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The evaluation of the Interfaces between Regenerative/Restorative Biomaterials used In Conservative Pulp Therapy

Tese no âmbito do Doutoramento em Ciências da Saúde, ramo de Medicina Dentária, orientada pela Professora Doutora Ana Luísa Moreira Costa e pelo Professor Doutor João Carlos Tomás Ramos e apresentada à Faculdade de Medicina da Universidade de Coimbra.

Dezembro de 2020

Faculty of Medicine of the University of Coimbra

THE EVALUATION OF THE INTERFACES BETWEEN REGENERATIVE/RESTORATIVE BIOMATERIALS USED IN CONSERVATIVE PULP THERAPY

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Doctoral Thesis in the Doctoral Program in Health Sciences, branch of Dentistry, under the supervision of Professor Ana Luísa Moreira Costa and Professor João Carlos Tomás Ramos, presented to the Faculty of Medicine of the University of Coimbra.

December 2020

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- Institute of Pediatric and Preventive Dentistry
- Laboratory of Mechanical Testing and Sample Preparation (LEMPA)
- Laboratory of Biostatistics and Medical Informatics
- Center for Research and Innovation in Oral Sciences (CRIOS) research line Oral biomechanics – Dental Medicine Area
- Laboratory of High-Resolution Cell Bio-Imaging

II. University of Aveiro

- Department of Materials and Ceramic Engineering (DEMaC)

SÍSIFO

Recomeça....

Se puderes
Sem angústia
E sem pressa.
E os passos que deres,
Nesse caminho duro
Do futuro
Dá-os em liberdade.
Enquanto não alcances
Não descanses.
De nenhum fruto queiras só metade.

E, nunca saciado,
Vai colhendo ilusões sucessivas no pomar.
Sempre a sonhar e vendo
O logro da aventura.
És homem, não te esqueças!
Só é tua a loucura
Onde, com lucidez, te reconheças...

Miguel Torga Diário XII
Coimbra, 27 de Dezembro de 1977

Ao João
e ao meu filho João

Aos meus Pais,
irmão João
e sobrinha Isabel

Aos meus Amigos

Acknowledgements
Agradecimentos

Ao Professor Doutor João Luís Maló de Abreu, pela obra que construiu e da qual, humildemente, tenho o privilégio de fazer parte. Será sempre um exemplo de dedicação à Medicina Dentária.

Ao longo de todo este tempo tive (tenho!) o imenso privilégio de ter a orientar este meu caminho duas pessoas que muito prezo, a Professora Doutora Ana Luísa Costa e o Professor Doutor João Carlos Ramos, que, de forma tão genuína e abnegada, partilharam comigo o seu conhecimento e o seu saber e me guiaram ao longo destes anos de trabalho, tornando possível o objectivo a que me (nos) propus e fazendo-me chegar, no final do ano de 2020, ao longe que tanto quis alcançar.

À Professora Doutora Ana Luísa Costa, para além de amiga, é um modelo de rigor científico e profissionalismo, incentivando-me sempre a superar as dificuldades e a manter-me com os pés no caminho, assentes em terra firme, apesar das "tempestades" que de quando em vez me empurravam para o devaneio da mente.

Ao Professor Doutor João Carlos Ramos, um exemplo de competência clínica e científica que tanto admiro, com um amor pela profissão que transmite a todos com quem se cruza, e que me ofereceu generosamente o que tanto faz falta hoje em dia: a partilha. Partilha de sabedoria, espírito crítico e avaliação clínica nas várias etapas de todo este processo.

Ao Professor Doutor Francisco Caramelo, pelo imprescindível auxílio na análise estatística deste trabalho e grande disponibilidade no esclarecimento de dúvidas, quer metodológicas, quer estatísticas.

À Professora Doutora Isabel Poiares Baptista, pela catarse semanal, essencial para manter o ânimo na prossecução deste projeto.

Aos meus colegas das Unidades Pré-Clínica 3, Clínica 3 e Clínica Integrada de Odontopediatria/Ortodôncia do Mestrado Integrado em Medicina Dentária da Faculdade de Medicina da Universidade de Coimbra, em particular às Dras. Ana Daniela Soares, Bárbara Cunha, Joana Leonor Pereira, Margarida Esteves e Sara Rosa, pelo apoio incondicional com que participaram neste trabalho.

Aos restantes colegas e funcionários da Área de Medicina Dentária da FMUC, pela amabilidade e consideração com que sempre me tratam.

À técnica Cláudia Brites, pela sempre pronta disponibilidade com que me auxiliou no estudo laboratorial.

Aos alunos do Mestrado Integrado em Medicina Dentária da FMUC, a partilha de conhecimento com todos vós é um constante incentivo para o meu desenvolvimento pedagógico-científico.

Ao Professor Doutor João Caramês, a alavanca constante para o brio e empenho na prática clínica, rejeitando o conformismo e a acomodação, trabalhando sempre no sentido de inovar e aperfeiçoar o conhecimento.

Ao Professor Doutor Duarte Marques, pelo caloroso apoio e por tornar e mostrar simples a dificuldade da minha prolongada ausência.

Ao Dr. Jorge Martins, à Dra. Luísa Poppe e à Professora Doutora Helena Francisco, pelo alento nos momentos de desânimo.

A toda a equipa do Instituto de Implantologia e da clínica IPB, que muito me orgulho de integrar.

Por fim, ao meu Pai, pela transmissão do espírito inquieto na procura do saber, superando as fraquezas e dificuldades com trabalho e perseverança.

Resumo

Introdução e objetivos

A utilização dos cimentos de silicato de cálcio tem vindo a ganhar maior relevância na prática clínica, possibilitando uma abordagem mais conservadora baseada na preservação e regeneração do tecido pulpar, inclusivamente em idade pediátrica.

Por forma a ultrapassar algumas desvantagens do agregado de trióxido mineral convencional têm surgido novos cimentos, de que são exemplo o NuSmile® NeoMTA (NuSmile Ltd. Houston, TX, USA) e o Biodentine™ (Septodont, Saint-Maur-des-Fosses Cedex, France), que associam à biocompatibilidade e bioatividade dos cimentos de silicato de cálcio, maior estabilidade da cor, melhores propriedades mecânicas e menor tempo da reação de presa; carecem ainda, porém, de estudos *in vitro* e *in vivo* que avaliem a implicação da sua interação com os materiais adesivos restauradores, fator esse determinante para o sucesso do tratamento regenerador/restaurador.

Objetivou-se estudar as interfaces adesivas entre estes dois cimentos e a subsequente restauração adesiva com resina composta, nomeadamente no que concerne à força de adesão e aos seus padrões ultramorfológicos. Para além do tipo de cimento de silicato de cálcio, foram avaliadas outras variáveis independentes como o tipo de sistema adesivo, o efeito da aplicação de uma camada adicional de resina hidrofóbica e o tempo de execução da restauração definitiva (imediata ou após 7 dias).

Materiais e Métodos

Procedeu-se à análise quantitativa da adesão entre os cimentos de silicato de cálcio e as restaurações adesivas com resina composta. Foram realizados testes por forças de cisalhamento em 320 amostras distribuídas por 16 grupos (n=20) em função da utilização dos dois cimentos de silicato de cálcio (NuSmile® NeoMTA e o Biodentine™), dos dois sistemas adesivos autocondicionantes (Clearfil™ SE Bond 2 e do Clearfil™ Universal Bond Quick - Kuraray Noritake Dental Inc.; Sakazu, Kurashiki, Okayama, Japan), da aplicação ou não de uma camada adicional de resina hidrofóbica e dos dois tempos de execução da restauração com resina composta, imediata ou após 7 dias de armazenamento. Todas as amostras foram armazenadas durante 48 horas numa incubadora a 37°C com 100% de humidade, previamente à realização dos testes de adesão numa máquina de testes universal.

As superfícies fraturadas foram examinadas sob microscopia ótica para classificação dos padrões de fratura. A análise estatística dos dados registados foi realizada na plataforma estatística IBM® SPSS® v26 tendo-se adoptado um nível de significância de 0.05. Complementarmente, procedeu-se à análise qualitativa das interfaces adesivas através da avaliação ultramorfológica por microscopia eletrónica de varrimento. Para o efeito foram realizadas 32 restaurações em molares decíduos naturais aleatorizados pelos 16 grupos de estudo. A penetração do sistema adesivo nos cimentos de silicato de cálcio foi avaliada por microscopia confocal de varrimento a laser em restaurações efetuadas em dentes artificiais, seguindo os mesmos protocolos dos 16 grupos de estudo, mas com a particularidade dos sistemas adesivos terem sido previamente marcados com Rhodamina B.

Resultados

Os resultados dos testes de adesão não revelaram diferenças estatisticamente significativas entre os dois cimentos de silicato avaliados (p=0.897). No referente aos sistemas adesivos, o Clearfil™ Universal Bond Quick apresentou uma melhor *performance* adesiva comparativamente com Clearfil™ SE Bond 2 (p<0.001), o mesmo podendo ser afirmado no respeitante à aplicação de uma camada de resina hidrofóbica (p=0.014). Por último, a restauração definitiva diferida apresentou melhores resultados nos testes de cisalhamento (p<0.001). A microscopia eletrónica de varrimento e a microscopia confocal de varrimento a laser evidenciaram a interpenetração entre os sistemas adesivos e cimentos de silicato de cálcio formando uma zona híbrida cuja profundidade e grau penetração dependeram do momento da restauração e da estratégia adesiva. A interpenetração entre os cimentos de silicato de cálcio e os sistemas adesivos foi maior nos grupos com restaurações definitivas efetuadas de imediato. A espessura da camada adesiva foi maior nos grupos com aplicação da camada adicional de resina hidrofóbica. No que concerne à difusão dos sistemas adesivos em profundidade para o interior dos cimentos de silicato, verificou-se uma penetração mais profunda no NuSmile® NeoMTA que no Biodentine™.

Conclusões

- Não se verificaram diferenças nos valores de adesão aos cimentos de silicato de cálcio testados, Biodentine™ e NuSmile® NeoMTA.
- O Clearfil™ Universal Bond Quick proporcionou valores de adesão mais elevados aos cimentos de silicato de cálcio que o Clearfil™ SE Bond 2.
- A aplicação de uma camada adicional de resina hidrofóbica sobre os sistemas adesivos aumentou os valores de adesão aos cimentos de silicato de cálcio.
- O valor de adesão aos silicatos de cálcio foi maior quando a restauração adesiva com resina composta foi efetuada após 7 dias, comparativamente ao grupo em que a restauração foi imediatamente efetuada após a colocação da base de silicato de cálcio.
- A avaliação ultramorfológica das respetivas interfaces adesivas pelas duas técnicas de microscopia revelaram a presença de uma camada híbrida, com uma interpenetração evidente entre os sistemas adesivos e os cimentos de silicato, mas cuja espessura, morfologia e nível de penetração variaram em função dos grupos.

Palavras-chave: tratamento pulpar vital; cimentos de silicato de cálcio; sistemas adesivos; adesão; microscopia eletrónica de varrimento, microscopia confocal de varrimento a laser; pulpotomia; proteção pulpar direta.

Abstract

Introduction

The use of calcium silicate-based cements has gained an increasing relevance in clinical practice, enabling a more conservative approach based on the preservation and regeneration of the pulp tissue, including in pediatric patients.

