxyapatite mineral de novo and has illustrated potential as an injectable scaffold for hard tissue engineering applications, regenerative treatment for early tooth decay (caries) [139, 148, 152-157]. It demonstrated visual enhancement in the aesthetics of carious lesions and improved the opacities present on X-rays post-treatment of proximal carious lesions [153, 158]. As P_{11-4} has the capability to restore impaired crystal structures in the depth of the enamel lesion, by operating as an immobilizing scaffold for attracting and dehydrating Ca^{2+} and

PO_4^{3-} ions (from the saliva), the ions stick to the matrix and drive de novo production of hydroxyapatite crystals [159]. It has been testified that P_{11-4} can be utilized to regenerate enamel [28, 156, 160-164]. Moreover, a report has described the benefit of using P_{11}-4 on composite resin bonding onto dentine with decay and an improved resin-dentine bond strength [165].

Researches mainly aim to provide a protocol with scientific basis to be applied in the daily life. The population behavioral diversity should be accounted when applying any new protocol as certain behaviors may affect the final outcomes. For example, one consideration is the use of fluoride with SAPs since it is a widely consumed agent by people. Fluoride exists naturally in our environment, but some people may ingest small quantities via dietary consumption (mainly through fluoridated water), fluoride supplements and inhalation. Fluoride can assist in enamel remineralisation and reduce demineralisation, which subsequently reduces the risk of developing dental decay. The application of fluoride - NaF and APF gels - before tooth-coloured restorative treatment was assessed in a trial and was not described to have any beneficial influence on the micro-SBS [166]. Therefore, it is reasonable to provide fluoride remedy and restorative treatment simultaneously. SAPs have been shown to be more effective than fluoride varnish when applied to enamel lesions. A systematic review was carried out to assess the clinical performance of SAPs compared to fluoride varnish in the management of early enamel lesions. When SAPs were used alone or with fluoride for non-classical remineralization, they had better results than fluoride varnish[167].

Another major consideration for public use is the safety of the product. Several studies have noted that P_{11-4} is safe for use in patients in vivo investigations, where there were no reported allergic reactions or adverse events have been reported when using it, or 12 months post-intervention [168, 169]. The product has been evaluated for biocompatibility within the human body [170], and according to ISO 10993 or equivalent, P_{11-4} did not raise any cytotoxic effects or any immunologic response [171]. It is important to note that the amount applied per patient is minimal, and even if the patient ingests P_{11-4}, being a peptide, it will be degraded into small forms of amino acids or secreted [172]. Moreover, patients who had been exposed to P_{11-4} had expressed satisfaction with the

product, and dentists expressed the easiness of using it as compared to applying a fissure sealant to a tooth or a filling [173]. Hence, P_{11-4} is both safe and acceptable to use by the dental team and the patients.

Additional considerations to the use of SAPs in the general population is its availability, accessibility, and affordability. P_{11-4} is available as a commercial product: Curolox Technologies (Credentis AG, Windisch, Switzerland) [174]. Both the commercial products Curodont ™ Repair, indicated for initial caries lesions and the professional remineraliser gel Curodont ™ Protect, indicated for post-bleaching orthodontic treatments, contain this peptide [175]. It can be purchased online (https://professional.vvardis.com/products/) for the price of €270 per 10 units (equivalent to £230.46). Therefore, it is already available, accessible, and affordable.

2.8 Research Question

In this study, one of the technologies discussed to help improve SBS includes the use of composite restoration with nano- and micron-sized inorganic minerals (that mimic the mechanical properties of sound vs MIH teeth) as one of the possible treatment options for MIH. We aim to investigate whether the use of SAPs P_{11-4} will be able to enhance the defective enamel of MIH teeth, and hence improve the shear bond strength of composite restoration to MIH teeth. The outcome of this new technology, if proven to be effective in improving SBS in MIH affected teeth could help patients with the condition worldwide.

CHAPTER 3: RESEARCH AIMS, OBJECTIVES NULL HYPOTHESIS

3.1 Aim

To investigate whether the application of SAPs P_{11-4} contributes to the improvement of shear bond strength of composite restorations for defective enamel lesions in teeth affected by molar incisor hypomineralisation (MIH).

3.2 Objectives

1-To evaluate composite shear bond strength of MIH affected enamel and compare it to sound enamel.

2-To evaluate composite shear bond strength to MIH affected enamel after treatment with SAP P_{11-4}.

3-To understand the failure mechanism at the restoration-enamel interface of sound enamel, MIH affected enamel and MIH affected enamel treated with SAP P_{11-4}.

3.3 Null Hypothesis

1. In vitro, the shear bond strength of composite restoration bonded to enamel in MIH teeth will not be different than the shear bond strength of composite restoration bonded to sound teeth.

2. The shear bond strength of composite restoration bonded to enamel following treatment with SAPs P_{11-4} in MIH teeth will not be different than the shear bond strength of composite restoration-bonded to untreated MIH teeth.

CHAPTER 4: MATERIALS AND METHODS

4.1 Specimen Size Calculation

Specimen size calculation was performed using an online web-based tool, https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html; based on the previously published research [165], the effect size (5.67), the standard deviation (SD) 12.03, the desired power (1-α, set to 0.80) and a significance level (type 1 error (β), set to 0.05). For the means of the two groups, data from a previous study was used [176], with a comparable experimental design that used SAPs $P_{11\text{-}4}$. The web tool has determined that at least 74 teeth (which we made to 75 to distribute 25 per experimental group) are required to detect a statistically significant result.

4.2 Research Ethical Approval

Ethical approval was granted from the Dental Research Ethics Committee (DREC) of The University of Leeds, reference 220921/SA/333 for collection of human sound and MIH teeth (first permanent molar teeth with yellow/ brown discoloured lesions) from School of Dentistry Tissue Bank (the form illustrated in Appendix A).

4.3 Teeth selection

The tissue bank is a university-managed resource that collects extracted teeth from appropriately consented patients of the local dental hospital. At the tissue bank, sample selection was conducted by the researcher, a trained professional dentist, based on the eligibility criteria (inclusion and exclusion) applied to each tooth sample. Before including a sample in the study, specific criteria were used. These criteria dictated that the teeth should be the first permanent molars, whether maxillary or mandibular. As we focused on MIH teeth clinically presenting with hypomineralised lesions, it was essential to ensure that the lesions met a specific size requirement for studying macro-shear bond strength, which should be greater than 1 mm^2. Consequently, lesions with a width and

height of 3mm were carefully selected for analysis (shown in Figure 7:C). In addition to the size criteria, the position and appearance of the lesion were also considered. The lesion needed to be located on a smooth surface, such as the buccal, mesial, distal, or lingual/palatal surface of the tooth. Furthermore, it should also show a whitish, or creamy, or yellow, or brown discolouration, as illustrated in Figure 7: A and B. Teeth were excluded from the study if they had no MIH lesion or were diagnosed with any other enamel or dentine defect. Additionally, teeth with gross caries lesions on any surface (examined using a dental probe and light), filled with restoration or root canal treatment, or those showing hypomineralised lesions or severe post-eruptive breakdown lesions exclusively on the cusps or occlusal surface were also excluded.

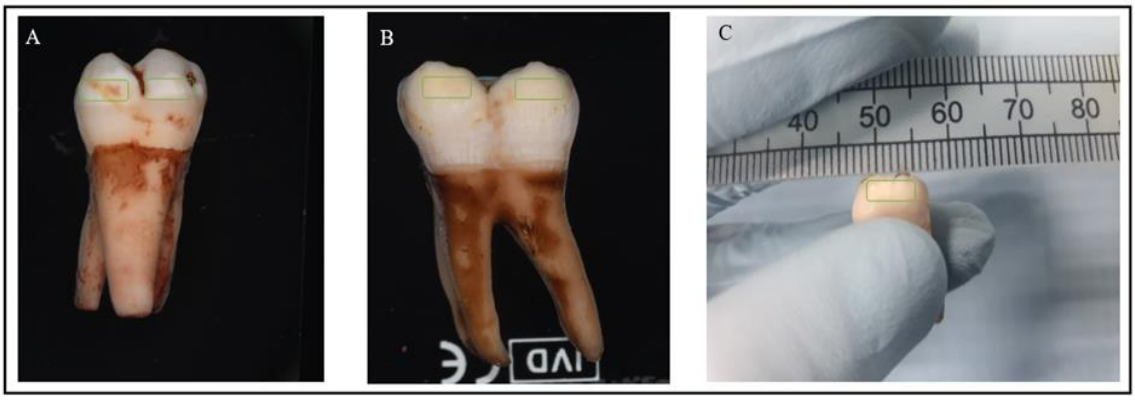


Figure 7 represented inclusion criteria of teeth selection process. Figure A, a depiction is presented featuring the teeth affected by Molar Incisor Hypomineralisation (MIH) during the process of tooth selection. The green box highlights the discoloured lesion (appearing white) on the smooth surface. Figure B further illustrates an MIH-affected tooth with a green box, emphasizing a chosen creamy discolouration on the smooth surface. Figure C showcases the selected lesion, with its dimensions measured using a rule, revealing a size exceeding 1 mm^2.

4.4 Acquisition and Storage of Tissue.

Teeth collected from the tissue bank in St. James's Hospital - Welcome Trust Brenner Building, 7th floor, Leeds, UK – were labelled with numbers and letters. These numbers and letters were used to identify the tooth and to maintain the tooth identification throughout the experiment.

Teeth were stored in thymol 0.05% w/v, which is prepared by mixing 0.05 g in about 5 mL of ethanol, and then top up to 100 mL with distilled water. Thymol

0.05% was used as a disinfectant and an antimicrobial, stored for 1 day. The teeth were cleaned from any remaining periodontal tissue, soft tissue, plaque, and blood using a toothbrush [177] and distilled water to reduce the bacterial , fungal and viral load after other storage media.[178].

4.5 Baseline Measurements

Several baseline records were carried out for all groups prior to division into three groups. Figure 8 illustrates a diagram summarising the baseline measurement for grouping teeth.

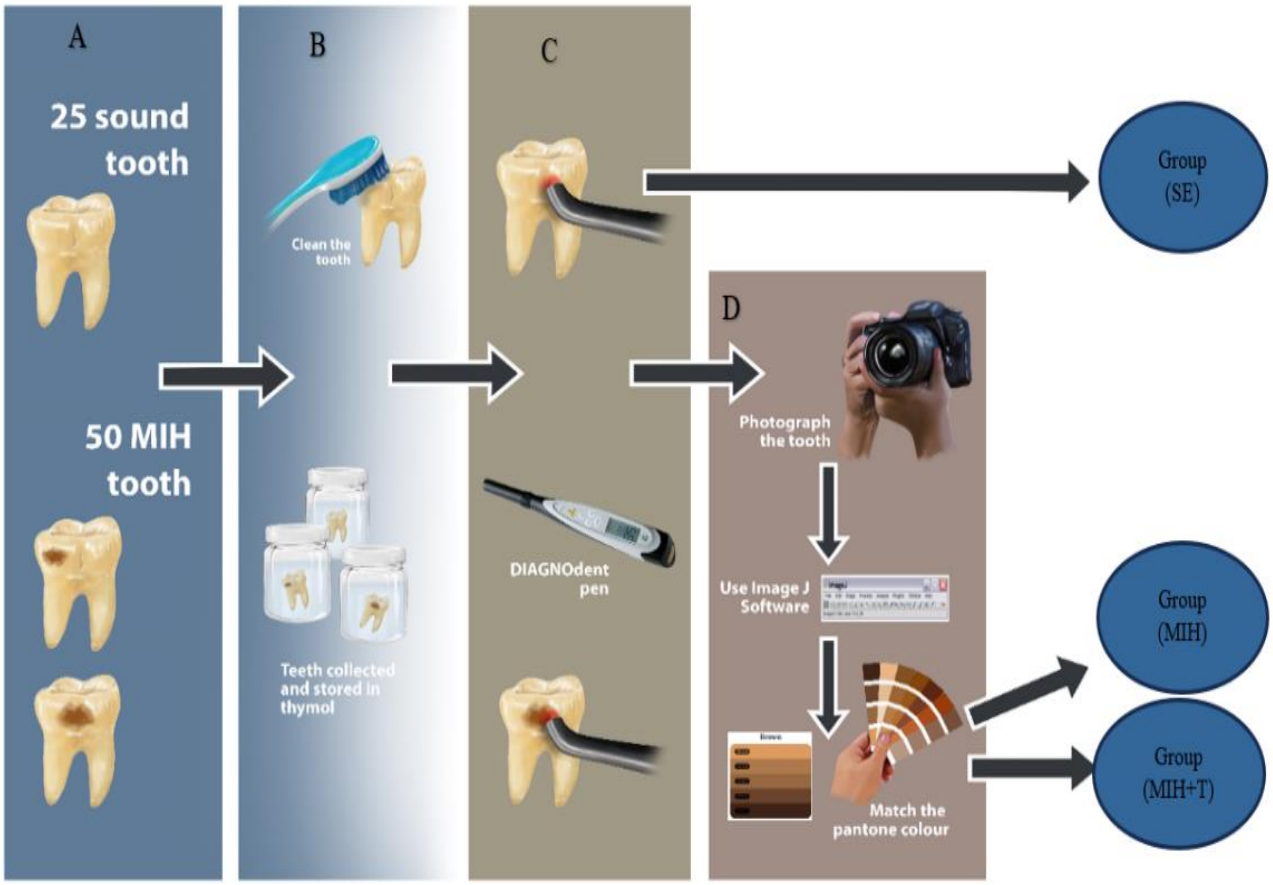


Figure 8 Diagram shows the baseline measurement Diagram for teeth grouping. A: Sound teeth and Molar incisor teeth total number is 75. B: cleaning and storage step C: DIAGNOdent records of all teeth. D: colour analysis for MIH teeth only.

4.5.1 Laser Fluorescence.

To determine the mineralisation in the teeth, particularly the hypomineralised lesions, a Laser Fluorescence (DIAGNOdent™ Pen IR 2190 kit, KaVo, Biberach, Germany) with a standard tool for calibration was used. This device has a fissure pin that touches the lesion to give a reading. The device's readings range from 0 to 99. The DIAGNOdent reading increases when the mineral density decreases in hypomineralised enamel [179]. Careful scanning and slight movement of the device's probe back and forth over the lesion increase the device's sensitivity and permit the identification of the maximum fluorescence's location [180]. For each specimen, 5 different points were selected in the lesion (right, left, top border, lower border, and central of the lesion) (shown in figure 9) in order to cover as much surface area as possible, and then calculate their average to be recorded. (The average values of the specimens shown in Appendix E).

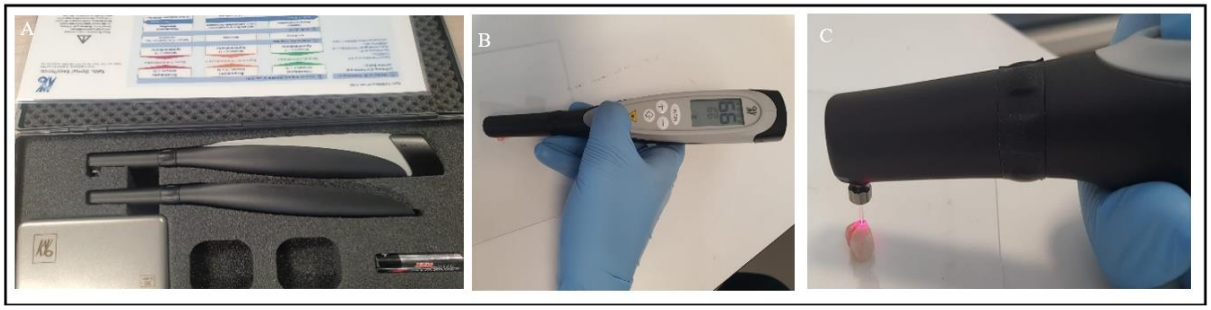


Figure 9 represented the Laser Fluorescence assessment step. In Figure A, an image is presented capturing the DIAGNOdent Device. In Figure B, a photograph is depicted illustrating the utilization of the DIAGNOdent, where in the fissure probe is positioned on the lesion to obtain a reading of the hypomineralised lesion. Figure C provides a close-up view of the Laser Fluorescence emitted by the DIAGNOdent on the lesion.

4.5.2 Imaging and Colour Quantification of MIH Lesion

4.5.2.1 Macrophotography Imaging

All the MIH teeth were photographed in the Leeds Dental Institute (LDI) photography department. The specimens were photographed at a magnification ratio of 1:2.5 using a Nikon D7500 Digital Single Lens Reflex (DSLR) Camera, Nikkor 105 mm Macro Lens and Sigma DG140 Ring Flash attached to a Kaiser

RS copy stand, with a flash output manually set at 1/2. An X-Rite colour checker passport was used to calibrate the colour and set an accurate manual white balance. Camera settings were set at Aperture or f/18, Shutter Speed 100, and ISO 100. These settings warranted a wide depth of field, reduced noise, and elimination of colour casts from ambient light. Each tooth images were saved on the memory card with folder name and number similar to the tooth collection letter and number to be identified later. (Photographing setup shown in Figure 10 A/B).

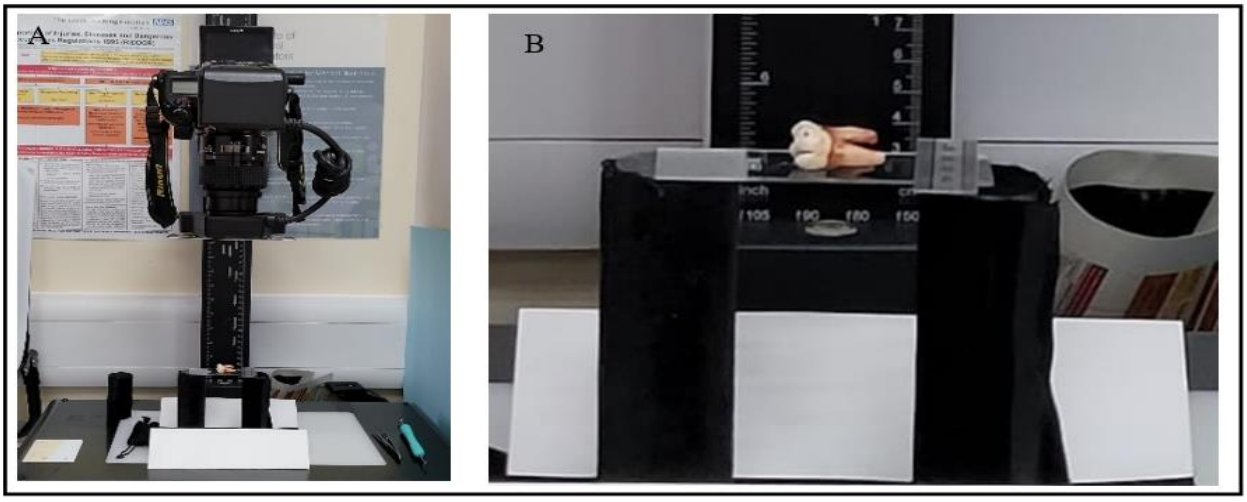


Figure 10 represented photograph setup. In Figure A, an image captures the imaging setup located in the Medical and Dental Illustration, University of Leeds. In Figure B, a depiction is presented where a tooth and a ruler are positioned on a glass slab, which is elevated by two elongated tubes concealed by a black sheet to provide support for the slab on which the tooth is situated.

4.5.2.2 Colour analysis

The photographs of the specimens were first transferred to image processing package software the program (Fiji image J version 2.9.0, NIH, Bethesda, Maryland, USA) [181]. Within this software, areas of hypomineralised discolouration were carefully selected to obtain their LAB mean values. These values included the L mean value for lightness and the darkness of the selected lesion, recorded directly from the photograph. Chroma was represented by a* and b*, without any specific numerical limits.

Once the LAB mean value was determined, it was entered onto an online site (https://hextoral.com/lab-to-hex-conversion/) to convert it to a HEX number. This HEX number, which represents a system that allows for a wide range of colours to be precisely defined and used in digital media, was then transferred to Microsoft Word/Excel (version 2402). There, it was displayed as a colour in the data collection sheet, illustrating the shade and severity of the discolouration, as shown in Appendix F. The detailed steps of the colour analysis process are illustrated in Appendix C, Figures A, B, and C.