In order to overcome some of the limitations of mineral trioxide aggregate, new cements have been developed, such as NuSmile® NeoMTA (NuSmile Ltd. Houston, TX, USA) and Biodentine™ (Septodont, Saint-Maur-des-Fosses Cedex, France), combining the biocompatibility and bioactivity of calcium silicate cements with improved colour stability, mechanical properties and shorter setting times. However, there are still limited *in vitro* and *in vivo* studies investigating the implications of the interaction between cements and the restorative adhesive materials, a factor that is crucial for the success of the restorative treatment.

The aim of the present study was to investigate the bond interface between the two cements and resin-based composites used as restorative materials, in particular regarding the shear bond strength and the ultramorphological pattern of this interface. Besides the different types of calcium silicate cements, other independent variables such as the type of adhesive system, the effects of the application of an additional hydrophobic bonding layer and the time of restoration (immediate versus delayed after 7 days) were further investigated.

Material and Methods

We conducted a quantitative analysis of the bonding strength between the two calcium silicate cements and the composite resin restorations. Shear bond strength tests were performed in 320 specimens divided into 16 groups (n=20) according to the use of the two tested calcium silicate cements (NuSmile® NeoMTA and Biodentine™), the type of self-etch adhesive system (Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick - Kuraray Noritake Dental Inc.; Sakazu, Kurashiki, Okayama, Japan), the application of an additional hydrophobic bonding layer and the two restoration times (immediate versus delayed after 7 days). All samples were stored for 48 hours in an incubator at 37°C and 100% humidity before performing the bond tests in a universal test machine.

The fractured surfaces were examined under a stereomicroscope for the classification of failure modes. The statistical analysis was performed using IBM® SPSS® v26 software, with a significance level set at 0.05. Additionally, a qualitative analysis of the bond interface was performed by evaluating the ultramorphology of the interface integrity by scanning electron microscopy. For that effect, 32 restorations were done in natural deciduous molars, which were randomly allocated into 16 groups. Furthermore, the adhesive system penetration into the calcium silicate cements was evaluated by laser scanning confocal microscopy in restorations performed on artificial teeth; the same protocol was followed for the 16 study groups, but with the particularity of the adhesive systems having been previously marked with Rhodamine B.

Results

The shear bond test results highlighted no statistically significant differences between the Biodentine™ and NuSmile® NeoMTA ($p = 0.897$). The Clearfil™ Universal Bond Quick presented a better bond performance compared to Clearfil™ SE Bond 2 ($p<0.001$), as with an additional hydrophobic resin bonding layer application ($p = 0.014$). The delayed restoration also presented better shear bond test results ($p<0.001$).

Scanning electron microscopy and confocal laser scanning microscopy showed the interpenetration between adhesive systems and calcium silicate cements forming a hybrid layer, which depth and degree of penetration depended on the time of restoration and the adhesive strategy. The interpenetration between calcium silicate cements and adhesive systems was greater in groups with delayed restorations. The thickness of the adhesive layer was greater in the groups with the application of an additional layer of hydrophobic resin. With regard to the interdiffusion of adhesive systems in depth into the silicate cements, a deeper penetration was observed in NuSmile® NeoMTA than in Biodentine™.

Conclusions

- The shear bond strength to Biodentine™ and NuSmile® NeoMTA was similar; however, both adhesives tested penetrated deeper in the NuSmile® NeoMTA, compared to Biodentine™.
- Clearfil™ Universal Bond Quick provided higher shear bond strength to calcium silicate-based cements evaluated, compared to Clearfil™ SE Bond 2.
- The application of an additional hydrophobic resin layer over the adhesive improved the shear bond strength of composite adhesive restoration placed over calcium silicate-based cements.
- The delayed definitive composite restorations placed after seven days provided higher shear bond strength than immediate restorations.
- The scanning electron microscopy and confocal laser scanning microscopy morphological evaluation of adhesive/Hidraulic calcium silicate cements interfaces revealed some important aspects. Both techniques have identified the interdiffusion and interlocking between the adhesives and calcium silicate-based cements, but with differences between the groups. Both adhesives penetrated deeper into the NuSmile® NeoMTA, compared to Biodentine™. Also, the penetration depth of the adhesives into the calcium silicate-based cements was higher in the group of immediate adhesive restorations, compared to those performed on the seventh day.

Keywords: vital pulp treatment; calcium silicate cements; adhesive systems; adhesion; scanning electron microscopy; laser scanning confocal microscopy; pulpotomy; direct pulp capping

List of abbreviations

3D	Three-dimensional
AAPD	American Academy of Pediatric Dentistry
ANOVA	Analysis of variance
BD	Biodentine™ (Septodont, Saint-Maur-des-Fossés Cedex, France)
BHT	Butylated hydroxyl toluene
Biodentine SE 0 1	Biodentine™ / Clearfil™ SE Bond / No extra HBL / Immediate restoration
Biodentine SE 0 7	Biodentine™ / Clearfil™ SE Bond / No extra HBL / Delayed restoration (7 days)
Biodentine SE 1 1	Biodentine™/ Clearfil™ SE Bond / Extra HBL / Immediate restoration
Biodentine SE 1 7	Biodentine™ / Clearfil™ SE Bond / Extra HBL / Delayed restoration (7 days)
Biodentine U 0 1	Biodentine™ / Clearfil™ Universal Bond Quick / No extra HBL / Immediate restoration
Biodentine U 0 7	Biodentine™ / Clearfil™ Universal Bond Quick / No extra HBL / Delayed restoration (7 days)
Biodentine U 1 1	Biodentine™ / Clearfil™ Universal Bond Quick / Extra HBL / Immediate restoration
Biodentine U 1 7	Biodentine™ / Clearfil™ Universal Bond Quick / Extra HBL / Delayed restoration (7 days)
Bis-GMA	Bisphenol A diglycidylmethacrylate
Ca^{2+}	Calcium ions
CH	Calcium hydroxide
CLSM	Confocal laser scanning microscopy
cm^2	Square centimeter
CQ	Camphorquinone photoinitiator
CRIOS	Center for Research and Innovation in Oral Sciences research line Oral biomechanics – Dental Medicine Area
DEMaC	Laboratory of High-Resolution Cell Bio-Imaging II. University of Aveiro - Department of Materials and Ceramic Engineering
EBPADMA	Ethoxylated Bisphenol A dimethacrylate
FMUC	Faculty of Medicine of the University of Coimbra
GIC	Glass ionomer cement
H_0	Null hypothesis
HBL	Hydrophobic bonding layer
HCL	Hydrochloric acid
HCSC	Hydraulic calcium silicate cements
HEMA	2-Hydroxyethyl methacrylate
ISO	International Organization for Standardization
kPa	KiloPascal
LED	Light-emitting diode
LEMPA	Laboratory of Mechanical Testing and Sample Preparation
MDP	10-Methacryloyloxydecyl dihydrogen phosphate

min	Minutes
MPa	MegaPascal
MTA	Mineral Trioxide Aggregate
mW	MilliWatt
N	Newton
NeoMTA SE 0 1	NuSmile® NeoMTA / Clearfil™ SE Bond / No extra HBL / Immediate restoration
NeoMTA SE 0 7	NuSmile® NeoMTA / Clearfil™ SE Bond / No extra HBL / Delayed restoration (7 days)
NeoMTA SE 1 1	NuSmile® NeoMTA / Clearfil™ SE Bond / Extra HBL / Immediate restoration
NeoMTA SE 1 7	NuSmile® NeoMTA / Clearfil™ SE Bond / Extra HBL / Delayed restoration (7 days)
NeoMTA U 0 1	NuSmile® NeoMTA / Clearfil™ Universal Bond Quick / No extra HBL / Immediate restoration
NeoMTA U 0 7	NuSmile® NeoMTA / Clearfil™ Universal Bond Quick / No extra HBL / Delayed restoration (7 days)
NeoMTA U 1 1	NuSmile® NeoMTA / Clearfil™ Universal Bond Quick / Extra HBL / Immediate restoration
NeoMTA U 1 7	NuSmile® NeoMTA / Clearfil™ Universal Bond Quick / Extra HBL / Delayed restoration (7 days)
nm	Nanometer
p	*p*-value
PD	Pediatric Dentistry
rpm	Revolutions per minute
s	Second
SBS	Shear bond strength
SEM	Scanning electron microscopy
TEGDMA	Triethyleneglycol dimethacrylate
TEM	Transmission electron microscopy
TGF-β1	Transforming growth factor β1
µm	Micrometer
VHN	Vickers hardness number.

Table of contents

Chapter 1. Background

1. Characterization of permanent and deciduous teeth

1.1. General characteristics

The diphyodont mammals have two dentitions, the deciduous and permanent dentition. Deciduous dentition is constituted by 20 primary teeth whereas the definitive dentition is constituted by 32 teeth, distributed in both arches. The dentition development happens in a complex process and involves the deciduous establishment, followed by shedding synchronized with development and eruption of permanent dentition (Fried et al., 2000).

Anatomically, the tooth consists of two parts, the crown and the root(s) separated by the cervical margin. The root is anchored in the alveolar (jaw) bone, while the crown, more precisely the clinical crown, is exposed to the oral cavity, has chewing or mastication functions that have an essential role in phonation. Beyond these, the teeth give structure, tissue support and shape to the face. The mineralized tissue (cementum and alveolar bone), epithelial tissue (gum) and connective (periodontal ligament) are important supportive tissues and constitute a complex structure, denominated by the periodontum (Figure 1.1) (Carvalho et al., 2013).

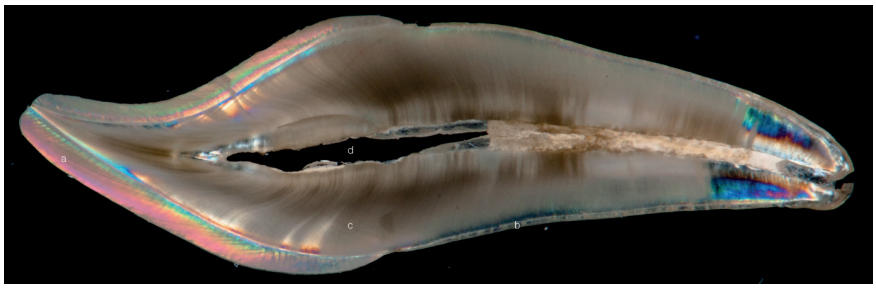


Figure 1.1. Tooth: a) Enamel; b) Cementum; c) Dentin; d) Pulp chamber.

Although teeth differ in size and shape in both dentitions, the histologic constitution is mainly similar. The external layer of the crown is the enamel and that of root is the cementum, while the line between both is the cemento-enamel junction (CEJ) (Nanci, 2013).

1.2. Enamel

The human tooth or more precisely, the crown, is composed by the pulp and dentin covered by enamel. The enamel derives from the ectoderm; is the most mineralized and hardest tissue in the human body and is composed by 96% of inorganic material and 4% of organic material and water (Cui & Ge 2007). Furthermore, this connective tissue has singular properties including wear resistance and stability over the lifetime of use within a physically demanding environment like the oral cavity (Habelitz et al., 2001). With age, the enamel becomes less permeable: behaves as a semi-permeable membrane in young enamel (allowing the slow passage of water and ions through the crystals), whereas in the old enamel the pore's size diminish and the surface thickness increase. On the other hand, one of its limitations is directly related with its formation process performed by the ameloblasts. These cells cover the entire

surface and, as the matrix produced becomes mineralized, they are blocked and died. Biologically, the result is a nonvital tissue that, when destroyed by caries, trauma or wear cannot be regenerated or replaced. However, it can be continuously and highly mineralized by ionic exchange with oral cavity environment, specially with the saliva (the surface layer is particularly mineralized by the fluoride topical application or incidentally exposure) (Nanci, 2013).

The mechanical and optical properties of a tooth are highly determined by its architecture, i.e., the mutual arrangement of its constituent units and their chemical composition (Zijp & ten Bosch, 1993; Zolotarev & Grisimov., 2001). The mineral phase consists primarily of calcium phosphate in a complex organization of hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$ (Zolotarev & Grisimov, 2001), which are carbonated, fluoridated, or incorporated with other ions like stroncium and magnesium during the enamel maturation (Nanci, 2013).

The fundamental microstructure units are the rods (prisms) and interrod enamel (interprismatic area). Primarily, enamel rods were described as enamel prisms due to its hexagonal or prism-like cross section form, however they don't have a regular geometry neither are prismatic: each rod is like a cylinder made by carbonated hydroxyapatite crystals tightly packed covered by a nanometre-thin layer of enamelin (Habelitz et al., 2001). Most of the times it has the same direction and lie parallel of the longitudinal axis of the rod, but deviate more and more as the distance increases from the center. In the final location the crystals tend to orientate perpendicular to the incremental lines (Risnes, 1998). These crystal units have a hexagonal symmetry in mature enamel, although in fully mature enamel have an irregular outline because they press to each other in the final part of their growth. The crystals that compose the interrod enamel and surround the rod enamel have different directions (Nanci, 2013). The literature suggests that in both phases the composition are identical; the only considerable difference is the orientation of the crystals (Nanci, 2013).

Finally, in the prismless layer the crystals are disposed parallel to each other and in a perpendicular direction, from the dentin-enamel junction (DEJ) towards the tooth surface (Fava et al., 1997; Nanci, 2013).

1.2.1. Deciduous enamel

The deciduous enamel differs from the permanent in several aspects such as chemical composition, mechanical properties and physiological aspects. Regarding to the composition, the calcium and phosphorus amount is lower in deciduous teeth when compared to permanent teeth. Derise *et al*. described that the mean concentration of calcium and phosphorus in the permanent teeth were 37.1% and 18.1% respectively (Derise et al., 1974; Oliveira et al., 2010) whereas in deciduous teeth they were 20% - 35% and 17.23% - 17.36% respectively (Fischer et al., 2013; Oliveira et al., 2010). However, aspects like the region where the enamel was collected, variations between individual teeth, the type, age, ethnicity and even the methodology should be considered in research's methodology (Oliveira et al., 2010).

Attending the fact that the mineralization degree is associated with crystals density, it might be considered that the global crystals density should be lower in primary enamel, compared with the permanent (Oliveira *et al*., 2010). Though, Wilson and Beynon reported that overall mineral density was lower in the peripheral layers and no significant differences were observed near to the dentinoenamel junction (Wilson & Beynon, 1989).

In summary, the two main differences are the relative to the low mineralizaton level and lesser thickness of the enamel in deciduous teeth - 80.6% in primary teeth enamel and 89.7% in permanent teeth

enamel - which may result in its whiter appearance and are linked with the reduced time available for enamel maturation (crown average growth is around 6-14 months, while the permanent teeth is 3 to 4 years (Oliveira et al., 2010; Mortimer, 1970; Wilson & Beynon, 1989).

The mineral content and crystals arrangement differences between both enamels might influence the mechanical properties observed in deciduous teeth (Kodaka et al., 1992; Low et al., 2008). Furthermore the chemical, morphological and physiological variations may seem also responsible for the different behavior of primary teeth under particular circumstances such as caries, erosion process and bond strength (Hunter et al., 2000; Marquezan et al., 2007; Wang et al., 2006).

1.3 Dentin-pulp complex

The core of the tooth is composed by the dentin and pulp, which fulfills the cavity inside the dentin and is a vascular and nerve tissue. While the root dentin is covered with cementum, 50% mineral and 50% organic matrix, predominantly type I collagen, the crown dentin is covered with enamel, the most highly mineralized tissue found in the body (Walker & Fricke, 2006).

Attending the intimate relationship of these tissues that are embryonically, histologically and functionally closely related, several authors prefer the designation of dentin-pulp complex (Orchardson & Cadden, 2001; Pashley, 1996). However, since the mature dentin and pulp are anatomically distinct and are easier to systematize separately, in this thesis these dental tissues will be described separately.

1.4. Dentin

The dentin derives from the mesoderm (Xu et al., 2009) and is the mineralized tissue of the bulk of the tooth providing both a protective cover for the pulp from microbial and other noxious stimuli and support to the overlying enamel. Furthermore, it allows relatively high forces without fracture (which can vary from 150–250N (Newton) on incisors to 660–1700 N on molars because it is more resilient, flexible and also compensates enamel brittleness (Gibbs et al., 1981; Marshall et al., 2012; Neill et al., 1989; Pruim et al., 1980; Tillitson et al., 1971). These two mineralized tissues, dentin and enamel, with different compositions and biomechanical properties, intermingle at the dentin–enamel junction (Walker & Fricke, 2006).

The mineral phase contains around 70% of the weight and 45% of volume, the organic matrix (primarily type I collagen) approximately 20% and 33%, respectively, while the remaining part is water (Arola et al., 2009; G. W. Marshall et al., 2012; Nanci, 2013; Xu et al., 2009). The water content varies from the dentin–enamel junction to the pulp because it's mainly located inside dentinal tubules and the diameter increases significantly from superficial to deep dentin (the wetness differs twentyfold from dentin–enamel junction toward the pulp) (Pashley, 1996; Zolotarev & Grisimov, 2001). According to Marshall *et al*., the composition of dentinal fluid is similar to plasma, but to date it has not been well-characterized (Marshall et al., 1997; Lee et al., 2014).

The main inorganic mineral of the dentin is hydroxyapatite crystals and the organic component is mainly collagen type I with a minor contribution from phosphoproteins, glycoproteins, and g-carboxyglutamate-containing proteins (Walker & Fricke, 2006).

A particular feature of dentin is the high density of tubules (the tubular width is largest near to the pulp and decreases close to DEJ or cementum) that cross its entire thickness, with exception of the superficial layers in mantle dentin, in the DEJ and adjacent to the cementum. Due to this tubular structure the dentin is highly permeable and a flow of dentinal fluid (outward) flows and microbial components (inward) movement may occurs (Tjäderhane et al., 2012). Because it contains the cytoplasmic extensions of the axons and the odontoblastic cell processes, dentin is a sensitive tissue and capable of producing more dentin (Walker & Fricke, 2006).

The dentine is formed and after that maintained, by terminally differentiated cells, the odontoblasts, which differentiate from the ectomesenchymal cells of the dental papilla, the formative organ of dentin that finally becomes the pulp. The differentiation process description is relevant to understand its contribution to several mechanisms, such as: the dentin–pulp complex innate immune defense and transmission, the regulation of pulpal pain and the odontoblasts recruitment for dentin repair (Nanci, 2013; Tjäderhane et al., 2012). The sequence of events starts with the expression of signaling molecules and growth factors emanated by the inner enamel epithelium. An acellular layer is formed and the undifferentiated papilla dental cells become progressively separated from the enamel. Consequently, as these cells acquire an elongated form and increased size (cytoplasm and protein-synthesizing organelles increase), they become preodontoblasts and odontoblasts successively; they occupy the accelular zone that gradually disappears. Simultaneously, the cells of the inner enamel epithelium reverse polarity, with the nucleus positioned away from this layer (Nanci, 2013).

On the other hand, the mineralized extracellular dentin is divided into intertubular and peritubular dentin. The intertubular dentin is formed by odontoblasts during dentinogenesis, through predentin mineralization and included most of the dentinal volume. Peritubular dentin is formed by mineralization inside the walls of dentin tubules within mineralized dentin and apparently is totally absent near the pulp in human teeth (Tjäderhane et al., 2012).

1.4.1. Types of dentin

Dentin can be classified in a wide variety of terms according to the site, function, origin, physiological aging and disease processes resulting in different composition, mineralization, and structure (Marshall et al., 1997). Although is not consensual in literature, the most common terminology is based on the formation sequence and dentin is categorized in various subtypes: dentin–enamel junction, mantle dentin, primary dentin, secondary dentin and tertiary dentin. On the other hand, tertiary dentin can be also divided into reactionary and reparative dentin (Tjäderhane et al., 2012).

Mantle dentin

The first dentin layer formed by secretory odontoblasts is denominated the mantle dentin and has 5 to 30 mm in thickness in human (Hayashi, 1992). In general, it differs from the rest of dentin types in the more irregular organic matrix, with Von Korff fibers frequently present, consist of bundled large-diameter collagen fibrils of type III, with a minor portion of type I (Pioch & Stachle, 1996), plus the different biochemical composition (Hayashi, 1992) where phosphoproteins are absent (Nakamura et al., 1985; Takagi et al., 1986). Regarding mineralization degree, several studies report that it is lower comparing with circumpulpal dentin (Tesch et al., 2001; Wang & Weiner, 1997), while others refer the mineral content does not vary

significantly (Tjäderhane et al., 1995). Though, it seems that the differences in the degree of mineralization are not limited to mantle dentin but may be more gradual (Tesch et al., 2001; Wang & Weiner, 1997).

Another difference from the circumpulpal dentin is the fact that the mantle dentine does not have dentinal tubules (Mjor & Nordahl, 1996) which doesn't result in a lack of permeability (Byers & Lin, 2003; Ikeda & Suda, 2006; Sognnaes et al., 1955). Plus, the collagen fibrils are thicker and perpendicular to the DEJ (Vinagre, 2014).

Dentin–enamel junction

Traditionally the DEJ has been assumed to be just a simple anatomical interface between enamel and dentin; however, recent studies have demonstrated that the DEJ may be much more than an inactive cutting-edge between these two different hard tissues layer: inner aprismatic enamel and mantle dentin (Goldberg et al., 2002). Actually, because of the high fracture toughness, beside the more resilient underlying dentin, it prevents enamel fracture during tooth function (Craig & Peyton, 1958).

The DEJ structure is commonly described as a scalloping and wavy interface (Habelitz et al., 2001; Marshall et al., 2003; Oliveira et al., 2001; Radlanski & Renz, 2006; Walker & Fricke, 2006; Whittaker, 1978), these scallops convexities are directed toward to the dentin and are deeper and larger at the dentin cusps and incisal edges, equalizing toward the cervical region (Marshall et al., 2003; Radlanski & Renz, 2007; Whittaker, 1978). Each scallop generally varies between 25–100-mm-diameter and contains micro-scallops with a nanolevel structure in it (Lin et al., 1993). This singular microstructure with collagen fibrils (with 80–120-nm-diameter crossing between two dissimilar mineralized tissues may be responsible for its increased physical integrity and mechanical interlocking performance (hardness and elastic modulus characteristics), both important to long-term tooth function (Lin et al., 1993; Leo Tjäderhane et al., 2012; Walker & Fricke, 2006). Furthermore, the progressive mineralization increase from the DEJ toward the pulp (Tesch et al., 2001; Wang & Weiner, 1997) and the mantle dentin may also contribute for the elastic properties of teeth (Tjäderhane et al., 2012).