4.6 Experiment Grouping

Baseline measurements were used to stratify the three groups appropriately (n=25 in each group). Firstly, DIAGNOdent was used to assess and confirm the mineralization of the dental enamel of 25 sound teeth and 50 MIH teeth. All 25 sound teeth (sound enamel) were in Group (SE), while the 50 MIH teeth (hypomineralised enamel) required further assessment to ensure equal distribution between Groups (MIH) and Group (MIH+T), ensuring that each of the groups had 25 teeth with similar lesion severities. Therefore, the second baseline measurement was conducted based on the subjective assessment of lesion colour (Darker shade and Lighter shade). Generally, the more severe the lesion, the darker its colour. The colour shades were therefore categorised into two groups: the Mild/Moderate severity group, which included white, creamy, and yellow shades, and the Severe group, characterised by brown shades. Both the (MIH) group and the (MIH+T) group had an equal distribution of specimens from the Mild/Moderate and Severe categories. To assess the consistency and repeatability of these colour shades, they were divided and evaluated at two different times in two days. taking into account the subjectivity of the researcher's perception. The flow chart is illustrated in Figure 7.

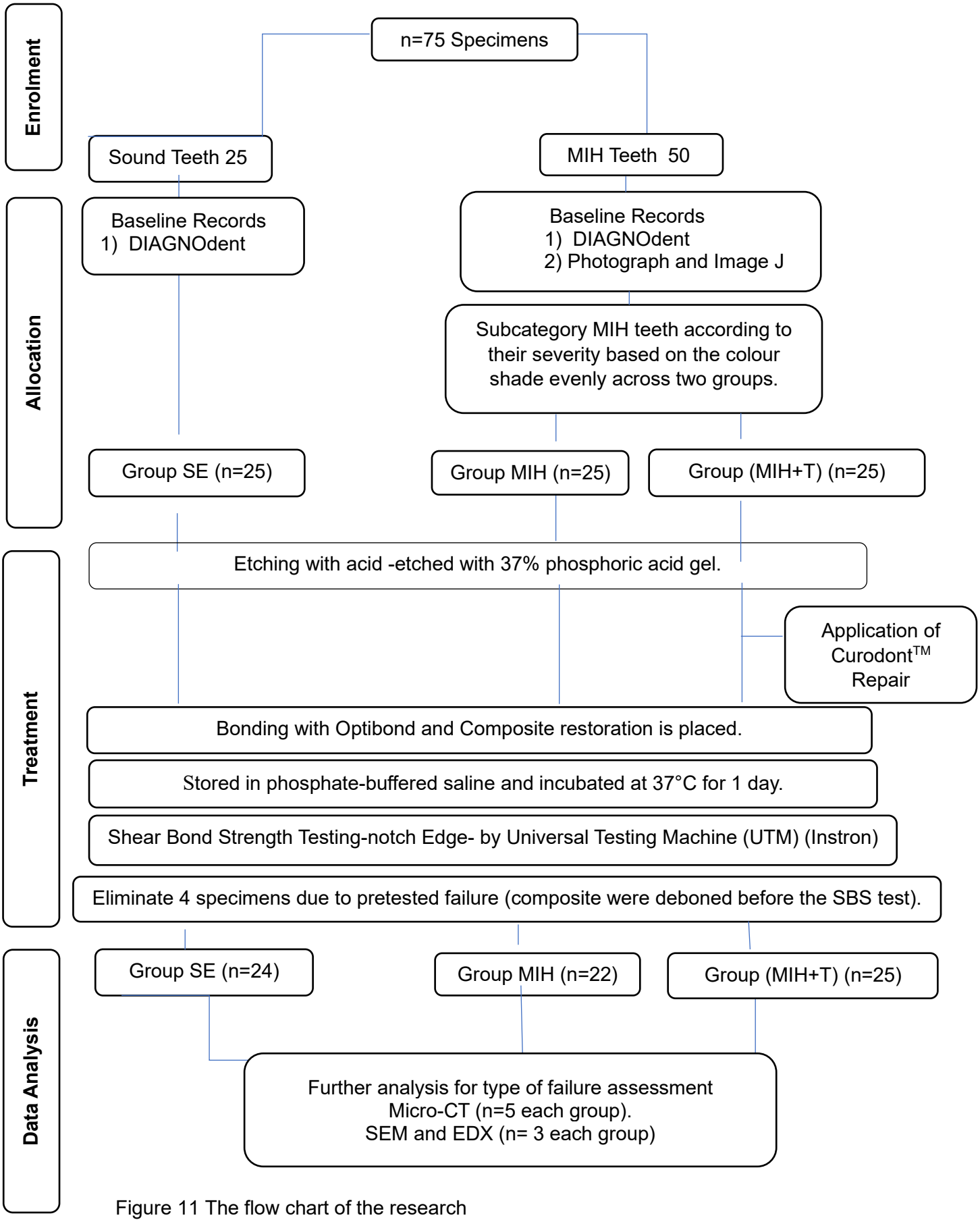


Figure 11 The flow chart of the research

4.6.1 Control Groups

-Group of Sound Enamel (SE): a positive control of 25 specimens, sound enamel bonded to composite restoration.

-Group of untreated MIH enamel (MIH): a negative control of 25 specimens, each one with hypomineralised enamel bonded to composite restoration.

4.6.2 Pre-treatment Group

- Group of MIH enamel with P_{11-4} SAP treatment (MIH+T): a treatment group of 25 specimens: hypomineralised enamel pre-treated with P_{11-4} and bonded to composite restorations.

4.7 Preparation of Enamel Specimens

4.7.1 Cutting the teeth.

Each tooth root was identified with a number and a letter to ease the identification of the crown surface during the cutting process. The tooth was mounted using compound impression - thermoplastic impression - to provide the tooth with support before placing it on the cutting holder. The Accutom machine (seen in Figure 12/A) was used to cut the roots below the cement-enamel junction and detach them from the crown, Using a 0.3 mm thick, water-cooled diamond-coated blade wheel on the Struers Accutom-10 cutter (Denmark) at a speed of 3000 rpm, the distance from the diamond cut-off wheel to the specimen holder was measured and set on the Accutom display screen for each specimen. The feed rate was 0.15 mm per second. The speed was constant for all enamel specimen, with only the distance varying. (illustrated in Figure 12/B). Sectioned roots were discarded as hazardous waste. The crown was evaluated and sectioned into parts to produce an enamel specimen with a 3 mm diameter of hypomineralised lesion (shown in Figure 12 B/C).

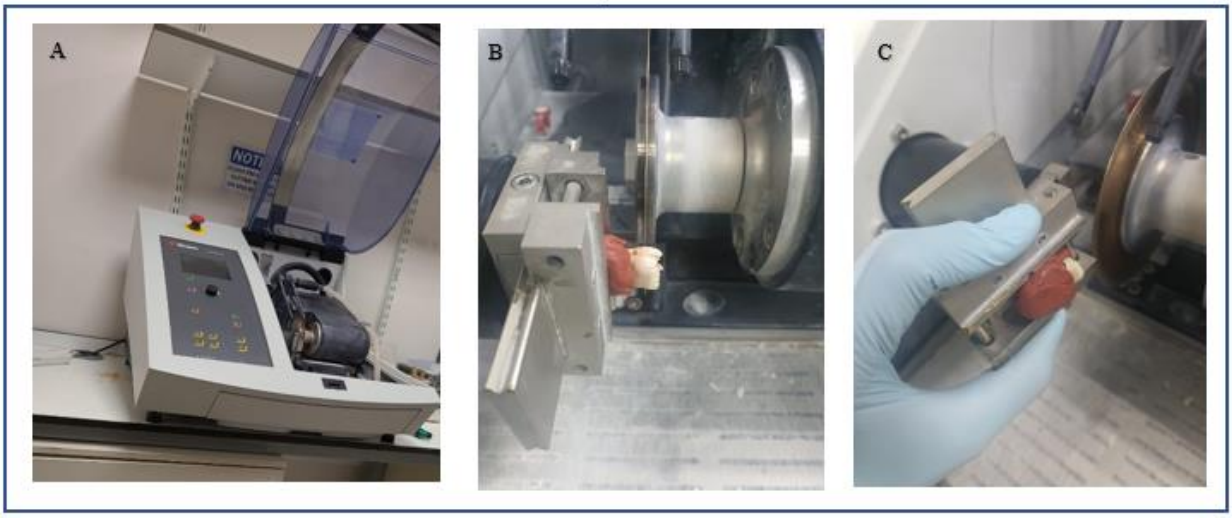


Figure 12 represented teeth cutting process. Figure A: Accutom machine B/C: tooth position and set up during the cutting root and crown cutting. (tooth impeded in the compound impression and secured in the specimen holder)

4.7.2 Resin Block Preparation

The sectioned crown was mounted on a resin block using a plastic square double-sided open-ended mould (diameter: 33mm, height:10.5 mm). The specimen was placed in a square mould supported by a glass slab as a base, with Vaseline as a separator medium, and applied to the corner of the square mould to ease the separation of resin and mould. Clear two components acrylic resin (ClaroCit, Struers, Ballerup, Denmark) were mixed following the manufacturer's instruction, weighting the powder and liquid in separate cups to get a ratio powder: liquid of 5:3. The powder and liquid were mixed with a wooden stick in a cup for one and a half mins until a colourless homogeneous with no visible streaks or unmixed regions and semi-fluid consistency achieved, then poured in the ring, until the resin is set. Once set, the glass slab was removed, creating a supported flat enamel surface suitable for bonding. Surfaces were ground manually using silicon carbide paper (400 and 600 grit for 2 min) sequentially to remove excess resin and to produce a flat enamel surface for bonding (steps illustrated in Figure 13). The resin blocks can be identified by a letter and a number as a reference record for the tooth.

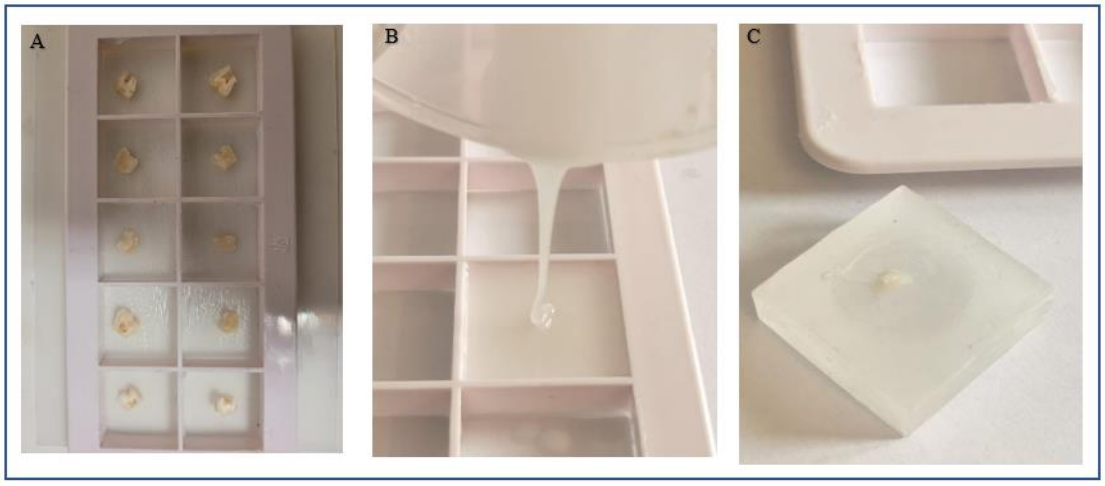


Figure 13 presented the resin block steps and procedure A: sectioned crown embedded in the mould the lesion faced downward B: Pouring of the resin in the mould. C: resin block with lesion of the tooth exposed.

4.7.3 Resin Composite Bonding Procedure

After preparation of the enamel specimen, 37% phosphoric acid gel was applied on the enamel surfaces using dispensing tip that is securely attached to the hub of the synringe for 20 seconds, washed with distilled water for 30 seconds using air water spray using triple way syringe, and air dried for 15 seconds. SAPs were applied only in the pre-treatment group (MIH+T) (explained in the 4.8.4 section). For all the groups, Optibond Solo was applied using a microbrush and gently air-dried for 10 seconds. Following that, a clear cylindrical plastic mould 2.5 mm x 3 mm was placed over the enamel surface and then light cured for 20 seconds so that the cylindrical tube would be attached to the tooth surface and used to produce resin composite cylinders bonded over the enamel surfaces. Filtek Supreme Universal (3M Oral Care) was used and placed in 2 increments, whereby each increment is photo-polymerised for 40 seconds using LED light with 1200 mW/cm^2 irradiance. Using a sharp lancet blade, the plastic mould was carefully removed, leaving a bonded resin composite cylinder perpendicular to the enamel. Application techniques and bonding procedures are described in (Figure 14 and Table 2).

4.7.4 Application of the SAP P_{11-4}

The Curodont™ repair is a commercial self-assembling peptide P_{11-4} that is supplied in freeze-dried form. Each Box had 5 units. Following the Manufacture instruction for its preparation (Credentis AG, Swiss). The first step was rehydration, using a 20-200 pipette with 50 mL of water and then placing it into a small plastic centrifuge tube equipped with a snap closure. The water was then extracted by graduated syringes made of transparent and plastic materials, ranging in size from 1ml to 60ml, and then the water was injected into the unit of the peptide (powder form), which features a central rubber component. Delicately insert the syringe into the receptacle and proceed to agitate the mixture, Allowing for subsequent reabsorption by the syringe. Each specimen received one droplet and waited for 5 minutes prior to the application of the bond and subsequent to the completion of the acid etching process.

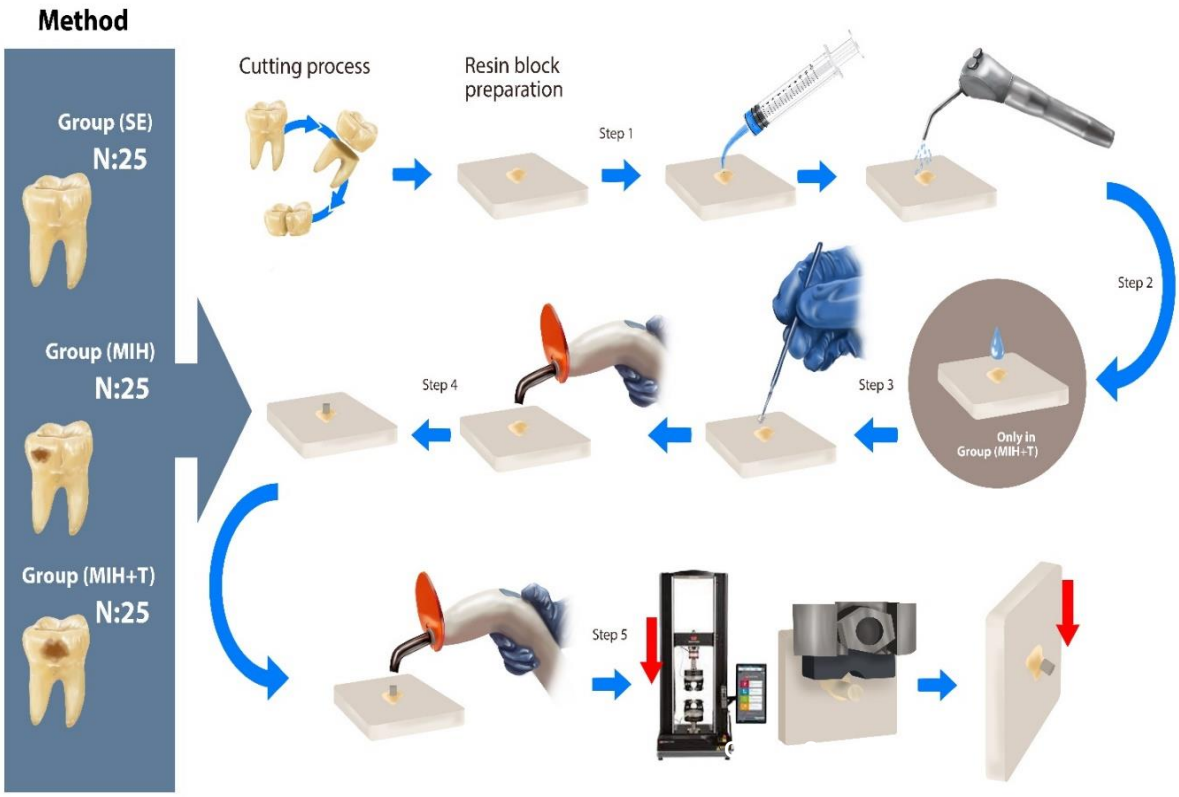


Figure 14 Representation of application technique and Bonding procedure

Groups	Enamel	Step 1	Step 2	Step 3	Step 4	Step 5
Group (SE)	SE	Etching: 20s acid -etched with 37% phosphoric acid gel, 30s wash with water and 30s dry with a 3-in-1 syringe	N/A	Application of Optibond: 10 s air dry with a 3-in-1 syringe then lightcured for 40 s using a light emitting diode (LED) light curing unit.	Composite restoration is placed on the etched and bonded lesion of the tooth. Any excess composite will be removed using a right-angled probe, 40s light polymerisation occlusally of the tooth.	All specimens were tested for shear Bond Strength Testing by Universal Testing Machine (Instron)
Group (MIH)	HE		N/A			
Group (MIH+T)	HE		SAPs (P_{11-4}) preparation: this step contains adding 50 ml of water to the powder and agitate them and use the syringe to add drop for each specimen. Curodont™ repair is used.			

Table 2 shows the application techniques and bonding procedures, SE: Sound enamel, HE: hypomineralised enamel, NA:No Application.

4.7.5 Specimens' storage before testing

The specimens were placed in phosphate-buffered saline (PBS) storage in the incubator at 37 °C for 24 hours prior to bonding testing following the ISO 29022 recommendation. The Universal Testing Machine (UTM) was then used to test the shear bond strength.

4.8 Macro Shear Bond Strength testing

The macro shear bond strength of all tested groups was evaluated according to the ISO recommendation 29022, Which outlined a standardised procedure to assess the adhesive bond strength between dental enamel and direct dental restoration [182]. A universal shear bond strength testing machine (INSTRON 3365, MA, USA) (Model 2530-416 /Serial no. 301083 -500N, Std. ISO7500, Class 0.5, certificate No: E182091222144002) equipped with a 2.38 mm wide semi-circular notched-edge shear crosshead (Ultradent, USA) was used.

4.8.1 Specimen Loading in the Machine

The loading of each specimen on the UTM was undertaken in several steps. First, each specimen was extracted from the PBS and put in the metal specimen container. The bonded tooth section was then mounted on a base clamp. The bonding surface faced the front of the base clamp, and the composite button was aligned vertically to the clamp. The base clamp was then placed into the UTM underneath its crosshead. The notch of the crosshead was positioned over the composite button against the tooth. The crosshead was lowered to the point that it barely touched the composite button. (Illustrated in Figure 15) It brushed the tooth surface while the rear of the test base clamp was pushed gently. The Test was conducted with a crosshead speed of 1.0mm/minute.