The DEJ may represent an area of continuous biological activity because several enzymes and growth factors, such as fibroblast growth factor-2 (FGF-2) and other potentially bioactive components remain stored there and may be released, exerting their effects at different locations (Goldberg et al., 2002). Additionally, the cross-talk between enamel and dentin continues throughout the dentin and enamel formation and mineralization. Attending the phylogenetic, developmental, structural and biological characteristics, several authors suggest denominating this complex structure as dentin–enamel junctional complex, instead of dentin– enamel junction (Goldberg et al., 2002).

Circumpulpal dentin – primary and secondary dentin

The primary dentin is the main portion and is responsible for the size and form of the tooth. The primary dentinogenesis happens rapidly during tooth formation; after that, the dentin formation continues in a much slower rate and the secondary dentin is formed as a result of physiologic stimuli (Walker & Fricke, 2006). Academically, it is assumed that this turning point occurs after the tooth erupts and when it becomes functional and root formation is complete however this is a wide time span (Linde & Goldberg, 1993; Nanci, 2013; Tjäderhane et al., 2012). The main differences between these two types of dentin are the curvature of dentinal tubules and the tubular structure which is less regular in the second dentin (Nanci, 2013).

Lastly, the tertiary dentin is produced as a reaction to an external stimulus – attrition, abrasion, erosion, trauma, caries, in a protective, reactive manner by increasing the dentin thickness, in order to protect the pulpal tissue (Tjäderhane et al., 2012).

Depending on the intensity and duration of the stimulus there are differences in the form and regularity of dentin and thus two categories have described: the reactionary dentin (has a more or less tubular continuity with the secondary dentin and is produced by original primary odontoblasts) and the reparative dentin (produced by newly differentiated replacement odontoblasts; generally atubular and may form a relatively impermeable barrier between tubular dentin and pulp tissue) (Lesot et al., 1993; Mjor, 1985; Smith & Lesot, 2001; Yamamura, 1985).

1.4.2. Histology of dentin

The knowledge of three-dimensional dentin structures is crucial to understanding the caries process and to define adequate therapeutic decisions – restorative and vital pulp treatments - attending to its implications on the pulp proximity, bonding behavior, design and cavity dimension (Tjäderhane et al., 2012).

Microscopically, numerous histologic structures can be identified in the dentin, such as dentinal tubules, peritubular and intertubular, interglobular dentin, incremental growth lines and finally the Tomes granular layer (Nanci, 2013).

Dentinal tubules

On a microscopic scale, the tubular structure of dentin makes it unique from other hard tissue in the body. The tubules are not only used for transportation of mineral salt to deposit at the calcified front at the mineral wall but also play an important role in transferring stimuli and irritants to nerve terminal at the surface of the dental pulp (Sikanta et al., 2017).

The tubularity is one of the most important dentin's features that is responsible for its mechanical properties, withstand masticatory forces, stimulus permeability and nutrients diffusion (Nanci, 2013; Tjäderhane et al., 2012). The tubules contain odontoblast processes, afferent nerve terminals and even processes of some immunocompetent cells and dentinal fluid, which is derived from the pulpal extracellular fluid (Rungvechvuttivittaya et al., 1998; Vongsavan & Matthews, 1992). The odontoblasts processes run in canaliculi, become larger as they get into the pulp and cross in a fairly direct—or slightly s-shaped—course through the dentin layer, less pronounced under incisal edges or cusps almost radially outward from the pulp toward the DEJ and cementodentin junction (CDJ) (Arola et al., 2009; Nanci, 2013).

The 3D phase-contrast microtomography showed with more detail that in fact, in the dentine 0,3 mm beneath the enamel the dentinal tubules tilt or even twist or curl (occasionally up to 90 degrees) whereas further into the dentin (approximately 0,5 mm) no tilting or curling was observed (Zaslansky et al., 2010). These curvatures seem to result from the crowding of and path by the odontoblasts, as they penetrate into the pulp and the space becomes smaller (Nanci, 2013). Exceptions are in the root dentin where no crowding is observed and the odontoblasts run straightforward and in the predentin where the odontoblasts processes are involved by unmineralized collagen fibers (Nanci, 2013).

The number and diameter of tubules are related with dentin permeability and increase towards the pulp: the permeability is greater in inner dentin than outer and in coronal dentin than root dentin (Orchardson & Cadden, 2001).

The dentinal tubules have also numerous branches and ramifications creating a copious anastomosing canalicular system (the number is higher in areas where the dentinal tubules density is lower) (Kagayama et al., 1999; Mjor & Nordahl, 1996). Mjör and Nordahl identified three types of tubular branches: major, fine and microbranches. Major branches (0.5 to 1.0 mm diameter) are abundant peripherally while fine branches (300 to 700 nm diameter) are abundant in areas where the density of the tubules is relatively low. Microbranches (25 to 200 nm diameter) extend at right angles from the tubules in all parts of the dentin (Kagayama et al., 1999; Mjor & Nordahl, 1996).

Peritubular dentin

After the formation of intertubular dentin the odontoblasts deposit on the inner surface of the dentinal tubules the peritubular dentin, resulting in a progressive reduction of its lumen (dentin sclerosis) that can be accelerated by external stimulus (Linde & Goldberg, 1993; Nanci, 2013).

Even though the exactly constitution and formation mechanism is unknown the peritubular dentin is composed by a hypermineralized collar, almost without collagenous matrix, which vary depending on the location: the thickness and tubular density per unit volume increase proportionally with the distance from the DEJ, towards pulp with peritubular dentin width presenting the inverse tendency; in the mantle dentin small amount is presented (Marshall et al., 1997; Nanci, 2013; Pashley, 1996; Tjäderhane et al., 2012; Zaslansky etal., 2006).

This highly mineralized sheath is also perforated, along with the tubular branches (Mjor & Nordahl, 1996), by many small pores and fenestrations, suggesting its importance in the active regulatory activity between the intertubular dentin and odontoblasts via tubular fluid (Gotliv & Veis, 2007).

Intertubular dentin

Primarily, the odontoblasts produce the intertubular dentin, which is a matrix formed by collagen type I fibrils randomly disposed and where the apatite crystals are deposited. It occupies the region between the dentinal tubules (Kinney et al., 2003; Marshall et al., 1997). The collagen fibrils have diameter of 50 to 100 nm, while the apatite crystals are around 5 nm thick and their remaining dimensions are influenced by the pulp distance [needle-like at the pulp and plate-like at the DEJ (Kinney et al., 2001)].

In the literature reviewed there is no consensus regarding the differences concerning nature, size and organization of the mineral phase between intertubular dentin and peritubular dentin; however, peritubular dentin seems to have a much higher mineral content, is almost collagen-free and a homogeneous distribution (Gotliv et al., 2006; Gotliv & Veis, 2007; Nanci, 2013; Weiner et al., 1999). When compared with enamel the collagen phase of intertubular dentin provides a lower modulus of elasticity and lower mineral content is related with a decrease in dentin microhardness (O'Brien, 2008). These structural differences result in properties differences, such as hardness, elasticity and fracture resistance (Kinney et al, 1996a, 1996b; Wang, 2005).

Interglobular dentin

Interglobular dentin is designated to describe areas of unmineralized or hypomineralized dentin that failed to fuse with mature dentin and is particularly identified in the circumpulpar dentin where the globular pattern of mineralizaton is observed (Nanci, 2013).

Incremental growth lines

The organic matrix of primary dentin is deposit rhythmically in a linear pattern of 4 µm increments per day and 12-hours cycle mineralization process in an inward and rootward direction, parallel to the pulp surface. These incremental lines run at right angles to the dentinal tubules (Nanci, 2013).

1.5. Deciduous dentin

In the examination of the similarities in morphology and composition of permanent and deciduous dentin, there has been an assumption that they have a similar histologic structure; however, the literature suggests that there are significant chemical and morphological differences between the two (Bordin-Aykroyd et al., 1992) which may influence tooth sensitivity, trauma type and susceptibility to caries (Sumikawa et al., 1999).

Primary dentin microstructure differs from permanent dentin in the tubule diameter where it appears to be greater and in the numerical tubule density where it appears to be larger (Hosoya & Tay, 2007; Pires et al., 2018; Sumikawa et al., 1999). Increases in tubule diameter and decreases in peritubular thickness are correlated in relation to their respective distances from the DEJ (Sumikawa et al., 1999).

Other authors state the opposite, that the decrease in tubule diameter and number (Kim et al., 2017; Koutsi et al., 1994; Mithiborwala et al., 2012). Different fields of investigation might affect both diameter and density of the dentinal tubules which may account for the differences between studies (Mjor & Nordahl, 1996).

Besides reducing the area of solid dentin and the bond strength, this tubular structure may contribute to triggering higher wetness. In addition, the increased number of larger tubules may also be responsible for a higher susceptibility to external stimulus transmission and the exposure of primary teeth pulp to noxious substances (Sumikawa et al., 1999).

1.6. Dental pulp

In terms of histology, the pulp is a soft connective tissue comprising four distinguishable layers, which include the odontoblastic zone, the cell-free zone of Weil (these two more prominent in the coronal pulp), the cell-rich zone and the pulp core, from the periphery to the core, respectively. The principal cells groups present are odontoblasts, fibroblasts, undifferentiated ectomesenchymal cells and macrophages (Nanci, 2013). The odontoblasts are post-mitotic, highly differentiated cells and constitute a distinctive layer with a tendency to become pseudo-stratified in the coronal area as a result of odontoblast crowding (Tziafas, 2010). They have a single layer in the radicular part, with highly polarized cell bodies and cytoplasmic processes that extend into the dentinal tubules, the amount of which corresponds to the number of tubules and ranges according to the type of tooth and its location (Kawashima & Okiji, 2016).

The phenotype reflects the functional state characterized by a sequence of cytological and functional changes (Tziafas, 2010) which vary from an active to a quiescent phase and are also dependent on the location. In a developed tooth, the cell bodies are columnar in the crown, cuboid in the midportion of the pulp and flatten in the apical area. Moreover, there are differences in the size and the odontoblasts are wider in the crown when compared to the root (Garrant, 2003; Nanci, 2013).

The tight junctions of the odontoblasts constitute a selective permeable barrier to water and small ions that regulate the passage of fluids and nutrients between the pulp and dentinal tubules and maintain a localized micro-environment inside it, which enhances matrix deposition and subsequent mineralization (Bishop & Yoshida, 1992; Turner et al., 1989).

The fibroblasts are the main cells group in the pulp, particularly numerous and concentrated beneath the odontoblastic layer in the coronal part (Garrant, 2003), where they constitute the cell-rich zone. Since their function is to form and maintain the pulp matrix, their histologic shape corresponds to their functional activity. Young pulp has a high number of fibroblasts with a large cytoplasm and a high amount of synthetizing and secreting organelles which, over time, become flattened into spindle-shaped cells with a dense nucleus (Nanci, 2013).

The undiferentiated ectomesenchymal cells represent a reservoir from which fibroblasts and odontoblast-like cells are differentiated (only the dental papilla cells possess the ability to differentiate into odontoblasts), as part of the wound healing process in the mature pulp (Tziafas et al., 2000) and are dependent on the stimulus (Nanci, 2013). These cells are observed in the cell-rich area and pulp core. They have a polyhedral shape with a large nucleus and display cytoplasmic extensions. The amount of these cells along with other cells in the pulp core diminish over time and this reduction, in parallel with other aging factors, decreases the regenerative potential of the pulp (Nanci, 2013).

Mesenchymal stem cells have been isolated from permanent and deciduous teeth and have the multipotency to differentiate into odontoblasts, chondrocytes and adipocytes when induced by specific growth factors (Kawashima & Okiji, 2016; Nanci, 2013).

The extra-cellular component of the pulp, the organic matrix, contains collagen (principally type I and III) and ground substance non-collagenous proteins (glycosaminoglycans, glycoproteins and water) (Nanci, 2013).

1.6.1. Deciduous pulp

Since the pulp in deciduous teeth has a lower longevity when compared to permanent teeth, its histologic structures never achieve the same degree of differentiation. Four distinctive zones are also present. In the odontoblastic layer the cells are more scattered with a tendency towards being pseudo-stratified. The cell-free zone of Weil is not obvious and the cell-rich zone is a non-continuous layer that is just present in the coronal part. Finally, the pulp core is comprises loose connective tissue, cells, vessels and nerves (Ferraris & Muñoz, 2004). In addition, the dentin secretion and pulpar repair activity of primary teeth decreases with aging (Borges et al., 2007).

Current data regarding microstructure and composition in deciduous teeth is still scarce and the available studies also show contradictory results and are very often the results extrapolated from studies performed on permanent teeth (Oliveira et al., 2010).

2. Restorative and Regenerative treatments

2.1. Ideal material

In general, the ideal endodontic material which can guarantee long-term treatment success should include some of the following characteristics: biocompatibility, radiopacity, be antibacterial, dimensionally stable, easy to handle, unaffected by blood contamination, resistant to dislodging forces, set in a wet environment, hard-tissue conductive (Ma et al., 2011; Shen et al., 2015) and block the communication pathways of bacteria and fluids between the root canal system and adjacent dental tissues (Caravia & Barbero, 2006; Wang, 2015).

More precisely, an effective endodontic material ensures optimal physical properties [short setting time, compressive strength, flexural strength, sealing, dimensional (Camilleri, 2011)] and color stability (Marciano et al., 2017, 2014), radiopacity (Islam et al., 2006; Vivan et al., 2009), insolubility in contact with fluids and should remain unaffected by moisture in its ability to seal (Cavenago et al., 2014; Fridland & Rosado, 2003). It should also have flowability and easy insertion (Duque et al., 2018; Duarte et al., 2012). In terms of its chemical and biological properties it should ideally have an alkaline pH, release calcium ions (Duarte et al., 2003), be bioactive (Gandolfi et al., 2010) and have cell attachment (Balto, 2004) and biocompatibility with the host tissues (Camilleri et al., 2004). Finally, it should be nontoxic, noncarcinogenic and nongenotoxic (Duarte et al., 2018; Parirokh & Torabinejad, 2010b).

Setting time is defined as the time required for a material to polymerize from a fluid to a hardened state and needs to be appropriate for each clinical approach (Zhejun Wang, 2015). In Pediatric Dentistry (PD) this characteristic takes on special importance due to the particularities of behavior management during treatment. Ideally, and according to Torabinejad *et al.*, the material should respect the working time of the procedure and set as soon as it is placed into the cavity; this would allow for the maintenance of dimensional stability and consistency following placement and also in reduce contact time of the unset material with vital tissues (Torabinejad, 2014).

Moisture is required for the hydration reaction and any modification in this complex process might influence the biological, chemical and physical properties of the resulting product (Camilleri, 2007; Torabinejad et al., 1995). For example, the degree of hydration and setting environment affect the microhardness and overall strength of the material (Nekoofar et al., 2010).

Another factor to take into consideration is that when a material is placed in stress bearing conditions (Torabinejad et al., 1995), its compressive strength fails at the point that uniaxial compressive stress is reached (Wang, 2015). However, these circumstances are not common for this kind of materials.

Another mechanical property is characterized by the capacity of the material to resist deformation under load and is known as flexural strength: the higher the value, the lower the risk of fracture in clinical conditions (Moshaverinia et al., 2010).

Hardness can be defined as the resistance to plastic deformation of the surface following indentation or penetration of the material (Wang, 2015). This is not a measure of a single property (Namazikhah et al., 2008), but is affected by other fundamental properties, such as yield strength, tensile strength, modulus of elasticity (Bentz, 2007) and crystal structure stability (Gilman, 1997). It can be used as an indicator of the setting process and the overall strength or resistance to deformation when compared

to baseline information (Namazikhah et al., 2008; Wang et al., 2015). An optimal value of the surface hardness is considered to be similar to natural dentin, which is between 60-90 VHN (Lai et al., 2003). Microhardness measures surface properties of materials has not been shown to have clinical relevance to the performance of MTA-type products (Camilleri, 2014).

In order to evaluate its sealing effectiveness, an ideal root canal filling and obturation material should have a high radiopacity to be clearly visible on radiographs so that can be distinguishable from the surrounding dental tissues or other materials (Torabinejad et al., 1995). The degree of radiopacity should follow international standard requirements that consider a minimal radiopacity to be the equivalent of 3 mm thick Aluminum (control material) (Silva et al., 2013). The measurement should be performed according to ISO 6876/2001 recommendations (Torabinejad et al., 1995).

The ability to fill difficult-to-access areas, such as the narrow irregularities of the dentin, is another physical property known as flow, which is fundamental to hermetic sealing. The flow rate is affected by particle size (inversely proportional to the size of the particles), temperature, shear rate, and time from mixing (Desai & Chandler, 2009). The greater the flow, the greater the ability to penetrate into irregularities. According to ISO 6876/2001, in a flow test, a disk with a minimum diameter of 20-mm should be obtained and moderate flowability is always preferable (Silva et al., 2013).

Biological properties

Several studies, both *in vivo* and *in vitro*, have suggested that the mechanism behind pulp healing, by means of dentinal bridge formation, is alkaline pH and calcium ion-release dependent (Holland et al., 2002; Okabe et al., 2006; Trope et al., 1992). These two interrelated variables are desirable in the setting reaction (Wang, 2015). Calcium hydroxide release should be adequate to maintain the alkalinity of the adjacent tissues (Namazikhah et al., 2008) and the capacity to stimulate osteoblasts activity is due to a high pH of the material (Koh et al., 1997).

In terms of close contact of pulp and periodontium tissues, the endodontic materials need to be non-toxic and biocompatible to guarantee viability of cells and the capacity to grow and populate on the surface (Gomes-filho et al., 2011; Wang, 2015; Zmener et al., 2012). When clinically applied to dental tissue, following the indications of the material, it is expected to not trigger any adverse reaction, such as toxicity, irritation, inflammation, allergy or carcinogenicity, which will guarantee an adequate host response (Al-Haddad & Aziz, 2016; Sun et al., 1997). It appears that biocompatibility is also guaranteed with the presence of calcium phosphate in the composition of the cementum, which is the principal inorganic component of the hard tissues (teeth and bone) (Al-Haddad & Aziz, 2016).

In order to block the access of bacteria and toxins to the root canal system (Wang, 2015) and protect the pulp from further leakage, an optimal biomaterial used in Endodontics, such as pulp capping, perforation repair or root-end filling should stimulate and modulate the biomineralization process to adequately seal the communication or the margin of a tooth defect. The biomineralization promotes the elevation of local pH, the release of mineral ions and induces the apatite-like structures precipitation in dentin over time (Zanini et al, 2012), and is expected to facilitate healing at the material-tissue interface (Carmona et al, 2009). The apatite crystals grow within collagen fibrils, promoting controlled mineral nucleation on dentin and triggering the formation of an interfacial layer at the material-dentin interface (Tay et al., 2007).

A bioactive material may be generally defined as "one kind of material that has been designed to induce specific biological activity'" (Camilleri, 2014). This broad definition may be specified in several applications, such as in the promotion of tissue regeneration after soft and hard tissue adhesion, the incorporation of growth factors that regulate cell proliferation and migration and to interact and promote an interfacial linkage with dental tissues (Amaireh et al., 2019; Bhadra et al., 2019; López-García et al., 2019; Vallittu, Boccaccini et al., 2018). Ideally, and particularly in the endodontic field, the biomaterials should have long-lasting antibacterial activity (Siqueira & Gonçalves, 1996) and stimulate odontoblast-like cell differentiation to enhance reparative dentinogenesis leading to improvements in clinical outcomes (Schröder, 1985; Wang, 2015). In a severe pulp exposure following underlying odontoblast layer destruction, the regeneration of the pulp-dentin complex should be initiated by progenitor cell recruitment and differentiation into the secreting cell, to induce dentin bridge formation. Ideally, the dentin bridge should have an organized tubular structure, which may provide a superior barrier when compared to the disorganized, amorphous calcified "dentin-like" tissue present in fast progressive caries lesions (Ricucci et al., 2014; Woodmansey et al., 2015). However, the clinical quality of dentin bridging remains unclear due to the fact that it can only be evaluated histologically (Walsh et al., 2018).

Nevertheless, an optimal material used for all endodontic purposes, from root filling to restorative or reparative procedures, with all the desired characteristics described above does not exist, even though bioactive endodontic cements fulfill most of the desired/essential properties. Parirokh *et al.* considered it more appropriate to designate these biomaterials by paying attention to the inclusion of materials with a variety of chemical compositions which have in common bioactivity (Parirokh et al., 2018) expressed by hard tissue conductivity (Moretton et al., 2000), calcium ion release (Parirokh et al., 2018) and hard tissue induction (Parirokh et al., 2010a). This latter process forms an apatite-like layer on the surface of the material when it comes into contact with physiological conditions *in vivo* (Hench & Wilson, 1984), or in a simulated body environment *in vitro* (Ducheyne et al., 1994), such as phosphate buffer saline (Parirokh & Torabinejad, 2010b, 2010a; Torabinejad, 2014).

2.2. Hydraulic cements

Thus far, the nomenclature used to identify materials based on tri/dicalcium silicate has confused the dental community since the terms used are non-specific such as, bioceramic and biosilicate, or even bioactive endodontic cements (Ha et al., 2017). In Dentistry, bioceramics are a wide subgroup and encompass ceramics for fixed prosthodontics (porcelain, alumina, zirconia, lithium disilicate) and ceramic implants (zirconia). Biosilicates include dental porcelain, bioactive and radiopaque glasses integrated as fillers in a variety of cements and restorative dental materials (Primus et al., 2019).

In general all of these are hydraulic dental cements, relying primarily on hydration reactions for setting, as opposed to the more usual acid–base systems used in Dentistry, but their solubility is relatively low (Islam et al., 2006; Torabinejad et al., 1995; Patent No. 5,415,547, 1995). They set and are stable under water (Fridland & Rosado, 2005) and reach their optimal physical and mechanical characteristics since they do not deteriorate when they become wet, can be employed in wet environments, and form calcium hydroxide as a by-product of the hydration reaction. Furthermore, they may also react with other components present, such as blood, tissue fluid, dentin, bone, irrigating solutions and restorative materials. These bring about changes in the surface chemistry with adverse effects on the material (Camilleri, 2020).