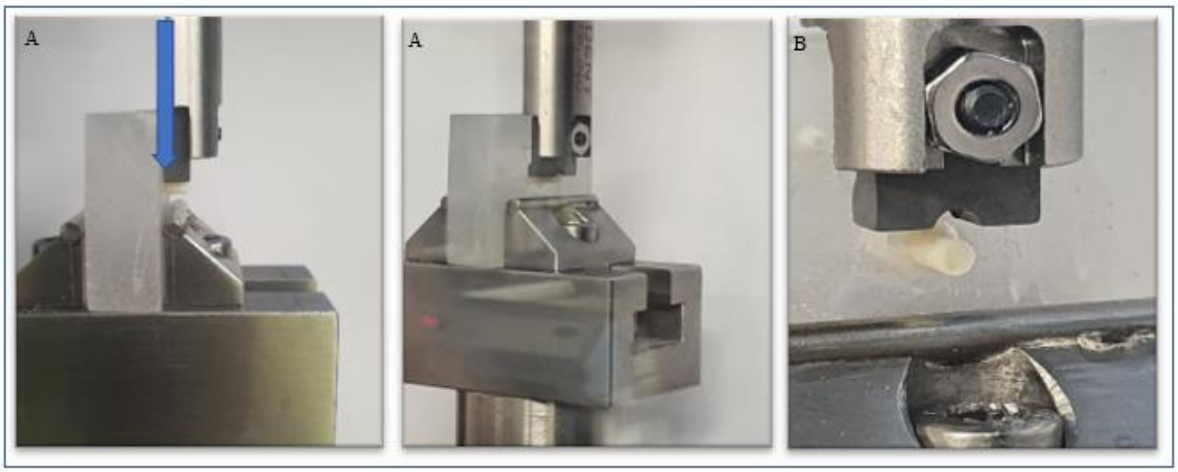
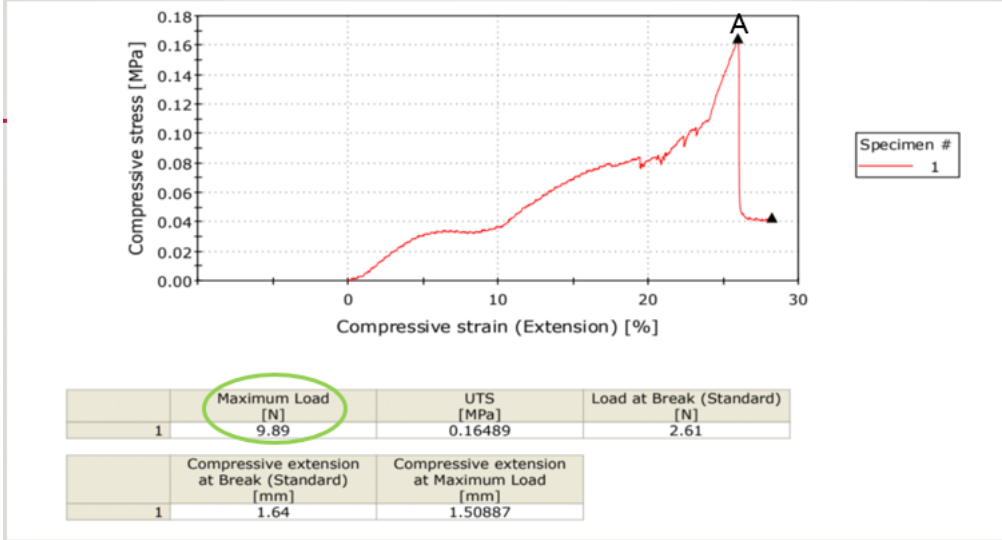


Figure 15 A: The diagram shows side profile the setup of the resin block containing the tooth in the clamp base and the direction of the force (blue arrow) was applied for composite debonding B: photograph of the debonding setup using the wide semi-circular notched-edge shear crosshead (Ultradent, USA).

4.8.2 Measuring the Force

The jig in the UTM was displaced relative to each tooth specimen at a rate of 1.0 mm/min until the maximum force, or load was reached before the specimen (composite restoration) broke. The force was recorded in the connected BlueHill program, which presents the force within a graph and in a table with numerical values (Table 3). The maximum force was presented immediately before the sudden drop from a rising line on the graph (shown in Figure 16), and this was the value used to calculate the SBS (by dividing the maximum load just before failure by the bonding surface area. Each measured force was tabulated in an Excel data-collecting sheet. (Appendix G)



	Maximum Load [N]	UTS [MPa]	Load at Break (Standard) [N]
1	9.89	0.16489	2.61

	Compressive extension at Break (Standard) [mm]	Compressive extension at Maximum Load [mm]
1	1.64	1.50887

Figure 16 depicted a line graph that illustrated the progressive increase in load (force, N) applied to the specimen. Point A indicated the peak load the specimen could withstand before composite deboned (broke), as shown by the drop in the line indicating specimen broke. Table 3, derived from the BlueHill Program, recorded force measurements (N) denoted by green circles for a specimen in the MIH group.

4.8.3 Calculating Shear Bond Strength (SBS)

The formula used to calculate the shear bond strength from the force and bonding area used in the experiment is [182]:

$\sigma = F/Ab$: where σ is stress (MPa), F is force (N), Ab is bonding area (mm^2)

The SBS was calculated within Excel by using the formula commands corresponding to the formulae above, with the surface area fixed at 4.4 mm^2.

4.9 Further analysis

The Data sheet collection was recorded with the values of DIAGNOdent and Shear Bond Strength (MPa) of each specimen of the three groups. In total, 15 Specimens from each group were randomly selected by placing them in a plastic container without knowing their DIAGNOdent or SBS. This method was used to reduce the risk of Bias. Analysis was carried out using Microtomography (micro-CT scans), and nine representatives carried out for Scan Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX). The Resin Blocks of these

specimens were minimised in size and reshaped into cylindrical shapes to be able to be fixed into the CT and SEM machines (illustrated in Appendix D).

4.9.1 Microtomography

Micro-CT is a non-invasive, widely used imaging technique that is used to assesses the tooth mineral density by indirectly assessing the organic content. It can create 3D images and can measure the mineral concentration of a tooth, as without the need to use using destructive measures [183]. Five enamel specimens from each group (SE), (MIH), and (MIH+T) were randomly selected for micro-CT scanning of mineral density using NEOSCAN_80, S/N=80D2316, Software Version=3.0.0.

4.9.1.1 Scanning and Reconstructions

For each specimen to be scanned, Hydroxy apatite standards were included which represent the 3 identified different densities as the following 0.25 g/cm^3 which correspond to low mineral density, 0.75 g/cm^3 as medium mineral density, and high mineral density with 2.9 g/cm^3. The rod was placed on the resin block next to the tooth and ensured that it was stable and in the same place during the scanning. The position of the lesion was checked visually so it can be identified easily in the analysis. The resin block was placed in the CT holder and then loaded in the machine chamber and tightened gently and door of the machine was closed carefully.

While the specimen was ready for scanning with the following parameters: Source Current (uA)=174, source Voltage was 92 (kv) current, Camera Exposure (ms) = 247, Filter=Copper (Cu) 0.25mm, Filter was used for optimisation purposes. Scan duration time was 38 min 43 seconds. The configuration of each specimen was recorded and saved in the file (BMP) titled by the name of the specimen and group.

Once the specimen had been scanned, the next step is the reconstructions of the image, which involved using the NeoScan programme to open the saved file

of the specimen. From there, every projection of the image was reconstructed into a 3D volume.

4.9.1.2 Data Viewer and Image J Fuji analysis

The first step was to upload the stack reconstructed image to the Data Viewer to be able to access the 3D view of the images. The adjustment in the orientation of each view was made to view the lesion in the right plane. The 3 views sagittal, coronal, and trans-axial were saved, and Image sequences were imported in Image J Fuji. Calibration of the Image was the first set for analysis the mean grayscale value, 15 circles of the standard rod were measured in order to calibrate with the known densities (lighter to darker radiolucency) 0.25, 0.75, 2.9 g/cm^3 respectively. After this step, the image scale setting was standardised for all the images to be able to measure the length of the selected area knowing that Image Pixel Size (um) = 9.

The surface of the tooth (area of the lesion after the de-boning test) was selected with free hand shape tool and the sub-lesion area was selected with multiple round selection tool in a dotted line. Saving the position of these selected areas as regions of interest (ROI) so their mean mineral density could be measured (g/cm^3). The mineral density of the whole scanned tooth was represented with a heat colour map (where each specific colour represents a specific mineral density for better visual presentation) (demonstrated in Figure 19 and Figure 20).
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4.9.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to assess the type of failure and qualitatively determine the failure mechanism at the restoration-enamel interface of sound enamel and hypomineralised enamel. The type of failure and assessment of the deboned surfaces were classified as follows:

1. Adhesive failure
2. Cohesive failure in enamel or in the adhesive

3. Mixed failure (partial cohesive and partial adhesive failures)

Specimens were gold-coated in an Agar Auto sputter coater before examination, and the probe current was 50 (the size of the beam is 50). This study used a Hitachi S-3400N SEM fitted with a tungsten electron source and secondary electron detector. This instrument was operated at an acceleration voltage of 10kV in a high vacuum at a nominal working distance of 10mm. Specimens were placed on 10 mm diameter pinned stubs with conductive tape.

4.9.3 Energy Dispersive X-Ray

Energy Dispersive X-Ray (EDX) analysis, using Bruker XFlash® 6 | 2x60 mm², was employed in conjunction with SEM to assess the elemental composition of a tooth and the Composite restoration. In this case, the elemental composition was Calcium (Ca), Phosphate (P), as they are tooth components and Silica (Si) as composite restoration components.

During EDX analysis, the tooth specimen's surface is stroked with an electron beam with energy of 10–20 keV. The energy from the X-rays emitted back from this action was measured [184]. The beam was moved across the teeth to form the image of the elements within each specimen [185] and a record was kept.

CHAPTER 5: RESULTS

This research involved testing a total of 75 specimens divided into three groups (SE), (MIH) and (MIH+T): (n:25) for each group. Each specimen underwent baseline mineral values assessment - using DIAGNOdent, and then the colour was assessed to classify the severity of MIH teeth- using photography and Image J. Four specimens have been excluded after premature failure and before SBS testing (the composite restoration deboned and broke from the tooth before placing the specimens into the UTM machine for applying the SBS testing). The macro-shear bond strength (SBS) was calculated following the method detailed in section (4.9.4). The SBS was calculated, and the results were tabulated in Microsoft Excel using its functions, representative specimens were selected from each group to assess the mineral density and the type of failure using micro-CT, SEM and EDX analysis. Further statistical analysis was conducted using SPSS software version 28 to determine the SBS's significance ($P \leq 0.05$) of the SBS between the three groups.

5.1 Specimen size in the study

A total of 75 specimens were tested in this research, based on a statistical power calculation of 80% with $P \leq 0.05$ being statistically significant using values from a previously published study. Based on that, it was concluded that a specimen size of (n=25) was required for each group. Due to premature failure, when the debonding of the composite restoration occurred before the SBS testing was applied, 4 specimens were eliminated (one specimen from Group (SE) and 3 specimens from Group (MIH)), resulting in 24 specimens in Group (SE), 22 specimens in Group (MIH) and 25 specimens in Group (MIH+T).

5.2 Baseline Records

5.2.1 DIAGNOdent Readings

The Median value for the DIAGNOdent readings for Group (SE) was 3.6 and interquartile range (IQR):2, Group (MIH) was 87.8 and IQR:11, and Group (MIH+T) was 98 and IQR:11. After checking the normality of DIAGNOdent values for all groups (normality test illustrated in Table 4), with the p-value <0.05, and therefore is not normally distributed. Mann Whitney test has been used, and the p-value = 0.028, indicating a significant difference between Group (MIH) and Group (MIH+T) (Box and whisker plot shown in and descriptive analysis and Mann Whitney test between MIH and MIT+T groups illustrated in Table 5). Outliers in the Box and whisker plot (Figure 17) would not impact the overall outcome, as DIAGNOdent assessment was only considered to ensure an even distribution between the experimental groups.

	Groups		
Shapiro-wilk	Group (SE)	Group (MIH)	Group (MIH+T)
Statistic	0.922	0.929	0.727
Degree of Freedom	25	25	25
Sig.	0.056	<0.001*	<0.001*
Normality Distribution	Normal	Not Normal	Not Normal

Table 4 represents the tests of normality for DIAGNOdent reading between the three group (SE), (MIH) and (MIH+T). *Significant P value <0.05

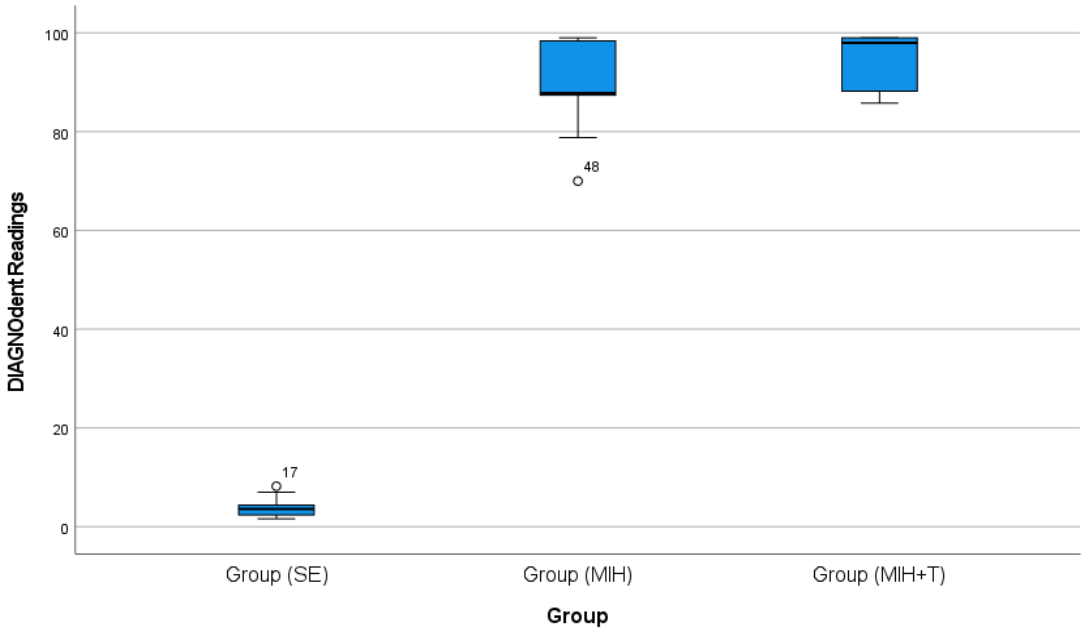

Figure 17 Box and whisker plot comparing the DIAGNOdent readings of the three groups (SE), (MIH) and (MIH+T). The DIAGNOdent reading of sound enamel (SE) group had lower values than both hypomineralised enamel in both groups (MIH) group and (MIH+T).

	Groups			
	Group (SE)	Group (MIH)	Group (MIH+T)	Total
N	25	25	25	75
Mean of DIAGNOdent readings	3.84	90.74	94.42	
SD	1.70	7.57	5.35	
Median	3.60	87.8	98	
Interquartile range	2	11	11	
Mann-Whitney test between group (MIH) and group (MIH+T) Asymp. Sig: 0.028*				

Table 5 Descriptive and statistical analysis of the DIAGNOdent readings for each group, and Mann-Whitney test between group (MIH) and group (MIH+T). *Significant P value <.05

5.2.2 Colour Quantification of MIH teeth

A total of 50 Hypomineralised lesions were identified. 18 were considered “severe”, as they had darker shades, and 32 were considered “mild /moderate” in severity, as they had lighter shades using Image J and an automatic conversion website tool (section 4.5.2.2). Both severe teeth and mild/moderate teeth were evenly distributed into Group (MIH) (9 severe and 16 mild/moderate) and Group (MIH+T) (9 severe and 16 mild/moderate). Intra-examiner reliability was evaluated for the categorization of teeth in to Mild/Moderate and Severe, with reassessment occurring at different day after the initial categorization. A Cohen’s kappa test was conducted to measure the intra-rater reliability of the tooth categorization, resulting in a value of $k=1$, $P<0.001$, indicating strong agreement for categorisation of the hypomineralised teeth of the two assessments.

5.3 Shear Bond Strength outcomes.

The average SBS was calculated for each of the three groups tested. Group (SE) had the greatest mean SBS (mean 15.8+/-12.9), followed by (Group (MIH+T)) with a mean SBS (mean 4.5+/- 1.4) and finally, Group (MIH) with the lowest mean SBS (mean 1.7+/- 0.8) (the descriptive analysis of SBS result illustrated in Table 6 and Box and whisker plot (Figure 18) comparing the SBS of the three groups (SE), (MIH) and (MIH+T)

	Groups		
	Group (SE)	Group (MIH)	Group (MIH+T)
N	24	22	25
Median (MPa)	11.73	1.59	4.44
IQR	6.4	1.1	2.1
Mean (MPa)	15.87	1.7	4.50
Std. Deviation	12.87	0.81	1.45

Table 6 represents the descriptive analysis of the Shear Bond Strength Values (MPa): groups (SE), Group (MIH) and Group (MIH+T).

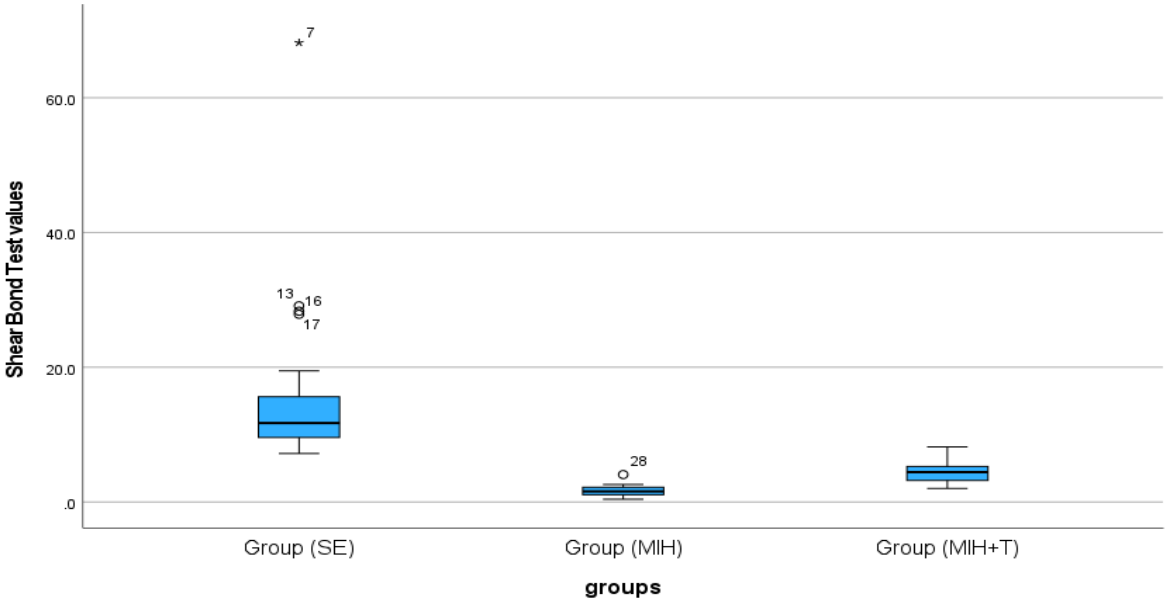

Figure 18 Box and whisker plot comparing the SBS of the three groups. The SBS has improved after adding SAPs to MIH teeth in (MIH+T) group compared to hypomineralised enamel without treatment in (MIH) group although not as high as the SBS of Sound Enamel (SE).

The locality, spread, and skewness of the SBS for each group are presented in the box and whisker plot. The median of Group (SE) is 11.73 MPa with the interquartile ranges (IQR) of 6.4. Group (MIH) median is 1.59 MPa with interquartile range (IQR) of 1.1, Group (MIH+T) median is 4.44 MPa with interquartile range (IQR) of 2.1. Anything outside these ranges is an outlier. The Figure indicated that outliers in Group (SE) appear very far from the median SBS (mostly requiring higher force and SBS), presenting normal human variations in sound teeth, and could have contributed to the wide range between each

interquartile. Less outliers are presented within group (Group (MIH)), while non-apparent in Group (MIH+T).

The outcome data was tested for normality using Shapiro-Wilk tests which indicated that Group (SE) was not normally distributed (p-value <0.05). However, Groups (MIH) and (MIH+T) were normally distributed (Test of normality represented in Table 7). Hence, the Kruskal Wallis test (non-parametric test) was used to compare between the 3 groups, which confirmed that there was a significant difference between the groups (p-value<0.05) (Kruskal Wallis test illustrated in Table 8). Furthermore, a pairwise comparisons test was used by conducting the Mann-Whitney test to compare Group (SE) vs Group (MIH) and Group (SE) vs Group (MIH+T) (Mann-Whitney was represented in Table 9). The pairwise comparison showed that there was a significant difference between (SE) vs (MIH), (SE) vs (MIH+T), and (MIH) vs (MIH+T). The Bonferroni correction was used to adjust the p-value for pairwise comparison, so the adjusted p-value was 0.0167.

	Groups		
Shapiro-wilk	Group (SE)	Group (MIH)	Group (MIH+T)
Statistic	0.606	0.925	0.969
Degree of Freedom	24	22	25
Sig.	<0.001*	0.096	0.625
Normality Distribution	Not Normal	Normal	Normal

Table 7 represents the tests of Normality for Shear Bond Strength between the Groups (SE) (MIH) and (MIH+T). * Significant P value <.05

	Groups		
	Group (SE)	Group (MIH)	Group (MIH+T)
Median (MPa)	11.73	1.59	4.44
Interquartile range	6.4	1.1	2.1
Kruskal Wallis test	60.09		
p-value	< 0.001*		

Table 8 represent the statistical analysis of Kruskal Wallis test for SBS between the three groups (SE), (MIH), (MIH+T). *Significant P value <.05

	Groups		
	Group (SE) vs Group (MIH)	Group (SE) vs Group (MIH+T)	Group (MIH) vs Group (MIH+T)
Mann-Whitney test (z-statistics)	5.08	5.92	5.54
P-Value	<0.001*	<0.001*	<0.001*

Table 9 represents the statistical analysis of the Mann-Whitney test Between Group (SE) Vs Group (MIH) and Group (SE) vs Group (MIH+T),*Significant P value <0.05

5.4 Further Analysis

5.4.1 Microtomography

X-ray micro-computer tomography was used to quantify the mineral density of five specimens from each group. Generally, between the three groups, the lowest value mineral density was 1.6 g/cm^3, and the highest was 3.2 g/cm^3 between all the specimens. Sound teeth had generally higher mineral density than hypomineralised lesions in the MIH teeth, with or without pretreatment of SAPs. The mean mineral density in sound teeth was the highest, with a value of 2.58 g/cm^3, while the mean for MIH teeth was 2.04 g/cm^3. In Group (SE), the mineral

density of sound teeth ranged from 2.2 to 3.2 g/cm^3, while it was similar between the other two groups of MIH: between 1.6 to 2.5 g/cm^3 in Group (MIH), and 1.7 to 2.5 g/cm^3 in Group (MIH+T). (Table 10 and Appendix H). There is no relation between the SBS and mineral density or type of failure.