Other authors considered the descriptor "hygroscopic" to be more correct than "hydraulic", since hydraulic refers to materials that react 'under water' and could be extended glass ionomer cements (GIC) and

related glass-based cements that set using acid-base aqueous reactions. On the other hand, the concept hygroscopic means that the material reacts with water, which would then exclude GICs (Ha et al., 2017).

More recently two classifications have been proposed for dental hydraulic cements in terms of clinical conditions (context and applications) and their constitution (chemistry and presentation).

The first, clinical conditions, is based on the nature of the environment, with a clear distinction between different conditions. Intracoronal is pulp protection, with a barrier for regenerative endodontic procedures (in contact with the dental pulp and coronal dentin). Intraradicular is root canal sealing, with apical plugs (in contact with treated dentin but limited amounts of fluid). Extraradicular, with root-end fillers and perforation repair materials (in contact with untreated dentin, with their surface completely in contact with blood and tissue fluids) (Camilleri, 2020).

Hydraulic cement chemistry is the foundation of the second classification, since it influences the behavior, properties and principally the hydration process. In particular, hydraulic calcium silicate cements are all dependent on cement chemistry, the modifiers used and whether the material is mixed with water or not (Camilleri, 2020) (Figure 1.2).

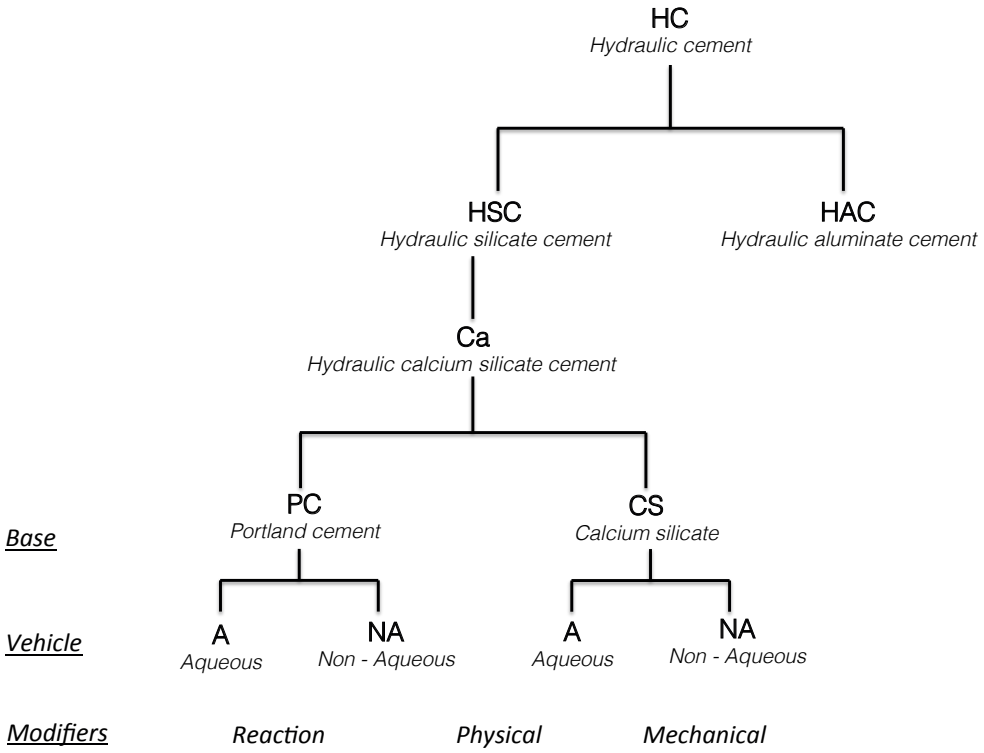


Figure 1.2. Classification of hydraulic calcium cements based on their chemistry (Adapted from Camilleri, 2020).

The first subgroup is hydraulic silicate cements, which are distinguished from calcium aluminates that are also being proposed as dental materials (Aguilar et al., 2012; Castro-Raucci et al., 2018; Oliveira et al., 2013). An additional subdivision is necessary - hydraulic calcium silicates-, due to the production of calcium hydroxide when reacting with water, which is clinically beneficial (Camilleri, 2020).

A further subgroup is necessary to differentiate calcium-based materials from other cement-like silicates due to the specific application of these materials in clinical Dentistry to replace the use of calcium

hydroxide. When reacting with water, hydraulic calcium silicates produce calcium hydroxide, which then makes the cement beneficial for several clinical uses (Camilleri, 2020).

Finally, the last subgroup refers to how the cements are presented as a powder when mixed with water or suspended in a non-aqueous vehicle and which depend on the diffusion of water from the surroundings for hydration to continue (Camilleri, 2020).

2.2.1. Mineral trioxide aggregate (MTA)

2.2.1.1. General description

Due to the relative biological and technical importance of Mineral Trioxide Aggregate (MTA), which has a wide range of clinical applications, its physical and chemical properties have been the subject of extensive studies since their initial development in the late twentieth century by Dr. Mahmoud Torabinejad and his coinventor Dr. Dean White, at Loma Linda University. Torabinejad and White registered two US patents with this (the initial United States patent #5,415,547, continued to #5,769,638) (Patent No. 5,415,547, 1995) Portland cement-based endodontic material, one century after Dr. Witte had published the first case report using Portland cement as a root canal filling. However, it was only in 1998 that MTA received approval for endodontic applications by the US Food and Drug Administration (Togaru et al., 2016). Finally, in 1999, ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) became the first commercially available MTA product in the United States (Darvell & Wu, 2011; Tawil et al., 2016).

MTA was only reported in the scientific literature in 1993 (Lee et al., 1993) as a Portland cement blended with a radiopaque powder. For instance, the Material Safety Data Sheet (MSDS) declares that ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) is mainly composed of Portland cement (75 wt%), followed by bismuth oxide (Bi_2O_3) (20 wt%), calcium sulfate dihydrate or gypsum ($CaSO_4 \cdot {}_2H_2O$) (5 wt%) and minor trace elements which may also be present as stated (Torabinejad, 2014). Since the first 20th century article, which introduced an experimental material known as "MTAggregate" (Lee et al., 1993) to the dental community, the term MTA has become so commonly used.

Several differences are reported between these two materials, particularly in terms of setting expansion, chemical composition, surface chemical composition, porosity, compressive strength, radiopacity and particle sizes (Asgary et al., 2004; Camilleri, 2007, 2008; Dammaschke et al 2005; Gancedo-Caravia & Garcia-Barbero, 2006; Islam et al., 2006; Komabayashi & Spångberg, 2008; Song et al., 2006), This resulted in Portland cement, not being used in Dentistry due to its heavy metal components (Bramante et al., 2008), inadequate radiopacity (Bortoluzzi et al., 2009; Vivan et al., 2009), relatively high solubility (Darvell & Wu, 2011) and wide range of particle sizes (Dammaschke et al., 2005).

2.2.1.2. Chemistry: manufacture / setting reactions / constitution

The original MTA patent, registered in 1995, stated that its constitution is 50–75 % (wt) calcium oxide and 15–25 % silicon dioxide. These two components together cover 70–95 % of the cement and when blended produce tricalcium silicate, dicalcium silicate, tricalcium aluminate and tetracalcium aluminoferrite (Patent No. 5,415,547, 1995).

Several techniques have been used to examine the chemical composition and material microstructure of un-hydrated MTA and to distinguish several materials from different manufacturers which

have been developed to improve clinical performance (Parirokh & Torabinejad, 2010a); these include scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), X-ray fluorescence and X-ray diffraction (Xrd) analysis, as well as Xrd with Rietveld refinement (Camilleri, 2014). The literature notes there are some differences in terms of the chemical composition of MTA, that may be caused by different liquids used to mix with the powder (Bortoluzzi et al., 2006; Asgary et al., 2004; Asgary et al., 2005; Asgary et al., 2006; Camilleri et al., 2005; Coomaraswamy et al., 2007; Özdemir et al., 2008; Song et al., 2006; Torabinejad et al., 1995) and the variety of equipment used to test its composition (Asgary et al., 2004, 2005; Camilleri, 2007; Camilleri et al., 2005; Dammaschke et al., 2005; Oliveira et al., 2007; Islam et al., 2006; Song et al., 2006; Torabinejad et al., 1995). Moreover, comprehensive and detailed information regarding tri/ dicalcium silicate manufacture is not available due to trade secret information protection (Primus et al., 2019).

Generally and independently of the manufacturers, MTA is a very fine powder which is mixed with sterile or distilled water in a 3:1 powder-to-liquid ratio (Torabinejad et al., 1993) to acquire a grainy, sandy consistency. The moisture/water is required for the setting reaction and affects setting time and solubility. A large amount of water increases both properties and also porosity and decreases radiopacity (Duarte et al., 2018; Fridland & Rosado, 2003, 2005). The ratio can be changed according to each clinical situation and is dependent on the area where the material will be used (Fridland & Rosado, 2003), from a stiffer mix in the pulp chamber to a more fluid consistency in the root canal (Camilleri, 2014). These differences don't appear to affect its clinical performance and no significant differences were observed in material expansion (Hawley et al., 2010) or in the histological outcome at different water/powder ratios when used as a direct pulp-capping material on healthy human pulp (Shahravan et al., 2011).

Furthermore, the mixture characteristics may be influenced by the mixing method (i.e. the amount of entrapped air), condensation pressure, pH and humidity of the environment, type of MTA, type of storage media or vehicle, the length of time between mixing and evaluation, thickness of the material and temperature (Aminoshariae et al., 2003; Chng et al., 2005; Coomaraswamy et al., 2007; Dammaschke et al., 2005; Danesh et al., 2006; Fridland & Rosado, 2003; Gancedo-Caravia & Garcia-Barbero, 2006; Hachmeister et al., 2002; Islam et al., 2006; Kogan et al., 2006; Lee et al., 1993; Lee et al., 2004; Saghiri et al., 2008; Sluyk et al., 1998; Storm et al., 2008; Walker et al., 2006; Watt et al., 2007).

Working time

The working time and the proper proportion of liquid to powder are important aspects in producing a grainy, sandy mixture, which is sometimes challenging to deliver and to compact effectively in the required location in the tooth (Shen et al., 2015).

In general, the instructions indicate a working time of around 5 minutes and estimate the setting time to be over 4 hours (Torabinejad, 2014). Some factors may influence these characteristics, namely covering the mixed material with moistened gauze to slow down evaporation thus increasing working time. Mixing MTA powder with anesthetic solution also increases setting time (Kogan et al., 2006). In this situation it is important to take into consideration the fact that local anesthetic solutions contain both chloride and sulphate ions, which again have a conflicting effect on cement hydration. In a higher sulphate solution, the cement may be subject to a sulphate attack and consequently excessive expansion and cracking is observed over time due to delayed ettringite deposition (Camilleri, 2014).

Different researchers have estimated a setting time of 165 minutes (Torabinejad et al., 1995), while others have quoted between 45–140 minutes for initial and final setting (Chng et al., 2005) or 50 minutes

(Kogan et al., 2006), or even 220–250 minutes (Ding et al., 2008). There are a number of conflicting reports in the literature which may be the result of different experimental methods, as they involve penetration of the cement by needles of various dimensions and/or weight (Torabinejad, 2014).

Setting reaction

These unique cements have great advantages, particularly in Dentistry, based on their hydraulic setting reaction. They can react with water, at room temperature (23 ± 1°C), to form a mass and are moisture tolerant (hydrophilic, hygroscopic) (Primus et al., 2019), thus complete moisture control is not essential (Juneja & Kulkarni, 2017).

After mixing the powder with water, calcium hydroxide (CH) and calcium silicate hydrate are initially produced. This mix forms a sticky colloidal gel (calcium silicate hydrate gel) that solidifies into a hard structure (Camilleri, 2007, 2008). In the scientific community there is some controversy regarding the production source for CH (Camilleri, 2008). Although it is the expectation that CH is formed from dicalcium and tricalcium silicate after mixing MTA powder with water, Dammaschke *et al.* described CH as a product of tricalcium aluminate hydrogenation (Dammaschke et al., 2005). Even then, it is widely assumed that calcium hydroxide production is the cause of the high alkalinity found in MTA following hydration (Camilleri, 2008). Because MTA is a hydraulic type of cement, it sets by reacting with water in an exothermic reaction, and is then stable in water (Torabinejad, 2014).

The hydration reaction is very slow and the time necessary is far beyond the duration of any reasonable one-visit schedule. Indeed, the tooth tissue is water-permeable, is exposed to saliva and gingival fluid coronally, tissue fluid pulpally and by periodontal fluid over the root surface. So, water will always be present for the setting reaction independent of the location or coverage by an impermeable restoration.

Nevertheless, covering with a moistened cotton pellet and sealing with temporary filling add water for hydration and helps to preserve cement integrity (Budig & Eleazer, 2008; Walker et al., 2006). It may also provide a barrier to mechanical washout while the material is fragile (similar to a glass-ionomer cement base). Setting under such conditions has been reported to improve mechanical and chemical properties (Lee et al., 2004; Torabinejad et al., 1995). Because of this, manufacturer instructions recommend the placement of the final restoration be delayed to allow for an initial setting period of not less than 4 hours with a moistened cotton pellet covering (Buchanan & Worner, 1945; Kahler, & Walsh, 2015; Ranjkesh et al.,2016; Sluyk et al., 1998).

Furthermore, it is important to critically evaluate the setting time claimed by manufacturers, since these are based on penetration tests (resembling the Gilmore needle), which cannot indicate the degree of hydration, and thus may be ambiguous, confusing and of limited value for monitoring the setting process (Darvell & Wu, 2011).

2.2.1.3. Cellular responses and physiological effects

In general, the MTA is osteogenic, inductive and conductive of hard tissue formation (Chen et al., 2009; Hakki et al., 2009; Reyes-Carmona et al., 2009). It stimulates cement-like hard tissue formation and is biocompatible which, due to its strong alkalinity, is bactericidal (Duarte et al., 2003; Holland et al., 2002; Tamburic et al., 1993) and shows osteoblastic adherence (Favieri et al 2008) with activated bone regeneration (Nascimento et al., 2008). These are confirmed by numerous *in vitro* and *in vivo* tests, such as general toxicity profile tests of potential materials in a cell culture, implantation and usage tests in

experimental animals according to accepted clinical protocols (AL-Rabeah et al., 2006; Balto, 2004; Bodrumlu, 2008; Camilleri et al., 2005; Camilleri et al., 2004; Deus et al., 2005; Gorduysus et al., 2007; Kettering & Torabinejad, 1995; Kim et al., 2008; Koulaouzidou et al., 2005; Laurent et al., 2008; Nakayama et al., 2005; Oviir et al., 2006; Pelliccioni et al., 2004; Pérez et al., 2003; Pistorius et al., 2003; Saidon et al., 2003; Takita et al., 2006; Thomson et al., 2003; Torabinejad et al., 1995b; Vajrabhaya et al., 2006).

While the mechanism behind the interaction between pulp-capping materials and pulp tissue remains uncertain, numerous hypotheses have been put forward, including the role of growth factors in angiogenesis, recruitment of progenitor cells, differentiation, and finally calcific barrier formation. The transforming growth factor-β1 (TGF-β1) is known to be involved as a key factor and has been shown to be involved in odontoblastic differentiation (Begue-Kirn et al., 1992).

2.2.1.4. Cytocompatibility and osteogenesis

The leaching of calcium and hydroxyl ions allows MTA to promote the regeneration and remineralization of the hard tissues, as well as enhances its sealing ability by deposition of calcium and phosphate crystals into voids and potential spaces between dentine and the root filling material (Gandolfi et al., 2013; Hickel et al., 2007; Martin et al., 2007).

Based on Sarkar and Bozeman, the most important physiochemical property of MTA in vital pulp therapy is the formation of an interstitial layer when it is placed in contact with dentin and/ or structures with a similar composition to hydroxyapatite (Bozeman et al., 2006; Sarkar et al., 2005); this allows for microleakage prevention and cell substrate attachment (Kang et al., 2015; Sarkar et al., 2005).

MTA releases calcium hydroxide as a by-product of hydration and Ca^{2+} ions when it is in contact with the connective tissue and causes an area of necrosis with carbon dioxide release; the crystals (calcium carbonate), which constitute the core of calcification, are formed by calcium hydroxide and carbon dioxide (Parirokh & Torabinejad, 2010a).

The bioactivity of MTA plays an important role in the mineralization process. The pH value is 10.2 after mixing and rises to 12.5 at 3 hours (Torabinejad et al., 1995). The alkalinity of the medium and Ca^{2+} release in the fluid surrounding MTA is beneficial to hard-tissue precipitation (Sarkar et al., 2005). In particular ions, Ca^{2+} enhances osteoblastic viability, proliferation and differentiation (Dawood et al., 2015; Duarte et al., 2018). Several authors have confirmed the synthesis of reparative dentin (Witherspoon, 2008) by odontoblast-like cells originating from the differentiation of progenitors, which are proliferated and pooled at the site of MTA capping (Accorinte et al., 2008; Asgary et al., 2008; Kuratate et al., 2008; Min et al., 2008). Likewise, the metallic ions dissolution in the setting process of MTA when placed clinically, may release dentine matrix components, such as non-collagenous protein, glycosaminoglycans, transforming growth factor (TGF-β1) and adrenomedullin which potentially mediate cellular activity in dentinogenesis (Tomson et al., 2007). Hence, until now it has not been clear whether these are direct effects of the MTA or indirect effects via hydrolysis products (Darvell & Wu, 2011).

2.2.1.5. Antibacterial activity

The literature states that there is an antibacterial and antifungal effect of MTA against some pathogens (Miyagak et al., 2006; Ribeiro et al., 2006; Torabinejad et al., 1995a; Yasuda et al., 2008). In particular, the alkalinity of the environment is unfavorable for bacterial growth and activity (Al-Hezaimi et al., 2006; Maeno et al., 2005; Poggio et al., 2015; J. F. Siqueira & Lopes, 1999) and accelerates apatite nucleation

(Gandolfi et al., 2013). Nevertheless, there are also conflicting reports that might be related to the different species of microorganisms that were tested, namely the source of the preparation material (Al-Hezaimi et al., 2005; Holt, Watts, Beeson, Kirkpatrick, & Rutledge, 2007; Miyagak et al., 2006; Mohammadi, Modaresi, & Yazdizadeh, 2006; Stowe, Sedgley, Stowe, & Fenno, 2004; Torabinejad et al., 1995a; Yasuda et al., 2008; Zhang, Pappen, & Haapasalo, 2009), as well as the concentration and type of MTA used in these studies (Al-Hezaimi et al., 2005, 2006, 2009). This effect seems to be also adversely influenced by lowering the powder-to-liquid ratio (Masoud Parirokh & Torabinejad, 2010b, 2010a).

2.2.1.6. Immune response

In addition to the physiological aspects described above, a number of animal studies have reported that MTA improves the adaptive humoral immune response to endodontic pathogens, stimulates the migration of neutrophils via the activity of mast cells and macrophages and has an anti-inflammatory effect via the suppression of the inflammatory cytokines (Gomes et al., 2008; Rezende et al., 2008). However, these still require the mechanism comprehension and validation in human models (Darvell & Wu, 2011).

2.2.1.7. Applications

MTA has come a long way from when its applications were limited to root perforations and as a retrograde filling material (Lee et al., 1993), and has gained wider acceptance in clinical practice due to its biocompatibility, physicochemical interaction with the local environment (Ma et al., 2011; Sarkar et al., 2005; Wang et al., 2012), sealing, sterilizing, mineralizing, dentinogenic and osteogenic capacities/performance (Kusum et al., 2015). Today it can also be used in direct pulp capping (Accorinte et al., 2008; Min et al., 2008), pulpotomy (Barrieshi-Nusair & Qudeimat, 2006), apical plug (Favieri et al., 2008; Saunders, 2008; Sübay & Kayataş, 2006), apexification (Chueh et al., 2009; Jaramillo et al., 2006) and apexogenesis (Huang, 2008; Jung et al., 2008). It has also been used in the treatment of horizontal root fracture (Er et al., 2009; Kusgoz et al., 2009; Yildirim & Gençoğlu, 2009), repair of resorptive defects, both internal (Altundasar & Demir, 2009; Meire & De Moor, 2008; Sari & Sönmez, 2006) and external root resorption (Gonzales & Rodekirchen, 2007; Gulsahi et al., 2007) and also to repair perforations at the root canal (Yildirim & Dalci, 2006) and furcation (Hashem & Hassanien, 2008; Ibarrola et al., 2008; Pace et al., 2008).

2.2.1.7.1. Vital pulp therapy

The primary aim of vital pulp therapy is to preserve the vitality of the dental pulp when it is exposed to caries excavation, trauma, restorative procedures, or anatomical anomalies. The principles that sustain this treatment option are based on cellular mechanisms involved in pulp repair and bridge formation, creating an environment that induces hard tissue formation by the remaining pulp cells and seals the exposure location and contributes to sustained pulp vitality (Moghaddame-Jafari et al., 2005; Schröder, 1985). Bacterial contamination occurs as a result of pulp tissue exposure and promotes an immune-component response followed by cell recruitment from the dentin-pulp complex and hard tissue formation by differentiated progenitor cells – reparative dentin (Torabinejad, 2014). In clinical practice the pulp capping materials are placed between the vital pulp and the external environment in order to protect the pulp and the exposure site (Moghaddame-Jafari et al., 2005; Schröder, 1985). A minor indirect trauma to pulp tissue without exposure stimulates existing primary odontoblasts to produce reactionary dentin. Therefore, the materials used in the vital pulp treatments should respect biological

principles such as adequate biocompatibility and bioactivity to promote dental pulp stem cell activity and pulp healing in permanent teeth (Gandolfi et al., 2015).

In short, vital pulp therapy techniques include five definitive alternatives, either in primary or permanent teeth. These are, from the least to most invasive, non-invasive stepwise excavation, indirect pulp capping (IPC), direct pulp capping (DPC) (Camilleri, 2014), partial pulpotomy (PP) (Cvek, 1978; Miyashita et al., 2016) and full/coronal pulpotomy (FP). Ultimately, treatment selection is dependent on the extent of remaining healthy pulp tissue and size of exposure (Camilleri, 2014; Catalá et al., 2018).