	Groups		
	Group (SE)	Group (MIH)	Group (MIH+T)
N	5	5	5
Mean of Mineral Density g/cm^3	2.58	2.04	2.04

Table 10 illustrates the mean values of mineral density of five specimens of each of the three groups (enamel surface and subsurface measured area) after the SBS testing.

To enhance the visualization of micro-CT mineral density values in the specimen images, a colour scale was employed to depict the mineral densities of the teeth. The mean value of mineral density of each group (represented in Table 10) was correlated with the values in the colour scale which illustrated in (top left side of) Figure 19 and Figure 20. For example, blue represented the mean mineral density value of 2.58 g/cm3 for sound enamel, while green represented 2.04 g/cm3 for hypomineralised enamel. The specimens were chosen randomly to ensure they represented the overall specimen population. Figures 14 and 15 were specifically selected to illustrate the general specimen, as they displayed the mean mineral density values.

A mineral density distribution in a sound enamel specimen in Group (SE) represented in Figure 19/A, the micro-CT scan image of a sound tooth specimen in axial view, coloured with heat map scale, showing the general distribution of mineral density within the dental tissue. The yellow selected area illustrates the surface which the composite area that has been broken after the SBS testing, and the blue square A1 shows the mineral density of the lesion surface (Dark Blue colour represented mineral density of 2.9 g/cm^3). Figure A2 is the maximised area of the blue square in figure A representing the lesion subsurface mineral density represented by a (multiple dotes) selected in a line. In Figure A it's obvious that the enamel surface (where the composite was deboned after the

SBS test) has higher mineral density when compared to the rest part of tooth which indicating stronger dental tissue.

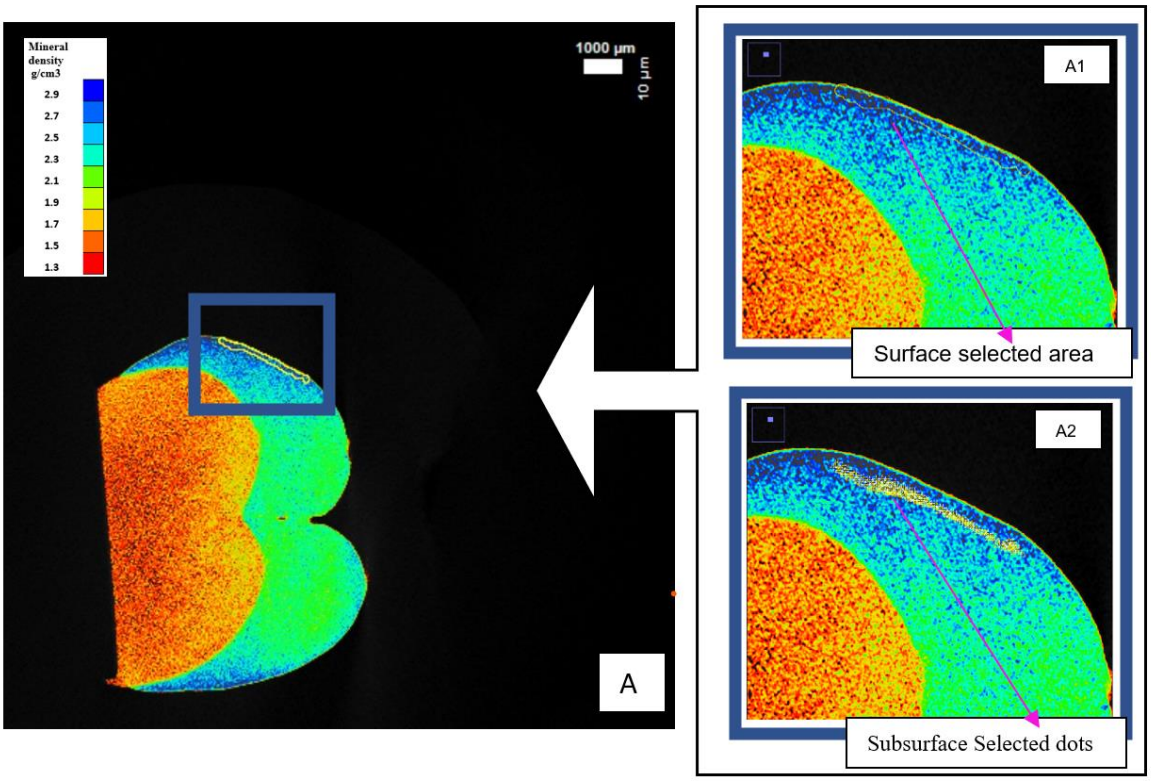

Figure 19 illustrates the Micro-CT Specimen of the sound tooth.

However, Figure 20 presents a micro-CT scan image of a hypomineralised tooth specimen in an axial view, showing the mineral density distribution within the enamel of a specimen from the (MIH) group. A coloured heat map scale vividly displays the mineral density within the dental tissue. The blue square in Figure B1 highlights a magnified area, showing the enamel surface after the SBS test in a yellow-selected area, representing the mineral density of a blend of colours from green to blue, corresponding to mineral densities ranging from 2.1 g/cm³ to 2.5 g/cm³, respectively. Additionally, remnants of composite restoration are visible on the surface (signifying leftover composite restoration), marked by red to yellow colours, indicating mineral densities from 1.3 g/cm³ to 1.9 g/cm³ similar mineral density of enamel closer to the dentine. Figure B2 illustrates the mineral density of subsurface area, with mixed green and blue hues indicating a density of 2.1 g/cm³ to 2.5 g/cm.

Overall, Figure 20 clearly shows that the hypomineralised enamel surface and the composite restoration exhibit a lower mineral density compared to the sound enamel depicted in Figure 19.

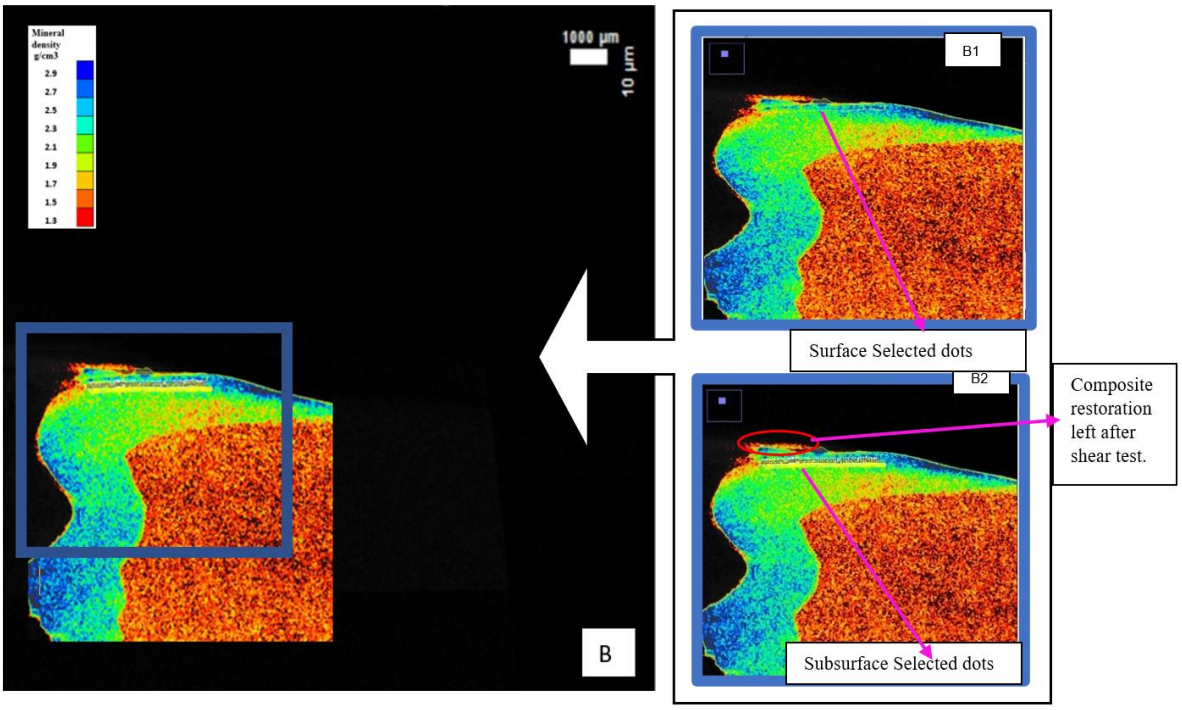

Figure 20 illustrated the Micro-CT Specimen of MIH tooth.

5.4.2 Scan electron Microscope and Energy Dispersive Xray

The type of failure was assessed using a Scanning Electron Microscope (SEM) and Energy Dispersive Xray (EDX) analysis. With SEM analysis, the specimens were viewed to assess where the deboned location has occurred and hence the type of failure; with the EDX analysis, the elements present at the surfaces were assessed (Calcium, Phosphate and Silica elements). In general, there are 3 types of failure**:** **Adhesive Failure**, which occurs between two different materials, such as between enamel and adhesive (bond) or between composite restoration and adhesive (bond). **Pure cohesive failure**, on the other hand, happens within a single material, meaning it could occur entirely within the enamel or within the composite restoration**. Mixed failure** involves a combination of both types, where both the adhesive bond and the cohesion within the enamel or composite restoration fail or any mixture of previously mentioned type of failures. (Adhesive (bond) and cohesive in enamel or adhesive (bond) and cohesive in Composite restoration).

Nine specimens in total were randomly selected for analysis using SEM and EDX. Three specimens from each Group (SE), Group (MIH) and Group (MIH+T),

generally, two specimens had pure composite cohesive failure and seven had mixed failure.

In Group (SE), an analysis of the three specimens revealed that two of them found with mixed failure, involving both adhesion and cohesion within the composite. One example of this mixed failure can be seen in Figure 21. The third specimen, however, displayed pure cohesive failure within the composite. This was confirmed by the presence of the element Silica (Si), identified using EDX, which indicated that the bonded area was entirely covered by the composite material. This pure cohesive failure is illustrated in Figure 22.

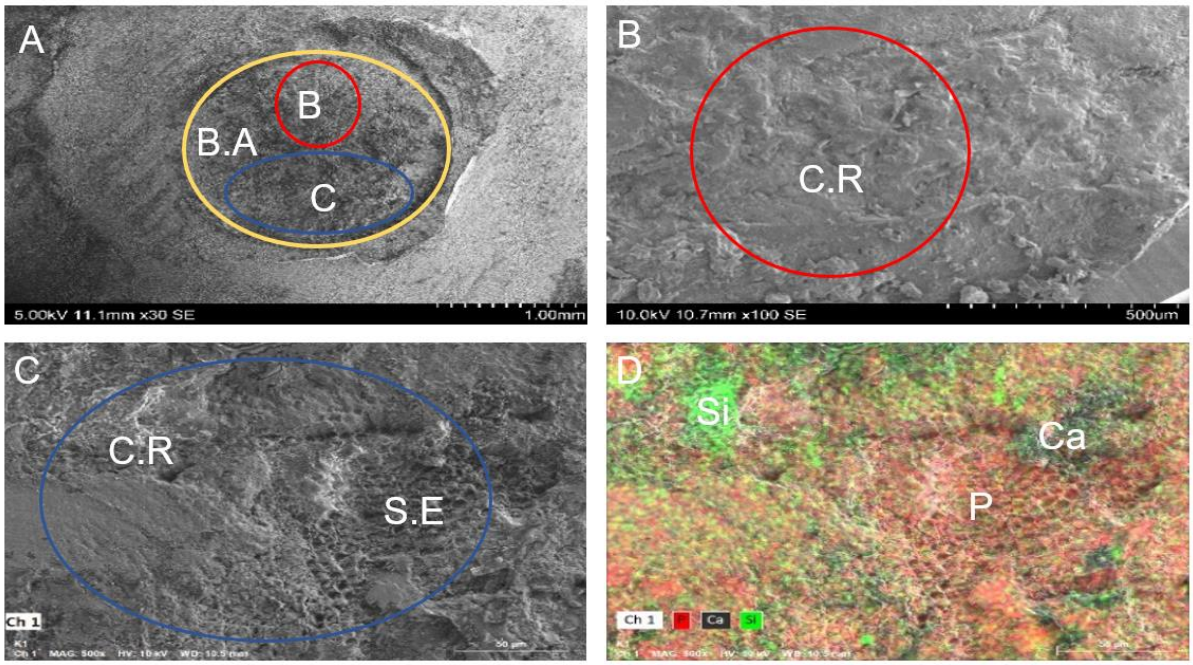

Figure 21 illustrates a specimen of sound tooth in Group (SE) scanned with SEM after SBS testing. In scan A: The yellow circle represents the bonding area (B.A) (at which the composite cylinder was bonded). In scan B, the red circle is the area showed the composite restoration (C.R) left on the surface. In scan C: another scanned area in the bonding area with X500 (higher magnification) showing the 2 different surfaces: the tooth (sound enamel (S.E)) and the adhesive (composite restoration(C.R)). In scan D, represents the EDX, which showed the elements of the surface: green represented Si: Silica (component of composite restoration), P: phosphate (red), and Ca: calcium (blue), which are components of the dental enamel). In this specmen, the type of failure is mixed faliure (adhesive and cohesive in composite restoration).

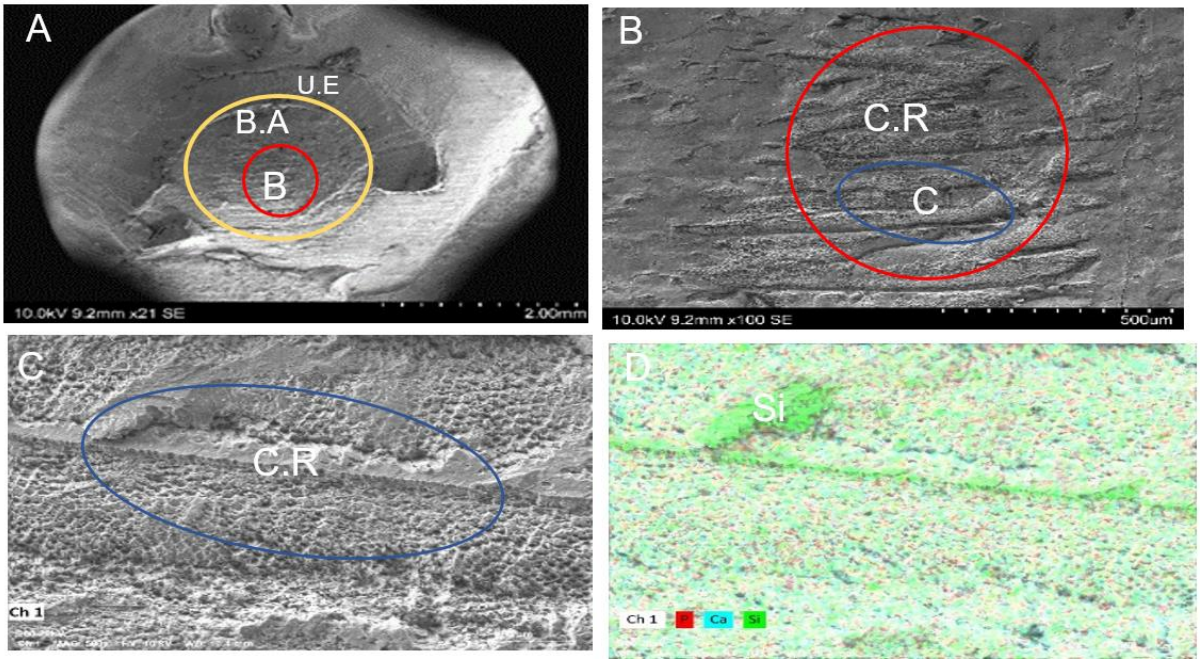

Figure 22 illustrates a specimen of the sound tooth in a Group (SE) scanned with SEM after SBS testing. In scan A.The yellow circle represents the bonding area (B.A: at which the composite cylinder was bonded) and the Unbonded enamel area (U.E), which is the surrounding enamel but not bonded to the composite. In scan B, the red circle is the area showed the composite restoration (C.R) left on the surface covering the enamel (enamel rod). In scan C: another scanned area in the bonding area with higher magnification showing the enamel (tooth) covered by composite restoration (adhesive). In scan, D represents the EDX, which shows the elements of the surface (green), Si: Silica (a component of composite restoration), P: phosphate (red), and Ca: calcium (blue) (components of the dental enamel). The type of failure is pure cohesive (cohesive in composite restoration).

In Group (MIH), one specimen has a pure cohesive of composite (illustrated in Figure 23), while the other two specimens have mixed failure (the Mixed failure of one of the specimens is shown in Figure 24).

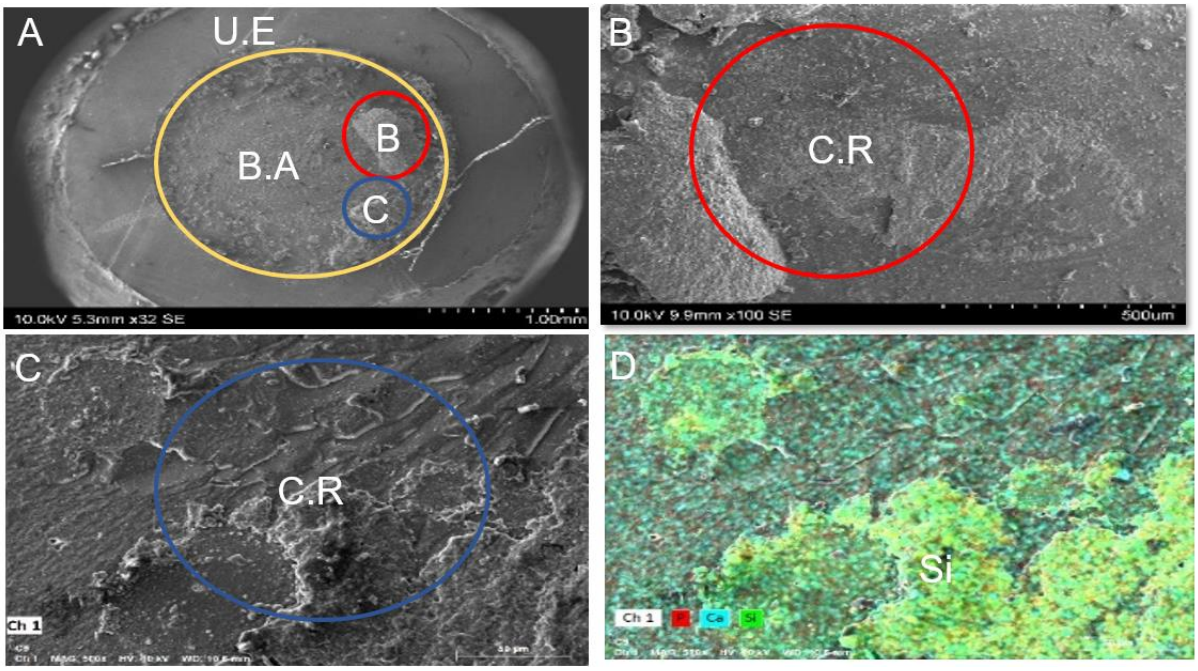

Figure 23 illustrates a specimen of hypomineralised enamel in Group (MIH) scanned with SEM after SBS testing. In scan A: The yellow circle represents the bonding area (B.A) (at which the composite cylinder was bonded) and, and U.E is the unbonded enamel surrounding the bonding area. In scan B, the red circle is the area (18B) showed the composite restoration (C.R) left on the surface. In scan C: another scanned area in the bonding area (18C) with higher magnification showing the enamel (tooth) covered by composite restoration (adhesive). Scan D represents the EDX, which showed the elements of the surface (green) Si: Silica (a component of composite restoration), P: phosphate (red), Ca: calcium (blue) (components of the dental enamel). The type of failure is pure cohesive in composite restoration.

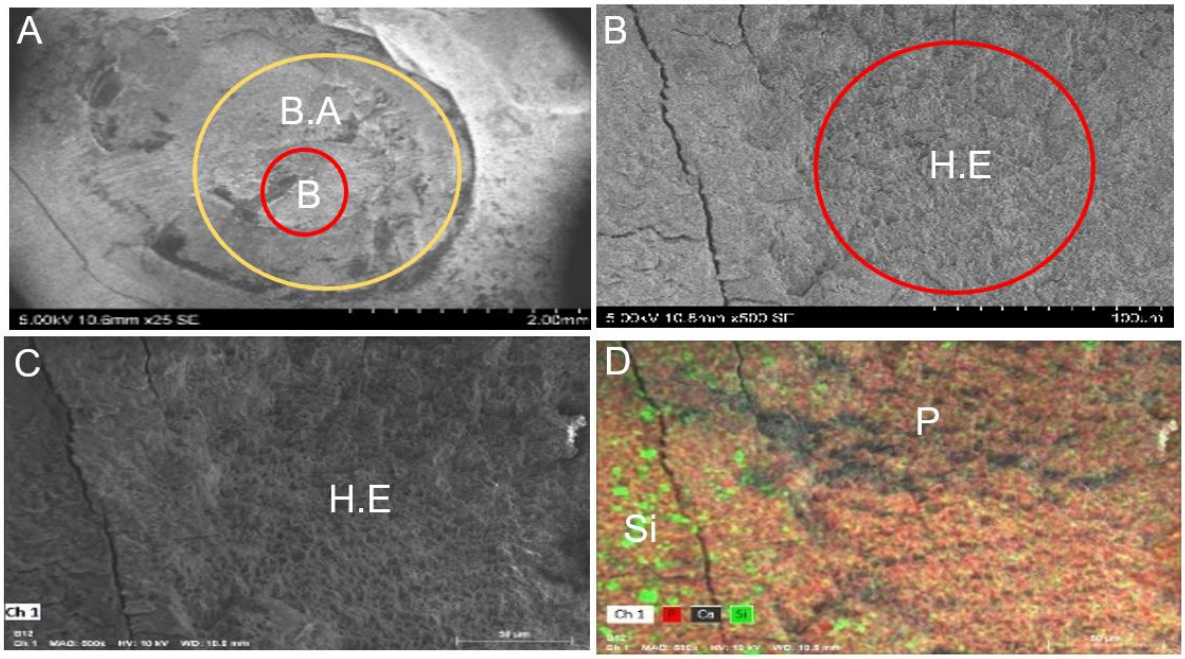

Figure 24 illustrates a specimen of hypomineralised enamel in Group (MIH) scanned with SEM after SBS testing. In scan A: The yellow circle represents the bonding area (B.A) (at which the composite cylinder was bonded). In scan B, the bonded area showed the hypomineralised enamel (H.E) exposed after the shear bond testing. In scan C: another scanned area in the bonding area with higher magnification showing the hypomineralised enamel H.E (tooth) and composite restoration (adhesive). Scan D represents the EDX, which showed the elements of the surface (green), Si: Silica (a component of composite restoration), P: phosphate (red), and Ca: calcium (blue) (components of the dental Enamel). The type of failure is Mixed failure (adhesive and cohesive in composite).

In Group (MIH+T), all three specimens exhibited mixed failures. For two of these specimens, the failures were a combination of pure cohesive failure within the composite and adhesive failure. This mixture of failures was evident from the surface colours of the specimens, which showed blue, red, and green, indicating the presence of Calcium (Ca), Phosphorous (P), and Silica (Si), respectively, as depicted in Figure 25. The third specimen also showed mixed failure, but it included adhesive failure and two distinct pure cohesive failures: one within the composite and the other within the enamel, as illustrated in Figure 26.

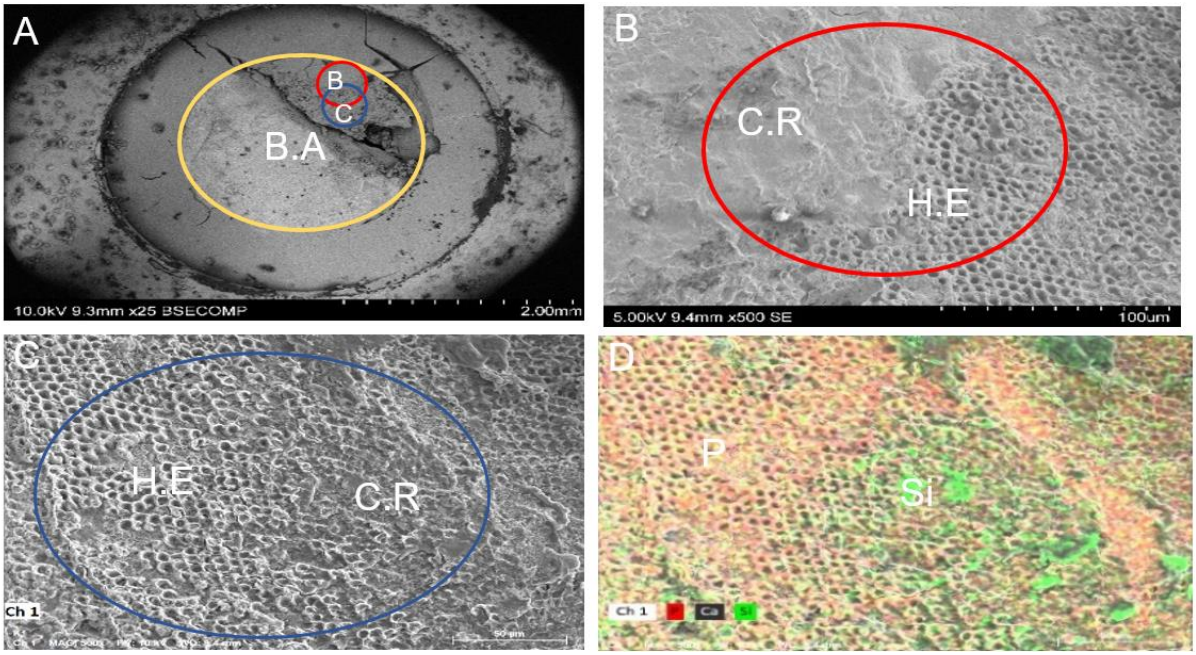

Figure 25 illustrates a specimen of a hypomineralised enamel in Group (MIH+T) scanned with SEM after SBS testing. In scan A: The yellow circle represents the bonding area (B.A) at which the composite cylinder was bonded) and U.E which area of surrounding enamel but not bonded to composite cylindar. In scan B, the red circle is the area showed the composite restoration (C.R) left on the surface and exposed hypomineiralised enamel (H.E) . In scan C: another scanned area in the bonding area with higher magnification showing the enamel (tooth) and composite restoration (adhesive). In scan D: elements of the surface (green), Si: Silica (a component of composite restoration), P: phosphate (red), Ca: calcium (blue) (components of the dental enamel). The type of failure is mixed failure (adhesive and cohesive in composite).

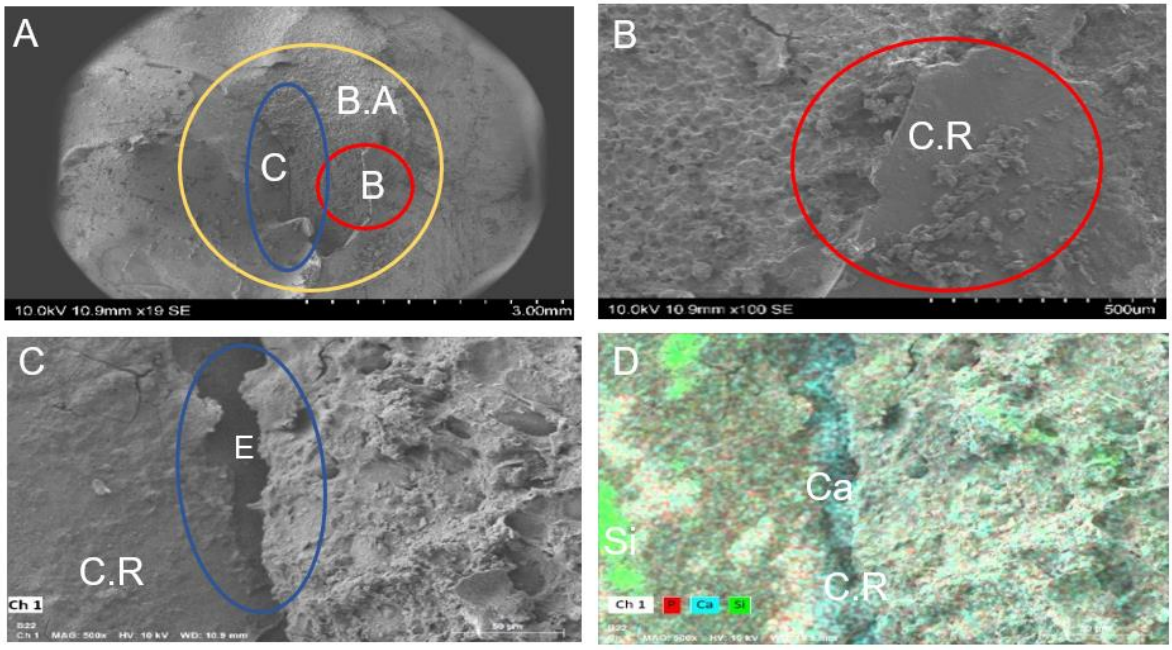

Figure 26 illustrates a specimen of a hypomineralised enamel in Group (MIH+T) scanned with SEM after SBS testing. In scan A:The yellow circle represents the bonding area (B.A at which the composite cylinder bonded). In scan B, the red circle is the area showed the composite restoration left on the surface). In scan C: another scanned area in the bonding area with higher magnification showing the enamel (tooth) partially covered by composite restoration (adhesive). In scan D represents the EDX, which showed the elements of the surface (green), Si: Silica (a component of composite restoration), P: phosphate (red), Ca: calcium (blue) (components of the dental enamel). The type of failure is mixed failure (adhesive and cohesive in composite and cohesive in enamel).

CHAPTER 6: DISCUSSION

6.1 Introduction

Molar Incisor Hypomineralisation (MIH) is a common developmental enamel defect that affects the first permanent molars and central incisors, characterised by reduced mineralisation and lower mineral content in affected teeth [21, 186]. Restorative dentistry for MIH-affected teeth presents multiple challenges [8], and the condition also has implications for aesthetic concerns (especially in anterior teeth [6]), bonding failure, tooth sensitivity, and physiological challenges for both patients and their families. To address these issues, it is crucial to develop targeted interventions that consider both the clinical and psychosocial aspects of affected individuals. Studies have shown mixed results from using 5% NaOCl and/or 37% phosphoric acid to pre-treat MIH-affected teeth to improve bonding. However, a single material that can improve sensitivity, bonding, and aesthetics for managing MIH lesions has yet to be found. According to B-Martins et al., the use of Self-Assembling Peptides (SAPs) as innovative approaches to enhance Shear Bond Strength (SBS) have offered a promising avenue for surface modification of the dentine tissue [186].The objectives of this research were to assess whether the application of a commercially available SAPs P_{11-4} to the, yet untreatable, hypomineralised teeth would improve the shear bond strength of composite restoration in MIH affected teeth. The results of SBS on a specimen of 25 MIH teeth post SAPs application compared to MIH teeth alone and sound teeth yielded valuable insights into the potential of this innovative approach for clinical dentistry for patients with MIH.

Considering that there is currently no clearly defined cause to prevent MIH development in children, or treatment to preserve the affected teeth, the application of SAPs which is already widely available and being used in the dental clinic, may become a novel approach. However, more consideration should be made before recommending its use clinically. The following discussion provides an overview of key findings, methodological considerations, implications, and potential future directions.

6.2 Study Design

An in vitro experiment was designed to be carried out in the lab to investigate whether the application of SAPs would enhance SBS in MIH teeth, and it was carried out using a rigorous and systematic approach.

The in-vitro study design allowed more environmental control. All teeth were cut on the same day and stored under the same condition, and a single force was applied to the tooth specimens, to assess the specific changes caused by that force. Randomization was not applied in this study. Though this might have introduced an element of selection bias, it allowed the assessment of the severity of each specimen before trying to evenly distribute them between Groups (MIH) and (MIH+T). Hence, the specimens in each tested group that contained MIH teeth were rather more standard. This in turn allowed for better comparisons between the groups and provided a solid foundation for evidence-based practices in dental research and clinical applications. While it is practical to apply such methods especially for initial assessment, further studies will need to be conducted before gaining the total clinical presentation.

6.3 Specimen Selection and confounders

Human teeth were obtained from the tissue bank with natural hypo-mineralised defects. This helped with the research on MIH teeth**.** Naturally, the gold standard of in-vitro studies is derived tissues [187]. Unfortunately, hypo-mineralised lesions cannot be artificially made in the laboratory owing to their composition and phenotype.

The collected teeth were collected after extraction from patients who attended the clinics at the Leeds Dental Institute, where teeth were extracted and sent to the tissue bank. Unfortunately, the specimens had no documented details about their patients of origin [188]. Some of the un-mentioned details, such as the patient's age, gender, ethnicity, prenatal conditions, exposure to PCBs, and daily fluoride use, may act as confounders in this research. According to the literature, MIH has been reported to be more common in boys [189], and to cause higher use of fluoride and (CPP-ACP) in attempts to control the tooth sensitivity. Not having enough data about the specimens might have caused an uneven

distribution of the specimens into groups. On the other hand, luckily, the extraction reason was considered. The research teeth were carefully selected from the Tissue Bank by choosing those that were extracted, for example, for orthodontic reasons, to avoid the use of carious MIH teeth.

Moreover, the teeth selected for the study were exclusively permanent molars, which are more susceptible to MIH. Particular attention was given to choosing lesions from the smooth surfaces (buccal, lingual, mesial, or distal) rather than the occlusal surface, as the latter can present challenges during the subsequent specimen preparation. Though lesions on the occlusal surface are more common in MIH molars, the enamel in this area is fragile and has post-eruptive breakdown areas, making it difficult to control preparation lesion size in laboratory settings. Finally, previous research has noted variations in enamel prism orientation across different tooth surfaces, with the buccal surface having well-defined prisms and the lingual surface having shallower prisms [190].Due to limitations in the laboratory setting, all surfaces, except the occlusal, were included in the study.

6.4 Specimen Size

The data were entered in a web-based tool and using a power of 80% which concluded that the specimen size of 74 was recommended. A specimen size of 75 was chosen to be able to have equal specimens in each of the three groups. Several challenges were faced while collecting MIH extracted human teeth to reach the desired specimen size, including a low inflow of extracted human teeth with MIH lesions in the Tissue Bank, limited availability of extracted teeth to share with other researchers, and a pause in the collection of extracted human teeth due to the COVID-19 pandemic.

6.5 Specimen Storage

Normally, the teeth specimens for in vitro studies need to be stored in 100% humidity [191]. Therefore, the study specimens were kept in liquid storage media following ISO 29022 at 37 °C. Considerations should be given to the type of

storage media used for the specimens, as different media may cause alterations in the mineral content or the mechanical properties of the specimens [192, 193]. These environmental alterations may affect the outcomes of mechanical testing carried out in vitro. Additionally, since the study investigated hypomineralised enamel and was sensitive to its mineral content, the use of an aqueous solution, though it can cause mineral leaching, was justified as it preserves the hard tissue's hydration [194].

Thymol 0.05% was chosen as the storage media; the thymol solution was made and has been widely used in studies investigating MIH teeth [195, 196]. According to previous studies, there were notable differences between specimens preserved in water/thymol and saline/thymol compared to those stored in ethanol and formalin [193, 197]

In this study, the teeth were stored dry before the assessment with DIAGNOdent. As the level of reproducibility is heightened in dry conditions, it is recommended to employ the drying method to detect caries-like lesions on unobstructed smooth surfaces, especially when utilising a DIAGNOdent device [198]. However, prior to SBS testing, they were stored in PSB for 24 hours in an incubator at 37 °C to ensure that they remained under moisture conditions before the composite breakdown testing following the ISO 29022. PSB was chosen as the storage media as it is non-toxic and acts as an isotonic buffer solution. It is commonly used in biological studies to maintain a constant pH and prevent cell rupture. This is owed to the fact that PSB mimics the human body's pH, osmolarity and ion concentration.

6.6 Teeth Grouping

The grouping of MIH teeth was conducted according to baseline measurements. Firstly, DIAGNOdent was used to determine the baseline mineral content of each tooth. Following this, colour quantification of the lesions in the specimens was undertaken to assess the severity of hypomineralised teeth and group them into (MIH) and (MIH+T groups). This colour analysis process ensured a fair distribution of MIH teeth in each group. Generally, MIH teeth, in comparison to sound teeth, had lower mineral content, indicating less calcium and phosphorus, while they tended to have higher organic content.

The main idea behind using DIAGNOdent is its ability to differentiate between the adsorption and scattering of the laser fluorescence radiation shot at the teeth specimen with carious lesions and the sound tooth tissue around it. The caries lesion, due to its high bacterial content, will give a higher fluorescence at the excitation wavelength [199]. Similar fluorescent findings may occur with MIH lesions, where there is an elevated amount of protein in the tooth enamel [200]. In demineralised or, in this example, hypomineralised enamel, a rise in DIAGNOdent values would indicate a decline in mineralisation or the loss of protein content. In other words, it would confirm the presence of hypomineralisation. When assessing a tooth**, Farah et al.** found that the reading provided by the DIAGNOdent does not necessarily imply a greater degree of hypomineralisation than that of a lower reading [180]. The mean DIAGNOdent values reported in the previous study have been summarised in Table 11 [6]. It may be valuable to note that the values reported in this study were somewhat similar to those of our research: sound tooth mean 3.9, MIH tooth mean: 90-94.

Enamel colour (code)	Number of samples	Mean (and range) LF	Mean (and range) MD (g/m^3)
Normal (1)	40	4.17 (1–29)	2.51 (2.26–2.81)
White/Chalky opacity (2)	22	26.6 (3–99)	2.24 (2.04–2.46)
Yellow opacity (3)	42	75.84 (40–99)	1.93 (1.68–2.4)
Brown opacity (4)	28	86 (73–99)	1.67 (1.52–1.74)
Posteruptive breakdown (5)	Not applicable	Not applicable	Not applicable

Table 11 the green box represents the mean and the range of the DIAGNOdent for each colour-code in the other study. No MD or LF could be recorded for the PEB. category. This Figure reproduced with a permission of the rights holder from [6]

Due to its low accuracy in assessing MIH severity, the study cannot solely rely on DIAGNOdent readings to distribute the specimens between groups evenly. An adjunctive second tool was adopted to avoid bias during grouping and overcome the previous device's limitations. It should be noted that DIAGNOdent readings weren't neglected, as they were confirmatory of the lesion severity when combined with the second tool, a colour sensor.

While spectrometers are conventionally regarded as the standard devices for colour measurement, colour sensors are deemed superior in applications prioritising repeatability over accuracy. A colour sensor, similar to the human eye,

monitor, or scanner, decomposes light into its RGB components. In this study, standardisation was carried out in all the photographing techniques, such as using the same camera, photo sets, and operator to ensure uniformity, repeatability and accuracy. The lesion 's calibration, measurement and accurate colour quantification were conducted using Image J, as referenced previously, rather than the subjective visual analysis of researchers, hence reducing human error.

Colour coding [6] and systems have been used in multiple studies to quantify tissue colour. One of the main ones used is the CIELAB (ΔEab) colour system [201], where the numerical values of the L*, a*, and b* can be determined, allowing for comparison between the specimens [202]. CIELAB was employed for colour quantification in this study, although an alternative method for quantifying shade, such as spectrometry Vita shade, exists, it was not utilised due to the need for further clarification, as indicated in the literature review.

In MIH teeth, It is noteworthy that darker shades correspond to more severe conditions,as previously noted [6]. To enable subjective assessment similar to those in a clinical setting and to evenly divide the 50 MIH cases into two groups, a standardised colour sheet was created, converting the LAB numbers to Hex shade - which can be identified in Microsoft programs as a standard colour. However, it is essential to acknowledge certain limitations, including the potential impact of the operator and the subjective selection of the shade.

6.7 Enamel Specimen Preparation

Each tooth root was identified with a number to facilitate the identification of the crown surface before the cutting process. When cutting the teeth into specimens with the Accutom machine, they were cut below at the root beneath the cement-enamel junction and detached them from the crown. This ensured that the maximum surface of the crown was included. The crown was evaluated and sectioned vertically into parts to produce a smooth surface enamel specimen with a 3 mm diameter hypomineralised lesion. These dimensions were chosen to allow the placement of the cylindrical resin while having enough space within the cylindrical area around the lesion. Using the Accutom machine for dental enamel cutting for the preparation of specimens for SBS testing has its advantages, as

it provides a controlled and precise cutting method to obtain an accurate reliable specimen with a standard size while minimising human error within a timely and safe manner [203]. The specimens were then placed into 3 mm by 10 mm moulds, and a resin mix was applied using a standardised method of the Struers manufacture instruction. The measurements of the mould allowed for the block to be easily fitted and placed into the UTM without causing an obstruction for the cross-head during the procedure while allowing the force applied to be perpendicular to the composite restoration.

6.8 Resin Composite Bonding

After preparation of the enamel specimen, the etch and rinse technique was the one adopted by the study, applying 37% phosphoric acid gel on the enamel surface prior to adhesive application. Unfortunately, the technique suffers from a major drawback: clinical variability as it is a multi-step procedure. Yet it was the technique adopted as it was reported to have higher bond strength values and to completely remove the smear layer when compared to other techniques [120, 204]

It is noteworthy that universal adhesive systems are widely used due to their ability to bond to different substrates in a comparable bond strength to single step self-etch adhesive systems. Additionally, they showed the ability to overcome some of the drawbacks of the simplified self-etch bonding systems. Many universal adhesives have the advantage of being used as multi-mode adhesives [205].

A universal adhesive was used by the study in etch and rinse mode, then composite was added to replicate the clinical scenario; MIH teeth are normally restored with composite. Filtek Supreme Universal (3M Oral Care) was the composite used by the study as per its manufacturer instructions it is indicated for use posteriorly.

6.9 SBS Testing

SBS testing is a commonly used test in the assessment of the adhesive properties. Macro-SBS test was used in the study rather than μSBS as the size of the lesions studied were (>1mm) [120, 121]. Furthermore, it is less technique-sensitive than μSBS and requires thin composite cylinders of specific diameter [122, 123]. During the test, the blade used had a notched-edge as it allows stresses distribution and concentration closer to the contact and interface than the knife-edge [91].

The reproducibility of SBS is the key to generalising results on an adhesive system and types of bond failure. The methodology was standardised throughout the testing process to ensure consistency and reproducibility and reduce confounding variables influencing the results, which may occur at different stages of specimen preparation. These variables may include unequal exposure to temperature, which may affect the SAPs' binding to the composite. The specimens were tested using the same UTM set-up and the same software program to assess SBS and analyse the force.

The wide range of techniques utilized in laboratory-based assessments of SBS may be a challenge when comparing between studies, especially since there are limited studies focusing on the bond strength of teeth affected by MIH. This can be due to the use of a smaller specimen size (hence not significant to show an effect), the use of different specimen preparation and storage, the use of different teeth (such as pre-molars, while in this study, molars were used), the bonding technique adopted, or the mode of bonding or de-bonding observed. Hence, although recent literature has proposed a standardised procedure, interpretation of results must be done cautiously [206, 207].

6.10 Improvements in SBS

Unfortunately, few studies investigated the SBS of MIH-affected teeth. The macro-SBS results obtained by our study in both sound and MIH teeth were comparable to the micro-SBS results previously published by **William et al.** and **Chay et al.** (Table 12). Though the two studies used the same teeth specimens;

molars, yet their teeth grouping rather relied on subjective methods, unlike our study which used DIAGNODent and ImageJ to quantify colour and assess MIH severity. They also used a wire loop to test the SBS instead of the notched-edge cutting blade that was used in this study [208, 209]. However, the results comparability reinforces the reliability of our results for sound and MIH teeth and for MIH teeth after SAPs application.

Our study found that SAPs P_{11-4} onto MIH teeth prior to composite restoration improved the SBS measurements to statistically significantly higher values compared to MIH teeth bonded to composite. However, this improvement didn't allow the SBS measurements to reach those of sound enamel. This improvement could be explained by the peptides' ability to self-assemble into a stable network when the environment is suitable. Self-assembly occurs through the formation of nanotapes, ribbons, and fibrils to fibers [159, 210, 211].

In this research, SAPs P_{11-4} is used as a surfactant [212]. By definition, Surfactants are molecules that can lower the surface tension of liquids, allowing them to spread more easily within surfaces [213]. In the case of SAPs P_{11-4}, its molecular structure enables it to adsorb positively at interfaces between different phases, such as the enamel and composite material. This adsorption reduces the forces at these interfaces, promoting better mixing and dispersion of the protein within the porous enamel of MIH teeth. This finding is considered study's primary finding as bond improvement minimises the risk of restoration failure, decreases the necessity for stainless steel crowns (SSC) potential tooth extraction, and recurrence of decay.

However, other studies improve the SBS by the ability of SAPs to increase mineral density [214] and their capacity to establish a stronger adhesive interface between the tooth surface and dental material [215, 216]

SBS (MPa) of Composite Restoration on Enamel	Previous Literature [209]	Previous Literature [208]	This Study
Sound Teeth	16.27 +/- 10.4	29.03 ± 6.75	15.9
MIH Teeth	7.08 +/- 4.9	22.05 ± 5.14	1.7
With SAPs on MIH Teeth	-	-	4.5

Table 12 SBS of Previous Literature compared to our Study.

6.11 Mineral Density and Type of Failure

Micro-CT scans were used to assess the mineral density of teeth of random specimens and were further backed up by SEM to understand the type of failure, whether adhesive, cohesive, or mixed.

Micro-CT used for dental mineral density assessment as it allowed the non-invasive reconstruction of the different areas [217]. Our results showed that the sound teeth had generally higher mineral density than MIH teeth, alone or with SAPs. This is in harmony with the CT findings of **Garot et al.**, which showed that the mineral density was reduced by 19 % ($p < 0.0001$) in hypomineralised teeth compared to sound teeth. In the case of MIH, it helped with the visualization of the deeper areas, where the milder forms of MIH may affect the tooth and may go unnoticed (at the dentine-enamel junction rather than the enamel surface) [218].

Scanning electron microscopy (SEM) and Energy Dispersive Xray (EDX) analysis were other imaging techniques used in this study. While SEM is limited in that, unlike the CT, it only assesses the tooth surface [217], it was used in this study to assess the type of bond failure. The study noted the most common type of bond failure a was mixed failure and pure cohesive failure in the composite.

The adhesive failure rates at the interface between the enamel and the composite occurred mainly in the hypomineralised group.

Generally, the low rates of adhesive bond failure could be implied by the high bond strength between the enamel and adhesive. The latter might be due to the bonding technique used, the etch and rinse technique, or the use of SAPs in MIH treated teeth, which supports their potential ability to improve bonding. The fact that the rates of adhesive bond failure were greater in the hypomineralised enamel (MIH) group might be explained by the nature of the lesions, which are more porous and susceptible to fracture, which hinders surface bonding.

High pure cohesive failures in composite in the mixed faliure could be related to the high bond strength between enamel and adhesive or a defective composite. The composite defects could be material related, such as low properties of the composite used, or operator dependent, such as improper incrementation technique or curing during the preparation steps. It should be noted that SEM results cannot be generalised, or highly interpreted, as only representative specimens were studied.

Finally, EDX analysis was used to assess the element content of the teeth. EDX shows higher calcium and phosphate percentages in the enamel of sound teeth than MIH teeth. This is similar to the findings of **Jälevik et al.** [219].

6.12 Clinical Implications

While the application of SAPs P_{11-4} has improved SBS in vitro, more considerations need to be made before recommending its use in the clinic to enhance SBS of MIH teeth in the complex oral environment of humans. Clinical trials need to be conducted to assess the performance of SAPs in a diverse patient population. Moreover, further research is required to develop practical restorative materials incorporating SAPs and their potential combinations with other dental materials. As technologies advance, perhaps customisation of SAPs can help address specific patient needs, such as the severity of enamel hypomineralisation.

Previous studies that have applied self-assembling peptides (SAPs) onto molar incisor hypomineralisation (MIH) teeth mainly focused on enhancing the bond

strength (BS) of restorative materials. SAPs demonstrated a promising clinical implication as BS improved significantly. It can be inferred that the enhanced BS may lead to improved quality of life for patients by potentially reducing the need for repeated restorations, enhancing treatment outcomes, and potentially alleviating dental fear associated with recurrent dental procedures.

Though the potential impacts on sensitivity, susceptibility to caries, or aesthetics were not studied, SAPs are assumed to carry significant clinical implications beyond BS alone. SAPs probably have the potential to reduce teeth sensitivity and caries recurrence in MIH-affected teeth. This is an indirect implication of the BS enhancement as the sealing properties of restorative materials improved using SAPs, hence reducing micro-leakage, and subsequently reducing the sensitivity and caries recurrence. Additionally, as defective, irregular, and discoloured enamel compromises aesthetics in MIH affected teeth, restorative materials may be required to replace this enamel. The enhanced BS through SAPs improved restorative outcomes by enhancing colour matching and increasing the resistance to staining or discolouration over time. Consequently, the aesthetic appearance of the affected teeth and overall patient satisfaction with their smile will improve.

The shear bonding strength of composite restorations plays a critical role in determining the longevity and success of dental treatments both in vitro and in the oral environment. In vitro studies assessing shear bonding strength provide valuable insights into the adhesive properties of restorative materials and their ability to withstand forces during mastication and daily oral functions. These findings help in selecting appropriate materials and techniques for bonding procedures in clinical practice. However, it is essential to consider the dynamic and complex conditions of the oral environment, including variations in temperature, moisture, pH levels, and mechanical stresses, which can impact the bonding performance over time. Therefore, achieving optimal shear bonding strength in vitro does not always directly translate to clinical success. Conducting studies that bridge the gap between laboratory findings and real-world oral conditions is crucial for ensuring the durability and longevity of composite restorations in clinical practice.

It was previously suggested by an in vitro study **Reynolds et al.** that the acceptable bond strength should lie between 6-8 MPa. [220].In the current study we found that the mean shear bond strength in MIH teeth treated with Saps was 4.5 MPa ± 1.4(SD). Even though this figure is slightly lower than the suggested value. It's worth noting that it was significantly higher than the average values reported for non-treated MIH samples. Suggesting that the treatment can improve the shear bond strength in MIH affected teeth.

This study serves as a valuable foundation for subsequent laboratory trials. However, it is insufficient to solely rely on this study to conclusively assert that it will enhance the longevity of restorations. Further validation through future clinical trials is necessary to justify the application of findings from this study in clinical practice.

6.13 Strengths and Limitations

The rigorous methodology involved a systematic approach that gave the results significant credibility and allowed other researchers to replicate and/or further build on the study. The study's strengths are mainly due to the novel approach of using SAPs to improve SBS in MIH teeth, offering a new perspective on a long-lasting global dental challenge. Looking at the type of failure as being most commonly cohesive failure helps better understand and further address it in future research, allowing this novel approach to be more effective and dependable. This research not only contributed to the existing body of literature but also introduced a promising pathway for more futuristic research and dental application.

Several limitations were acknowledged in this research. Firstly, the sample size used is small; it was planned to use 75 specimens, with an intended distribution of 25 specimens in each group; the sample size fell below the recommended threshold of 25 per group as determined by the power calculation. This limitation raises concerns about the potential for Type II error, where the study may have insufficient statistical power to detect true effects or differences between the groups. This can impact the study by increasing the likelihood of failing to identify

significant findings that actually exist in the population. As a result, there is a possibility that important relationships or effects related to the variables under investigation may go undetected, leading to inaccurate conclusions or underestimation of the true effects. To address this limitation and enhance the study's robustness, future research endeavours could consider increasing the sample size to improve statistical power and the chances of detecting meaningful differences or associations between the variables of interest. In addition, this may not be generalizable globally, especially when the cause of MIH is still unknown. Hence, different populations may have different vulnerability and severity of disease, react differently to SAPs, and have different availability of SAPs. The specimens collected from the tissue bank had no patient data, such as gender and age, which may act as confounders.

In addition to the limitation of the treatment protocol in the research is utilizing SAPs only and it might be worth to consider pretreatment surface using of sodium hypochlorite (NaOCl) for protein removal before applying SAPs. Another point of view is the study may be limited by the diverse types of lesions associated with molar incisor hypomineralisation (MIH) and the natural variation in affected teeth. This variability could impact the generalizability of the findings to different clinical cases. Furthermore, relying solely on shear bond strength testing as a single assessment method may limit the comprehensive evaluation of the mechanical properties and performance of the composite materials. Incorporating additional tests could provide a more thorough understanding of the material's suitability for bonding to hypomineralised enamel.

6.14 Future Directions

A few points have risen while doing this research that may help with the considerations for future studies. The specimen selection could include more data about the patient’s demographics, their habits, the reason for tooth extraction, the severity of the condition, and the prognosis of the condition to decrease bias that may result from the confounders that may result from the confounders that may arise due to the subjectivity of the oral examination in the clinic (which is based on oral hygiene, sensitivity to pain, presences of dental caries, PEB, and x-rays). Additionally, when possible, including a larger

specimen size, may increase the study's statistical power. Moreover, while there were significant improvements in SBS, other factors of SAPs use, such as re-mineralization and long-term durability of the material, need to be investigated by allowing the material to be applied with conditions for re-mineralization (waiting longer on the SAPs to take effect). While the primary outcome of the study was focused on the SBS, more studies could be conducted to explore the other mechanical pressures exerted on the tooth in the normal human chewing process. Future studies could include a fourth group where researchers could assess the effect of the acid etch use with SAPs.

6.15 Null Hypothesis

The 1st null hypothesis that there is no significant difference between the shear bond strength of composite restoration in MIH teeth compared to sound teeth was not accepted.

The 2nd null hypothesis, that there is no significant difference between the shear bond strength of composite restoration in MIH teeth following treatment with SAPs $P_{11\text{-}4}$ compared to untreated MIH teeth, was not accepted.

CHAPTER 7: CONCLUSIONS

The use of SAPs P_{11-4} on MIH teeth prior to composite restoration demonstrates potential in enhancing macro-shear bond strength by means of augmented adhesion. While this study added to the expanding body of literature on the different uses of biomimetic materials for dental restorations, the results require additional investigation through clinical studies to evaluate the practicality and durability of using gr P_{11-4} to improve the adhesive strength of composite restorations on hypomineralised enamel surfaces within the complex oral environment.

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Appendix A: School of Dentistry Skeletal Tissue Bank Application Form

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Version 4 26 April 2016

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:
The effect-of self-assembling peptide on bonding strength of composite restoration of defective enamel.

Application number:

Lead applicant name: Sarah Aljuwaihel

Co-applicant name(s) including supervisor(s): Dr Richard Balmer, Dr Robert Davies, Dr Asmaa Taie

Can animal samples be used for this research project: YES/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:
For this study, human teeth diagnosed with MIH are required, which were extracted because of poor prognosis for either elective or ortho treatment. Human teeth will be used as there is no synthetic alternative that allows for the tissue replication to be able to assess the bond strength of SAP in hypominerlised enamel tissue.

Extracted teeth will be used in 2 groups: The first group is the negative control group, which assesses composite restoration bond strength of MIH teeth, while the second group is the tested group, to investigate self-assembling peptide's effect on the composite restoration bonding strength in MIH teeth. In addition, sound teeth are required as a positive control group, for both evaluation of the standard bond strength of composite restoration on sound teeth, and as a comparison with the other MIH groups.

Type of tissue samples required:

☐ Bone ☒ Teeth ☐ Other (please specify) _____

Type of teeth and numbers required:

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):

40 Molar ☐ Premolar ☐ Canine ☐ Incisor ☐ Any

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

Any human first permanent molar diagnosed clinically with MIH

Condition of teeth:

20 Sound 20 Other (please specify) **MIH teeth**

Provide further details here if required:
Overall total of 75 slabs are needed for this research as total sample size. The total number of teeth required will depend on how many outcome samples (or 'slabs') will be obtained from each tooth.

☐ Fresh ☒ Dry

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

Part of tooth required:

☐ Pulp ☐ Root ☒ Crown

Will any part of the teeth requested be useable by other researchers?
No because after sectioning the tooth the root might be damaged or destroyed and is disposed.

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

Brief summary of your project in lay terms:
MIH teeth are unhealthy, highly sensitive teeth that breakdown easily and hence need to be restored and covered. One of the options to provide coverage is to place composite, however, the weak bonding strength of composite on MIH teeth is an issue. To solve issue SAPs will be used, and their effect on bonding strength will be invested.

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, it is a part of my Professional Doctorate in Paediatric Dentistry

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):
Bioengineering and Biomaterials Laboratory, Level 6, Worsley Medical and Dental Building, Leeds Dental Institute and, Level 7, Wellcome Trust Brenner Building, St. James

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):
After Sectioning the teeth and preparing of 75 slabs then mounting in resin blocks where they will be stored in artificial saliva with labelled container with investigator's name, date of collection and the same ID on it, in the cold room.

How will the tissue be disposed of?

☒ Clinical waste ☐ Returned to Tissue Bank

If disposed by clinical waste, provide details:
Following university regulations - Hazardous/sharp waste.

How is the proposed work to be funded?
University of Leeds, funded by student research fees

How was the work scientifically reviewed? (e.g. details should include name(s) of peer reviewer(s) and/or supervisor(s) Dr Richard Balmer, Dr Robert Davies , Dr Asmaa Taie

How will the results of the work be disseminated?

☒ Peer reviewed scientific journals ☒ Conference presentation

☒ Internal report ☐ No plans to disseminate results

☒ Other D Paed Dent Thesis

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):

☒ Completed Tissue Bank application form

☒ 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): Sarah Aljuwaihel

Signature: 

Date: 22/09/2021

Supervisor approval:

Supervisor (print name): Robert Davies

Signature: 

Date: 22.09.21

Appendix B: Tables **summarise the materials and tools/ instrument used in this study**.

Material	Type /Shade	Cure mode	Composition	Manufacturer	Lot number
Filtek Supreme Universal Capsules	-Universal nano-filled restorative material -Shade A2	Light cure	The resin system contains bis-GMA, UDMA, TEGDMA, bis-EMA, and PEGDMA. The inorganic filler loading is about 78.5% by weight (63.3% by volume). The fillers are a combination of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler of same size.	3M oral care ESPE	2109-01
Optibond Solo Plus	-Single Component Total-Etch Dental Adhesive	light cure	Bis-GMA, HEMA, GPDM, initiator, ethanol, fumed silica, barium aluminoborosilicat	Kerr corporation	8564474

			e and sodium hexafluorosilicate.		
37% PHOSPHORIC ACID gel	-Acid etch -Blue colour -Easily visible		Thixotropic gel of 37% PHOSPHORIC ACID	Denvtech	03228 84
ClaroCit kit	-Universal acrylic mounting resin -Shade clear	Curing mode cold	- 500 ml Liquid: ≥75 - ≤90 methyl methacrylate, ≥10 - <20 acetone, ≥10 - ≤25 tetramethylene dimethacrylate 800 g Powder: dibenzoyl peroxide -Required consumables	Struers	40200 072
Curodont™ Repair	SAPs P_{11-4} in Freeze dried form	-	Self-assembling Peptide P_{11-4}, made up of Amino acids.	Credent is AG	10061 98

Tools /Instruments	**Type**	**Manufacturer**
DEHP Plastic Filling Instrument 153	Composite Filling Instrument	DE healthcare
Micro brush Applicator Super Fine White 400pk	Micro Applicator	
Blood lancets, VITREX® Steel	Cutting instrument composition: hardened surgical steel	Vitrex Medical A/S

	sterilization gamma radiation to DIN EN 552/556	
Octagonal Handle - Autoclavable 9 Probe SE- Dental periodontal probe	Hand measuring instrument	UnoDent
Afantti Mini Rotary Tool	Micro Sander instrument	Afantti
WHPromLang Squares Moulds	Moulding instrument	WHPromLang

Appendix C: Image analysis steps and quantification of the MIH lesion Colour.

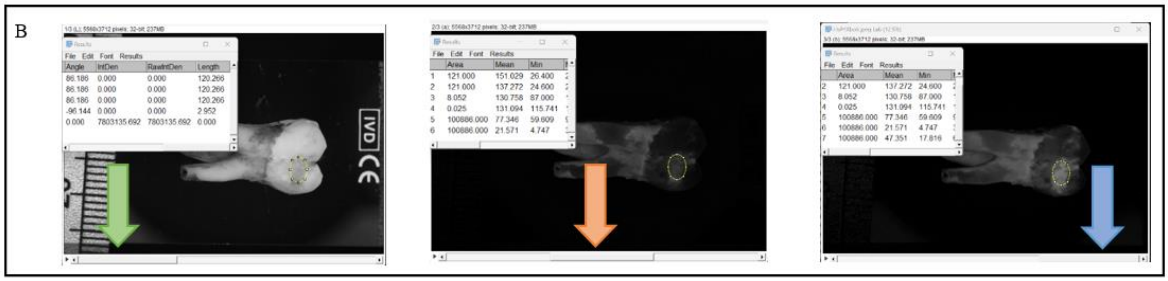


Figure A: The set the scale of the Image (Pixels to mm), measure the width and length of the hypomineralised area in the tooth.

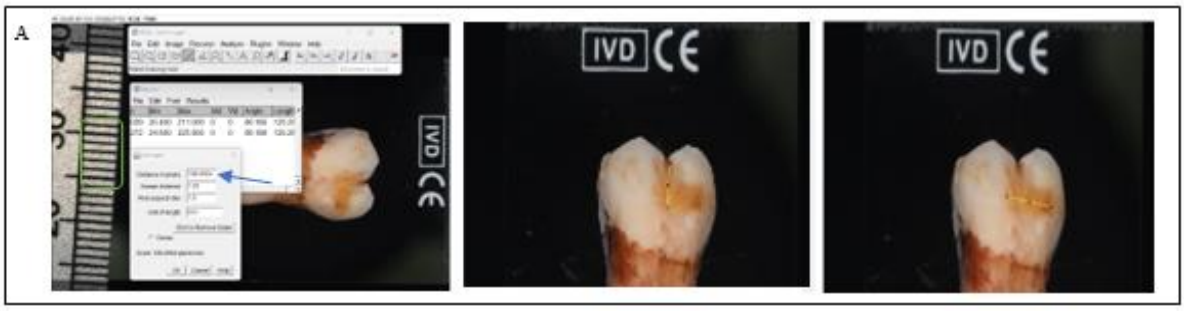


Figure B The green arrow represents the L value, the orange arrow shows the A value, the blue arrow is the B value.

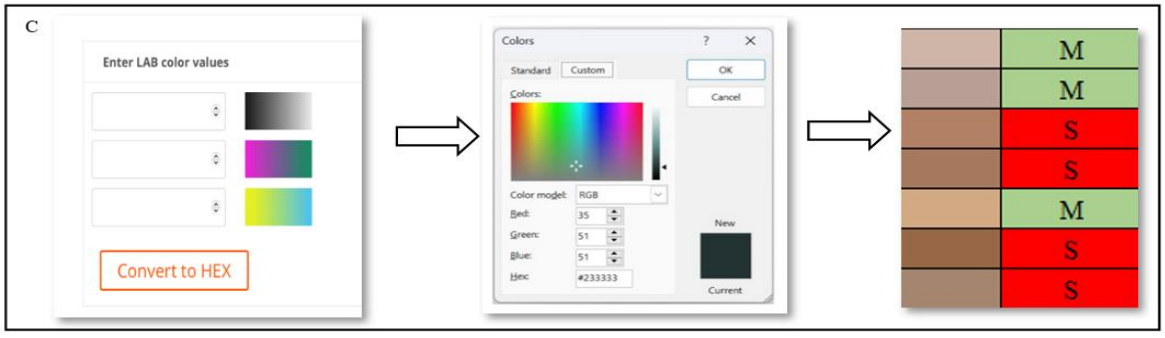
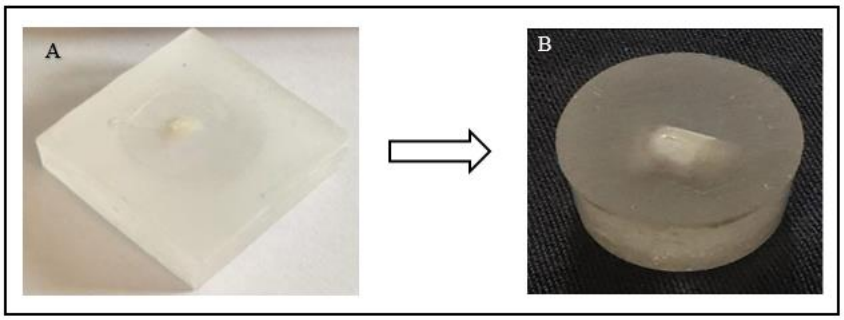


Figure C The LAB values were entered in the automatic conversion tool to get Hex value then Hex value entered in colour selection window in the Microsoft Excel sheet to create the color shade for every lesion. M: Mild/moderate severity level, S: severe level.

Appendix D: Reshaping the resin block before Micro CT and SEM analysis.



Figures B represent the preparation of the resin Block size before Micro-CT and SEM scanning.

Appendix E: Data collection sheet of the three groups of the DIAGNOdent Readings

Positive Control Group (SE)	Diagodent reading	Negative Control Group (MIH)	Diagodent reading	Tested Group Group (MIH +T)	Diagodent reading
1	3	**1**	87.4	**1**	87.4
2	4	**2**	87.8	**2**	90.2
3	6	**3**	98.6	**3**	99
4	1.6	**4**	98.8	**4**	87.8
5	4	**5**	87.2	**5**	99
6	7	**6**	99	**6**	85.8
7	3.2	**7**	98.8	**7**	99
8	3.8	**8**	87.6	**8**	87.6
9	5.8	**9**	87.8	**9**	98
10	3.5	**10**	87	**10**	89.4
11	1.6	**11**	78.8	**11**	99
12	3.6	**12**	86.4	**12**	98.4
13	2	**13**	87.6	**13**	88.2
14	4	**14**	98.4	**14**	97
15	6.2	**15**	98.2	**15**	99
16	4.4	**16**	83.2	**16**	99
17	8.2	**17**	87.8	**17**	99
18	2	**18**	87.8	**18**	99
19	2	**19**	99	**19**	98.8
20	2.4	**20**	98.2	**20**	99
21	3.6	**21**	99	**21**	98.8
22	3.4	**22**	88	**22**	88
23	2.4	**23**	70	**23**	87.8
24	4.4	**24**	88.2	**24**	88.6
25	4	**25**	99	**25**	97.8
Mean	3.84		90.74		94.42

Appendix F: Data collection sheet of the colour shade of the groups (MIH) and (MIH+T)

MIH teeth			
Negative Control		Tested Group	
Group (MIH)	Color shade	Group (MIH+T)	Color shade
1	M	1	M
2	M	2	M
3	S	3	S
4	S	4	M
5	M	5	S
6	S	6	M
7	S	7	S
8	M	8	M
9	M	9	S
10	M	10	M
11	M	11	S
12	M	12	M
13	M	13	M
14	S	14	M
15	M	15	M
16	M	16	S
17	M	17	S
18	S	18	S
19	S	19	M
20	S	20	M
21	M	21	S
22	M	22	M
23	S	23	M
24	M	24	M
25	M	25	M
M :Mild/Moderate Severity Level and S : Severe Level			

Appendix G: Data collecting sheet of the SBS values of the three groups of the study.

Positive Control S. E		Negative Control H. E		Tested Group H.E with SAPs	
Group (SE)	**SBS (MPa)**	**Group (MIH)**	**SBS (MPa)**	**Group (MIH+T)**	**SBS (MPa)**
1	11.1	**1**	1.6	**1**	5.3
2	9.6	**2**	2.2	**2**	5.3
3	10.6	**3**	1.1	**3**	4.3
4	7.4	**4**		**4**	2.8
5	19.5	**5**		**5**	3.2
6	9.7	**6**	4.1	**6**	3.6
7	68.2	**7**	1.7	**7**	4.2
8	9.5	**8**	2.2	**8**	4.6
9	12.4	**9**	1.5	**9**	6.8
10	7.2	**10**		**10**	3.2
11	7.5	**11**	1.2	**11**	2.7
12	10.4	**12**	0.4	**12**	3.2
13	29.1	**13**	2.4	**13**	5.8
14	11.7	**14**	2.6	**14**	6.4
15	14.5	**15**	1.1	**15**	5.1
16	27.9	**16**	2.6	**16**	4.6
17	28.3	**17**	1.9	**17**	5.3
18	14.9	**18**	2.1	**18**	5.2
19		**19**	0.8	**19**	5.8
20	7.7	**20**	0.9	**20**	3.0
21	9.6	**21**	1.3	**21**	4.3
22	12.9	**22**	1.1	**22**	4.4
23	16.4	**23**	1.3	**23**	2.0
24	13.2	**24**	1.3	**24**	3.4
25	11.8	**25**	1.7	**25**	8.2
Mean	**15.8**		**1.7**		**4.5**
	excuded samples due to pretest faliure				
	samples were randomly selected for mineral density analysis using micro-CT				

Appendix H: Data collection sheet of the mineral density of randomly selected teeth.

	Positive Control		Negative Control		Tested Group with P_{11-4}	
	Mineral density g/cm3	SBS MPa	Mineral density g/cm3	SBS MPa	Mineral density g/cm3	SBS MPa
	2.3	68	1.9	1.6	1.9	4.6
	2.6	14.5	2.3	0.4	2.5	3.6
	2.6	28.3	1.6	2.2	1.7	3.2
	3.2	14.9	1.9	1.5	1.8	4.4
	2.2	7.7	2.5	1.7	2.3	2
Mean	2.58		2.04		2.04	

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Figure 1: Enamel synthesis explained.

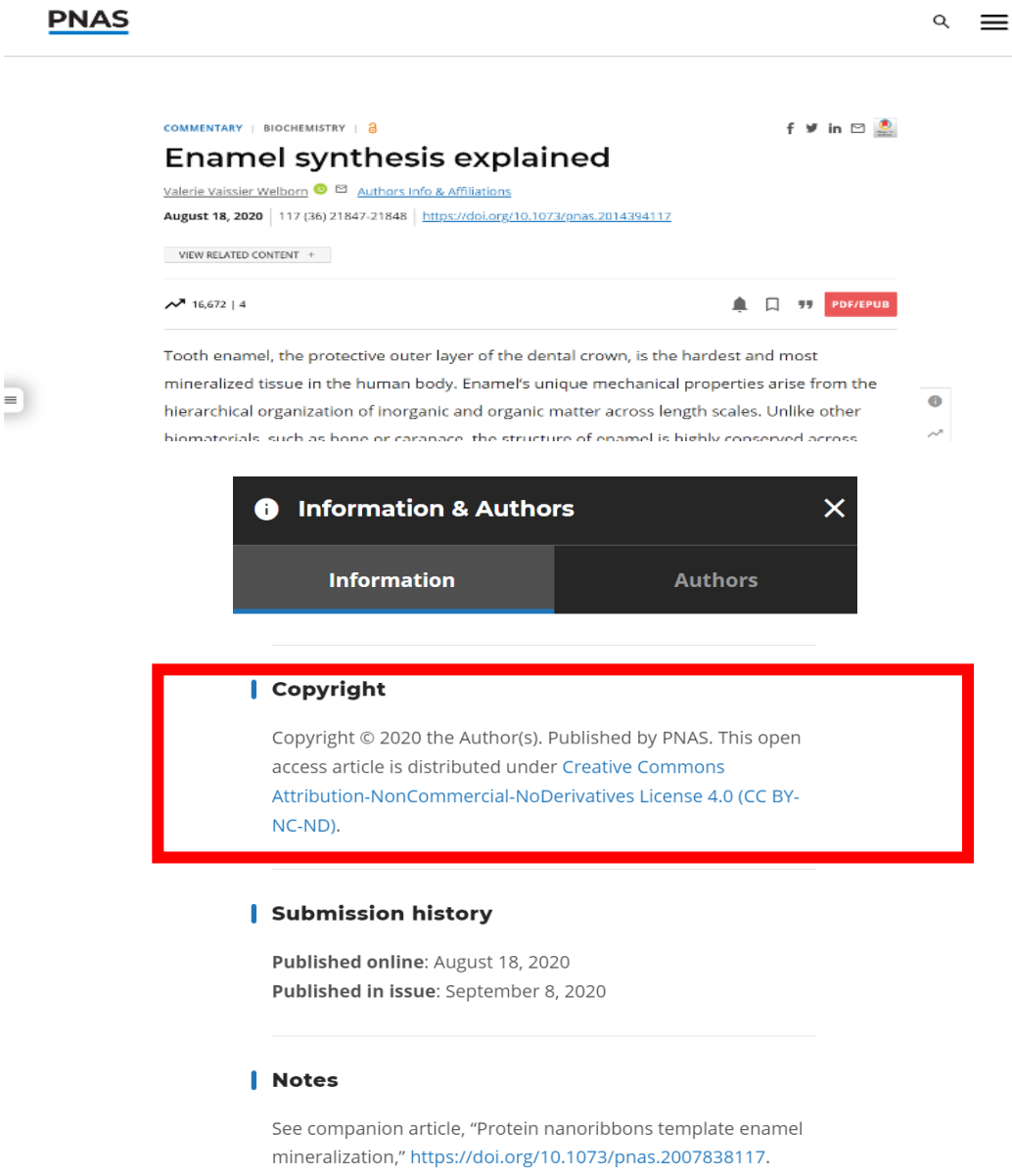


Figure 2 : The classification and instances of enamel defects.

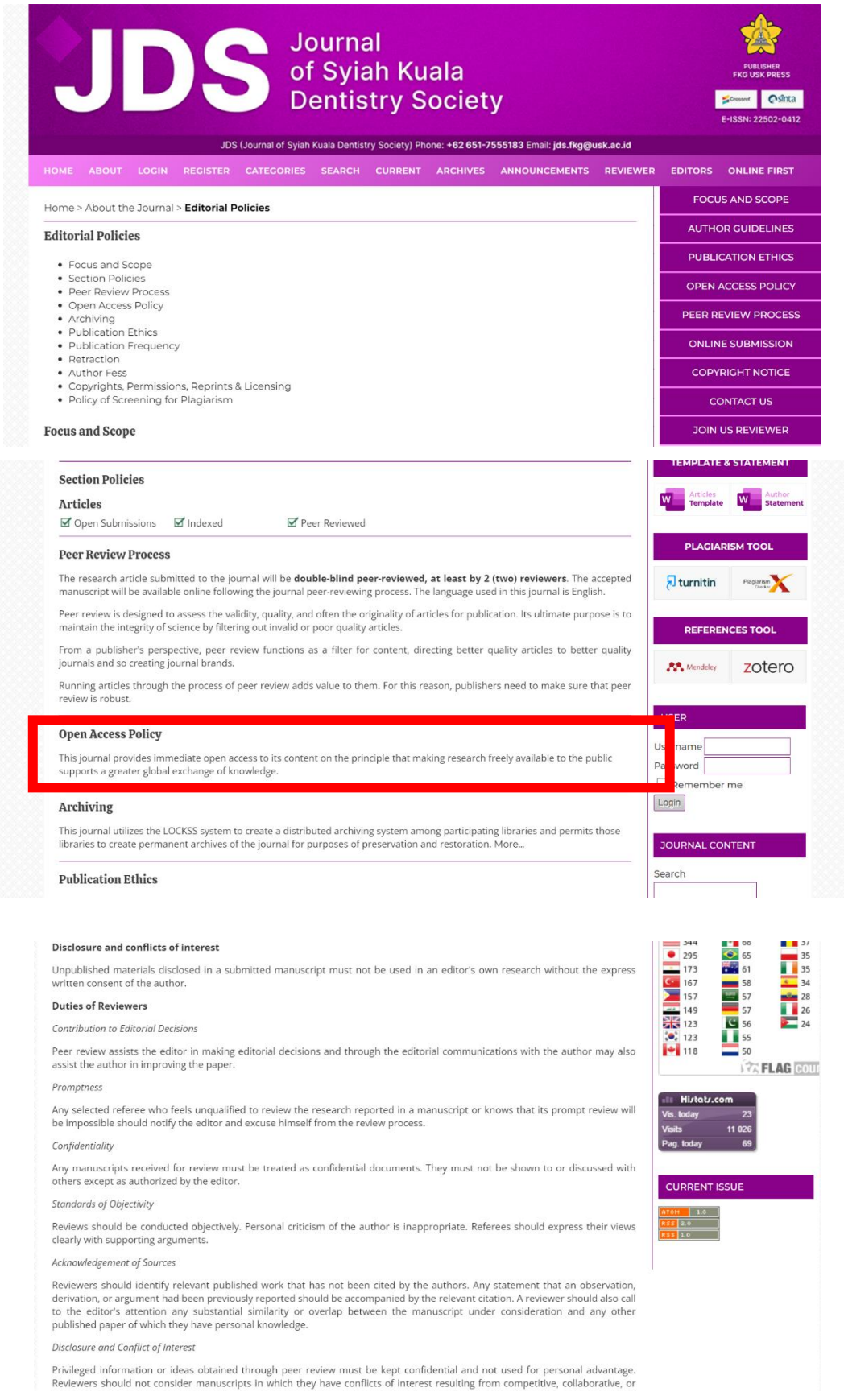
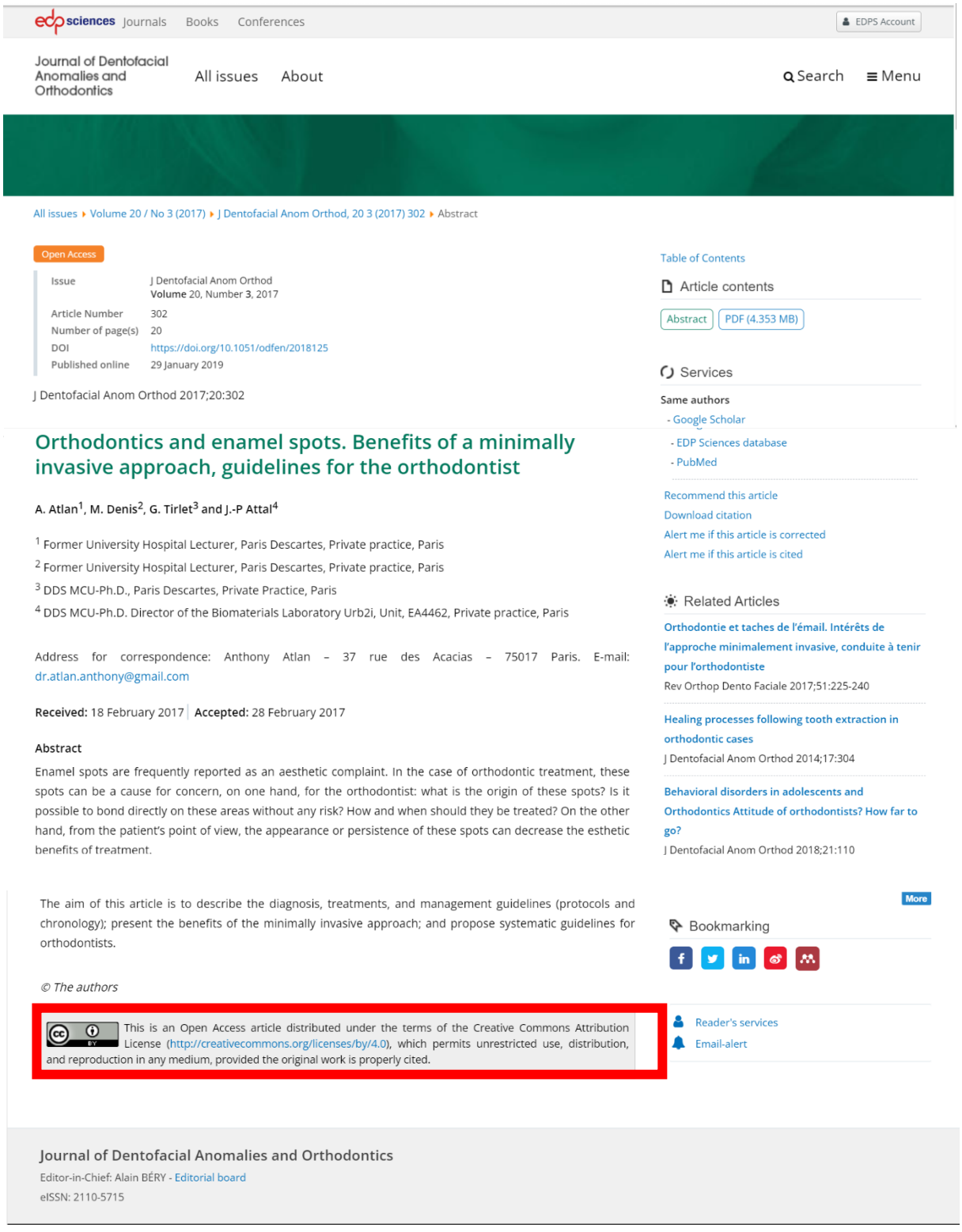


Figure 3: Clinical Presentation of different type of enamel defect.



Orthodontics and enamel spots. Benefits of a minimally invasive approach, guidelines for the orthodontist

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Abstract

Enamel spots are frequently reported as an aesthetic complaint. In the case of orthodontic treatment, these spots can be a cause for concern, on one hand, for the orthodontist: what is the origin of these spots? Is it possible to bond directly on these areas without any risk? How and when should they be treated? On the other hand, from the patient's point of view, the appearance or persistence of these spots can decrease the esthetic benefits of treatment.

The aim of this article is to describe the diagnosis, treatments, and management guidelines (protocols and chronology); present the benefits of the minimally invasive approach; and propose systematic guidelines for orthodontists.

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Figure 4: Amelogenesis imperfecta clinical presentation.

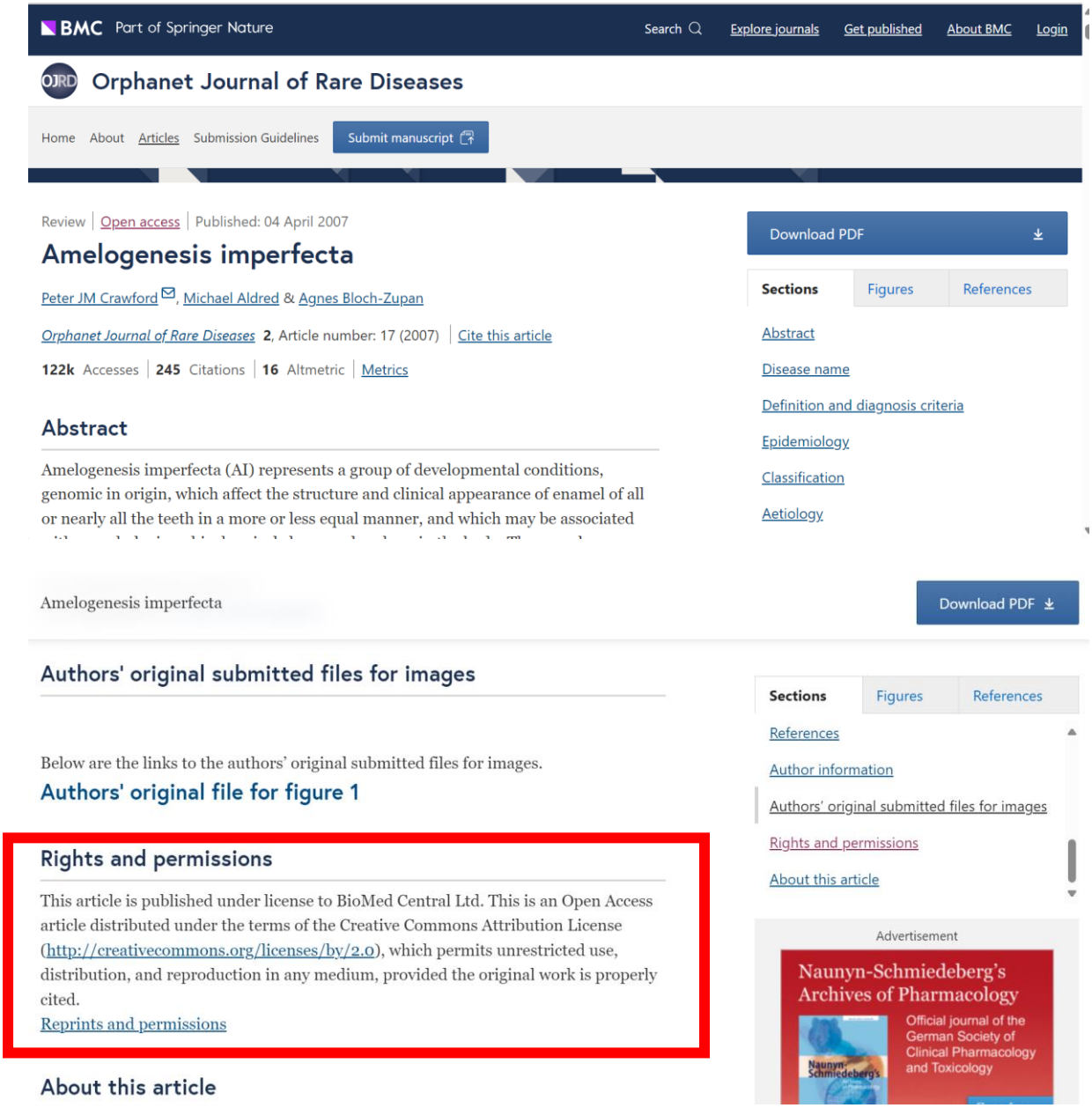

Figure 5: The Peptide Structure and folding.

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Expected presentation date	May 2024
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Figure 6: P_{11-4} demonstrating the colocalization of peptides in dimeric tape-like substructures and their dissociated monomeric forms.

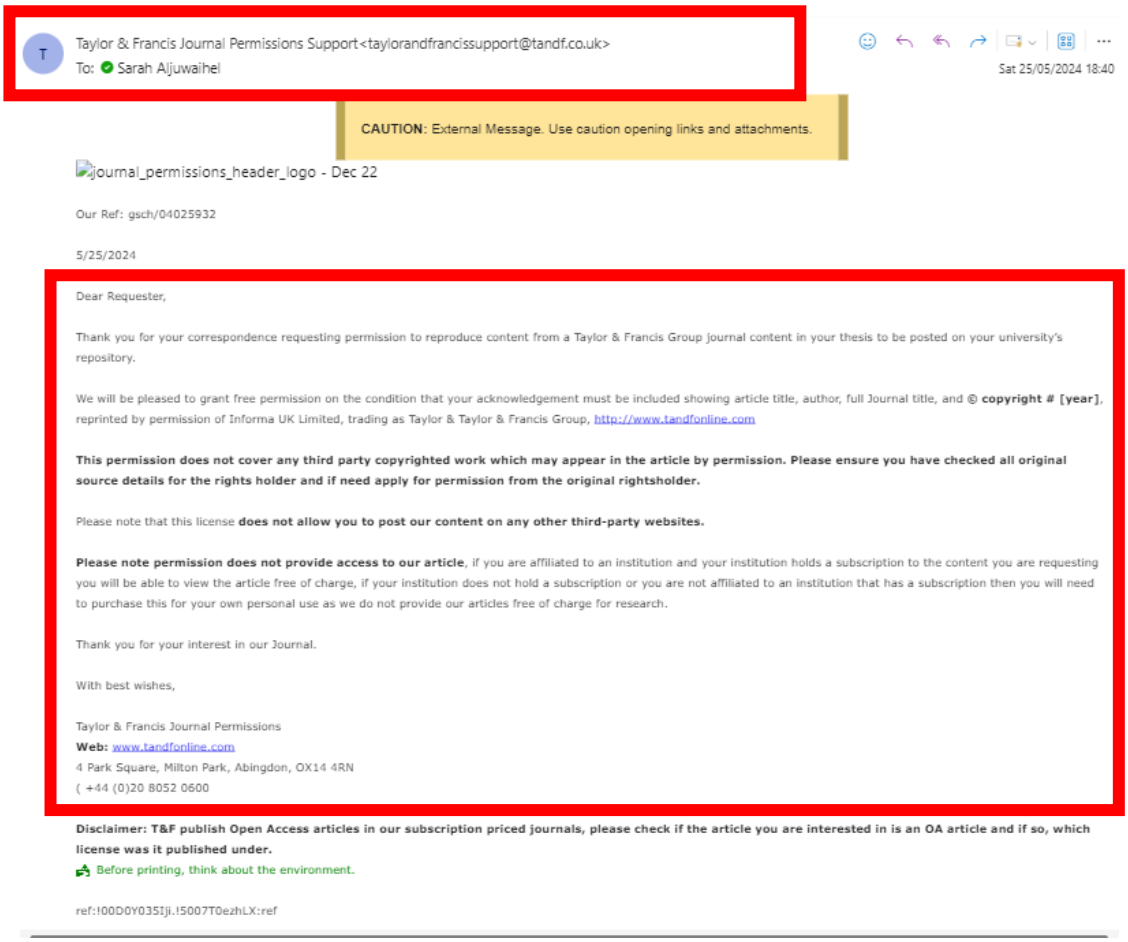

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Table 11: Mean and range of Laser Fluorescence of other study.

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Institution name	University of Leeds
Expected presentation date	May 2024
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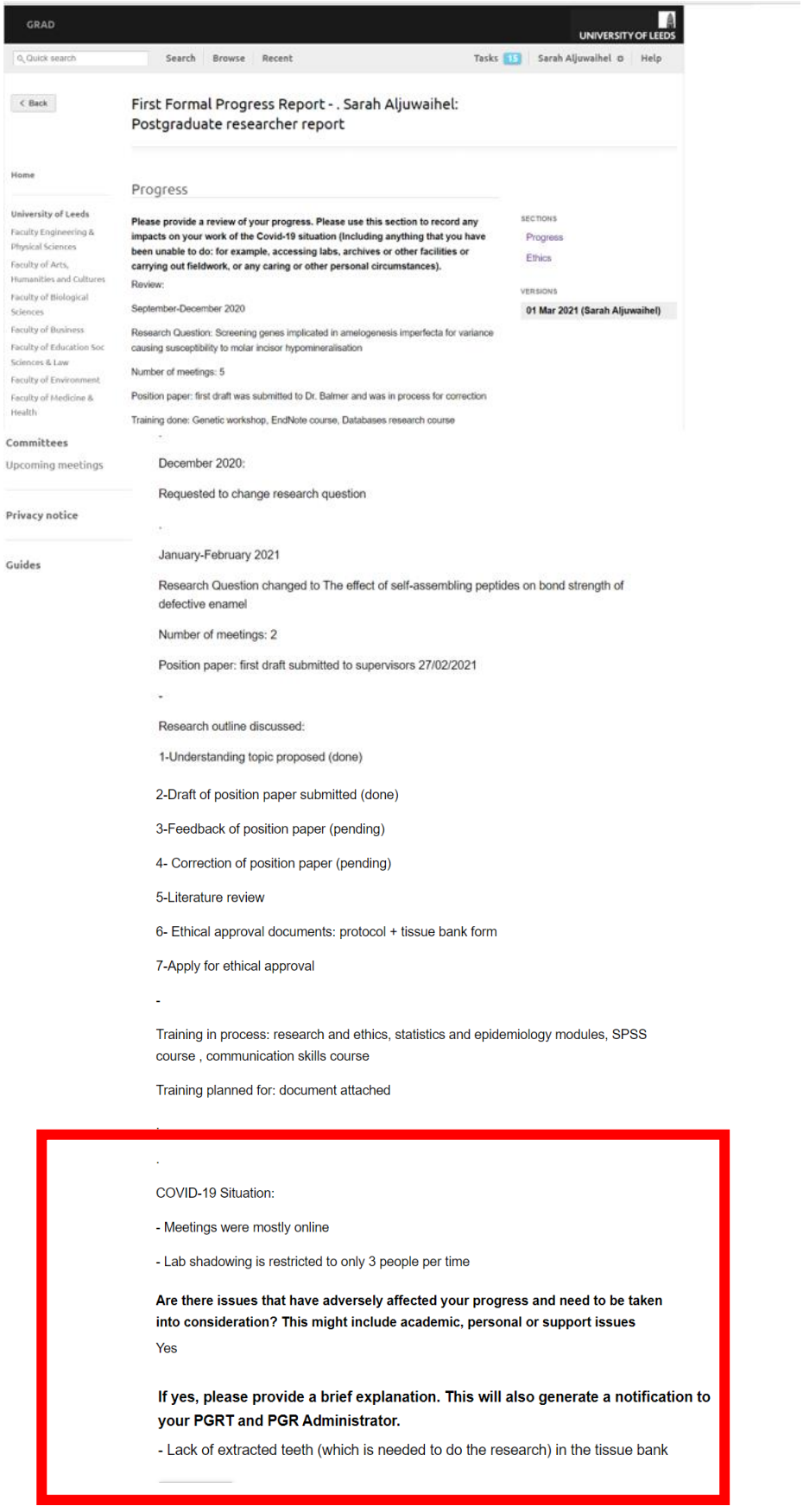

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Review:

September-December 2020

Research Question: Screening genes implicated in amelogenesis imperfecta for variance causing susceptibility to molar incisor hypomineralisation

Number of meetings: 5

Position paper: first draft was submitted to Dr. Balmer and was in process for correction

Training done: Genetic workshop, EndNote course, Databases research course

SECTIONS
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01 Mar 2021 (Sarah Aljuwaihel)

-

December 2020:

Requested to change research question

.

January-February 2021

Research Question changed to The effect of self-assembling peptides on bond strength of defective enamel

Number of meetings: 2

Position paper: first draft submitted to supervisors 27/02/2021

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Research outline discussed:

1-Understanding topic proposed (done)

2-Draft of position paper submitted (done)

3-Feedback of position paper (pending)

4- Correction of position paper (pending)

5-Literature review

6- Ethical approval documents: protocol + tissue bank form

7-Apply for ethical approval

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Training in process: research and ethics, statistics and epidemiology modules, SPSS course , communication skills course

Training planned for: document attached

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COVID-19 Situation:

- Meetings were mostly online

- Lab shadowing is restricted to only 3 people per time

Are there issues that have adversely affected your progress and need to be taken into consideration? This might include academic, personal or support issues

Yes

If yes, please provide a brief explanation. This will also generate a notification to your PGRT and PGR Administrator.

- Lack of extracted teeth (which is needed to do the research) in the tissue bank

Annex I: Covid-19: Assessment of Learning Outcomes and Covid-19 Impact Statement

Covid-19 Guidance

The University has prepared the following guidance for PGRs, Supervisors and Examiners on the assessment of learning outcomes. The Quality Assurance Agency for Higher Education (QAA) has produced advice on Doctoral Standards for Research Students and Supervisors which it may be helpful to read in conjunction with this guidance.

It is recognised that in some cases the research plans, and the thesis submission, may have to change from what was originally intended. This might include changes to the methodology, experimental design, plans for data collection, or refining the scope or the emphasis of the original research project. However, the ***quality*** of the thesis is expected to be equivalent to that produced at other times. The Learning Outcomes and criteria for award are unchanged. Flexibility may be considered, if appropriate, on the ***quantity*** of material expected in the thesis, whilst ensuring that the quality of the submission is preserved, and that the examiners still have a sufficient body of research to assess that the criteria for award and learning outcomes have been met. This may not always be easily quantifiable, but Supervisors, in consultation with Directors of PGR Studies and Heads of Graduate Schools, are encouraged to consider how best to interpret this for their disciplines. The Regulations to Ordinance X, and the Programme of Study entries, set down the normal maximum length for each thesis submission, but the University does not specify a minimum length for any of its research degrees. The maximum limits are not intended to be interpreted as a requirement for the length of a thesis, and in certain disciplines this may normally be considerably shorter.

PGRs are invited to submit a Covid-19 impact statement alongside their thesis[25], which describes any impact of Covid-19 on their research plans and thesis submission and what changes to the research project design/plans had to be made SARAH Aljuwaihel

a. How the Covid-19 pandemic impacted the research project.
b. What steps were taken to mitigate against the disruption.
c. Any decisions taken to change direction or focus, or re-design the research plans in response to Covid-19.

The impact statement will be shared with the examiners with the thesis submission. Examiners will be asked to consider this statement, and to be flexible in considering how a PGR may demonstrate they have met the learning outcomes and what constitutes such evidence. Examiners should be sympathetic to any Covid-19 related circumstances that may have impacted

[25] A discussion of this might also be included in the thesis, if appropriate and the Supervisor(s) will be best placed to advice on the most appropriate location and form for this

the research or necessitated a change of direction or emphasis from that which may originally have been planned, whilst still ensuring that learning outcomes for each award have been met, based on the quality of the thesis submission and the PGR's defence in the oral examination. Examiners are invited to include comments in relation to a Covid-19 impact statement provided by the PGR in the relevant section of the joint report form. A PGR may have used the impact statement to document how their ability to work was affected during the pandemic due to academic or personal circumstances[26]. In some cases, adjustments to individual research projects may have been needed to enable PGRs to complete to their original timetable. Any changes needed to the scope of emphasis of the project will be described in the statement and should be considered in accordance with the guidance above. In other cases, these mitigating circumstances will have been dealt with by additional support and time (via a suspension or extension of studies) prior to submission of the thesis, to allow more time to complete the original project plans.

The University has an established position on mitigating circumstances which is set out in this document. Any mitigating circumstances which may have affected the PGR during their period of study should never lead the examiners to award a research where the work is not felt to be of the appropriate standard. To be eligible for the award of the degree, all PGRs must complete an oral examination and the thesis submitted for examination must meet the stated criteria for award of the degree and the specified learning outcomes must be met. While the thesis may be shorter or contain fewer case studies than might otherwise be expected as a result of the difficulties encountered, it should still meet the criteria for award.

The focus should be on the ***quality*** of the thesis submission, together with the PGR's defence in the oral examination. In the case of doctoral awards, for example, the examiners will be expected to reach an academic judgement on whether there is a sufficient quality – and quantity – of original research, with the appropriate rigour of analysis and independent critical ability and matter suitable for publication to have met the learning outcomes for award. In considering 'matter suitable for publication' at doctoral level, the expectation is that the thesis will contain original work which is of publishable quality in appropriate, peer-reviewed journals (or publication in other form as appropriate for the field of research). When commenting on the extent to which the thesis contains matter suitable for publication, Examiners are invited to comment on work which has already been published and/or may comment on parts of the thesis which could form the basis of an appropriate publication following some reworking.

Recommendations for award ultimately remain an academic judgement for the appointed examiners. All recommendations for award are considered at the next meeting of the Graduate Board's Progression and Examinations Group. The Graduate Board has delegated authority to the Progression and Examinations Group to ratify examination results and award research degrees, subject to it being satisfied that the criteria for the award of the degree have been met.

[26] PGRs are reminded that the statement will be shared with their examiners are strongly encouraged not to include personal or sensitive information in their statement. The statement should instead focus on the impact of the pandemic on their research project and any changes required in response to this

Covid-19 Impact Statement Template **available on website**

Doctoral College & Operations
(Progression & Examination)
Enquiries: rp_examinations@adm.leeds.ac.uk

UNIVERSITY OF LEEDS

Covid-19 Impact Statement

The University recognises that there will be some situations where progress will have been affected by the Covid-19 pandemic. In some cases there may have been an impact on the research project to such an extent that adjustments needed to be made to a PGR's individual research plans. This might include changes to the methodology, experimental design, plans for data collection, or refining the scope or the emphasis of the original research project.

PGRs are invited to upload to GRAD alongside their thesis an impact statement which describes any impact of Covid-19 on their research plans and thesis submission. This document will be shared with the Examiners. PGRs are reminded that the statement will be shared with the internal and external examiner(s) and are strongly encouraged not to include personal or sensitive information in their statement. The statement should instead focus on the impact of the pandemic on their research project and any changes required in response to this.

Name of PGR	SARAH Aljuwaihel

Please use the sections below to describe any impact of Covid-19 on your research project. Your statement should focus on how your research project was impacted and any changes you had to make as a consequence E.g. changes to the methodology, experimental design, plans for data collection, or refining the scope or the emphasis of the original research project.

How the Covid-19 pandemic impacted the original research project plans.

1-Lab closures and restricted access and reduced number of people allowed in the lab at any given time with social distancing measures lead to slowing the pace of research and delayed in equipment training and delayed experiments. 2- Illness and slowing and remote work, Delays in Research Progress, increase stress and burn out 3-lack of extracted teeth in the tissue bank.

What steps were taken to mitigate against the disruption.

Online meetings, small sample size.

Any decisions taken to change direction or focus, or re-design the research plans in response

Lab schedules, safety protocols, flexible deadlines, online training, virtual lab simulation, public awareness

PGR Signature/Authorisation:	Sarah Aljuwaihel 
Supervisor Signature/Authorisation:	R Balmer 

Please save this document as "Impact Statement" and upload this to GRAD alongside your thesis submission for examination. **This document** **aminers.** Statements can be accepted after thesis submission (by email to rp_examinations@adm.leeds.ac.uk) but examiners may not be able to take the statement into consideration if they receive it too close to the date of the viva.