Indirect pulp cap

In indirect pulp-capping in deep lesions a reactionary dentin is formed by the proximity of the capping to the pulp and indirect communication (Simon et al., 2013). When tri/dicalcium silicate cements set, a hydrated calcium silicate matrix is produced, where calcium hydroxide solution is surrounded, which is favorable to the alkalinity of the environment (Primus et al., 2019).

Direct pulp cap

Direct pulp capping (DPC) is performed on an accidental (mechanical or traumatic) pinpoint exposure to preserve pulp vitality and induce mineralized tissue formation (American Association of Endodontist, 2013; Camilleri, 2014).

Pre-existing pulpal conditions are critical for the short-term outcome of DPC, including adequate blood supply, the severity of inflammation, obtaining hemostasis, disinfection of the exposure site, size of the pulpal exposure (Jang et al., 2015) and include the biocompatibility properties of pulp-covering agents and provision of an adequate seal (Katge & Patil, 2017). A biocompatible radiopaque base may be used in contact with the exposed pulp tissue, such as with MTA or CH (American Academy of Pediatric Dentistry, 2017). MTA reduces some of the disadvantages of CH, such as the absorption of the capping material, mechanical instability and subsequent inadequate long-term sealing ability due to leakage (Dammaschke et al., 2010) and has the advantage of being able to set in the presence of blood and tissue fluids since it is a hydrophilic and hygroscopic cement (Torabinejad et al., 1995).

As a result, a several number of clinical trials have revealed that the MTA outcome is more stable over long-term periods, when compared to calcium hydroxide pulp capping, even exhibiting a time-dependent decline in cumulative success; nevertheless, both have exhibited similar performance for short periods (up to 3 or 6 months) (Cho et al., 2013; Hilton et al., 2013; Mente et al., 2014). Dentinal bridging with the tri/dicalcium silicate materials may be supported by the maintaining of the high pH achieved. Calcium hydroxide transforms more rapidly to inert calcium carbonate (lower pH), compared to the calcium hydroxide embedded in the tri/dicalcium silicate matrix (Heward & Sedgley, 2011).

Pulpotomy

Pulpotomy involves partial or total amputation of the affected or infected exposed coronal pulp in order to reach healthy tissue and preserve vitality and function (American Academy of Pediatric Dentistry, 2017). In permanent teeth this clinical procedure is indicated in pulp exposure due to trauma or caries and with no evidence of radicular pathology. This situation is particular indicated in immature

teeth since it guarantees apexogenesis (Torabinejad, 2014). In the literature this treatment option in symptomatic permanent teeth (including with irreversible pulpitis) has long been considered paradoxical and a matter of debate among investigators. Nevertheless, more recent studies have described its clinical success (Torabinejad, 2014) (Annex II).

MTA and other calcium silicate cements have similar action mechanisms to CH, however, they induce odontoblastic differentiation of dental pulp stem cells and produce more uniform and thicker dentin bridge formations with less inflammatory response and less necrosis of pulp tissue (Bortoluzzi et al., 2015; Margunato et al., 2015) (Annex II).

Deciduous teeth

This treatment is indicated for pulp exposure in deciduous teeth in which the inflammation and/or infection has been judged to be restricted to the coronal pulp (Torabinejad, 2014).

Depending on the type of material used the treatment approaches include devitalisation, preservation or regeneration of the pulp tissue (Camilleri, 2014). Historically, formocresol was the most common global treatment approach. However, a number of clinicians and researchers have cited, its mutagenic, carcinogenic, toxic and allergenic properties (Duggal, 2009) which has led to increased criticism and decreased usage (Maroto et al., 2007).

Today the modern PD has shifted the objective of pulpotomy from devitalisation to revitalisation/ using biomaterials that induce calcific barrier formation on the inflamed pulp and guarantee a biological seal (Camilleri, 2014; Mehrdad et al., 2013).

MTA has been reported to provide successful results in pulpotomy treatment of primary teeth due to antimicrobial activity, high success rates, dentinal bridge formation, preservation of healthy pulpal tissue and a lack of root resorption (Aeinehchi et al., 2007; Oviir et al., 2006; Torabinejad, 2014). Based on randomized clinical studies in humans MTA should be considered the new gold standard (Nair et al., 2008; Paranjpe et al., 2011; Paranjpe et al., 2010; Simancas-Pallares et al., 2010), due to its biological properties, namely in promoting the formation of a reparative dentin bridge by odontoblast-like cells (Accorinte et al., 2008; Darvell & Wu, 2011; Min et al., 2008), the preservation of the pulpal tissue, its high clinical success rates (67–98.5%) and also presenting lack of post-operative internal root resorption, inflammatory response and irritation of the pulp (Shayegan et al., 2008) (Annex II).

Moreover, a recent meta-analysis comparing MTA to FC in 30 clinical articles from 7 databases reported greater clinical success with MTA (95 %) than FC (success rate 87 %) (Junior et al., 2015) and a retrospective review of primary molar pulpotomies consistently shows better performance for MTA products over formocresol as long as 48 months following treatment (Ghoniem et al., 2018).

2.2.1.8. Potential problems

Color stability

The causes of discoloration are still a matter of debate. However, the interaction of bismuth oxide with collagen present in tooth tissue and sodium hypochlorite, which is routinely used during root canal therapy, has been described as the main contributing factor. The use of sodium hypochlorite as an antibacterial agent before the application of the pulpotomy agent in particular, has been shown to improve the success of MTA pulpotomies for observation up to 12 months (Akcay & Sari, 2014). Thus, the problem

of darkening teeth over time would be solved with new alternatives with reduced or no bismuth oxide. White MTA was introduced in order to prevent tooth discoloration, although several studies still confirm color changes (Belobrov & Parashos, 2011; Felman & Parashos, 2013). Alternatives, such as calcium tungstate, zirconium oxide (Marciano et al., 2014), were introduced in new calcium silicate cements (Duarte et al., 2018; Ramos et al., 2016). However, a clinical report of partial pulpotomy stated that even Biodentine™ (Septodont, Saint-Maur-des-Fossés Cedex, France) (BD), which contains no bismuth oxide, created perceptible darkening over time, although less than the original ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) bismuth oxide-containing tri/dicalcium silicate (Uesrichai et al., 2019).

Furthermore, MTA is a dynamic material with permanent interaction with tissues and fluids over time. In particular, the contamination with blood may interfere with the morphology of the set material, reducing calcium ion release and radiopacity (Vallés et al., 2013).

Setting time

Another drawback of MTA is the long setting time (Parirokh et al., 2018; Parirokh & Torabinejad, 2010a; Torabinejad et al., 1995), which may compromise permanent restoration in the same visit, with major importance in PD daily practice. The alternative to performing one-visit treatment is to cover the MTA with a glass ionomer cement (GIC) base, under the permanent restoration (Palma et al., 2018; Torabinejad, 2014).

Mechanical properties

The washout (tendency of a freshly prepared cement to disintegrate/be removed/disappear when is in early contact with blood or other fluids after the MTA is placed *in situ* is another disadvantage (Camilleri, 2014).

The compressive strength of MTA is between 45 and 98 MPa (Islam et al. 2006; Nekoofar et al. 2010), flexural strength 11–15 MPa (Walker et al. 2006; Aggarwal et al. 2011) and microhardness 40 and 60 VHN (Danesh et al. 2006; Nekoofar et al. 2007; Namazikhah et al. 2008; Nekoofar et al. 2010a,b; Kang et al. 2012) of MTA is lower than dentin. Thus, MTA seems to be unsuitable for long-term use when applied as a sole restorative base or to replace dentin after an indirect or direct pulp capping (Torabinejad, 2014; Danesh et al., 2006; Islam et al., 2006; Namazikhah et al., 2008; Walker et al., 2006).

Despite the clinical and commercial success of ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA), for the past two decades the marketplace has grown to include over twenty commercial hydraulic tri/ dicalcium silicate dental products to overcome the drawbacks and improve the overall handling characteristics, setting, washout resistance and material costs (Dawood et al., 2015; Walsh et al., 2018).

With the aim of increasing flowability and important properties, such as appropriate radiopacity and setting time, color stability, alkaline pH, release of calcium ions and biocompatibility (Marciano et al. 2017), novel formulations of tri/dicalcium silicate products have been introduced into the market. With the exclusion of resin-based products these all have these features in common: hydraulic setting (reaction with water), the creation of alkaline pH (>7), calcium ion release, bioactivity, relatively slower setting time compared to MTA and gradual strengthening by hydration over approximately 4 weeks. The radiopaque components vary between the many products. Presentation of the products is wide-ranging, and the indications are also broad (Primus et al., 2019).

2.2.2. NuSmile® NeoMTA

2.2.2.1. General description

New calcium silicate-based restorative cements that offer alternatives to MTA have been developed and commercially marketed and have strived to improve on its limitations in terms of its tooth discoloration, poor handling properties, as well as slow setting time (Dawood et al., 2015).

NuSmile® NeoMTA (NuSmile Ltd. Houston, TX, USA) is a newer, promising alternative to MTA with enhanced properties. It was initially developed by Carolyn Primus, who also founded Avalon Biomed inc. in 2011, Fradenton, FL, USA. Later, NuSmile Ltd announced its acquisition of substantially all of the assets of this company, a manufacturer of advanced mineral trioxide aggregate (MTA) products (Avalon BioMed, 2016).

2.2.2.2. Chemistry: manufacture / setting reactions / constitution

When comparing ProRoot® MTA to NuSmile® NeoMTA, the latter has a finer particle size which is responsible for its different clinical performance. NuSmile® NeoMTA has a decreased setting time, increased ion release, increased water sorption, decreased porosity (Camilleri et al., 2013), improved handling and placement (Gandolfi et al., 2015; Gandolfi et al., 2014). The gel has been developed to confer washout resistance (Formosa et al., 2013; Siboni et al., 2017; Walsh et al., 2018). The radiopacifier agent is tantalum oxide, rather than bismuth oxide, to prevent post-procedural tooth discoloration, which is the only difference between MTA Plus and NeoMTA Plus. Other than this, they are indistinguishable (NuSmile Ltd, 2018).

Propylene glycol may be used as a solvent, does not interfere with biological properties (Duarte et al., 2012; Holland et al., 2007) and increases the adhesion of the biomaterial. The association with propylene glycol using different ratios was evaluated in terms of physical and chemical properties. A 20% propylene glycol / 80% distilled water mix encouraged the manipulation of MTA, pH, calcium release, and flowability, causing minor changes in setting time (Duarte et al., 2012).

2.2.2.3. Cellular responses and physiological effects

NeoMTA® and MTA Plus® (Avalon Biomed Inc., Fradenton, FL, USA. Later Nusmile Ltd) showed cell viability and a high degree of cell proliferation and adhesion (Chng et al., 2005) with a promising equivalent biological response to ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) (Camilleri, 2015; Gandolfi et al., 2015).

2.2.2.4. Applications

This new calcium silicate based cement has been marketed particularly for use in pulpotomies because it doesn't stain tooth structure (Camilleri, 2015); however, it has a wide range of applications and may be used as restorative or endodontic cement (Duarte et al., 2018), with varying powder-gel ratios, from a thin consistency to a thick putty-like consistency (Siboni et al., 2017).

2.2.2.5. Potential problems

The formulations with powder-liquid separated allow some freedom to add 1-2 extra drops to achieve the desired consistency and is hard to replicate due to operator variability (Darvell & Wu, 2011).

2.2.3. Biodentine™

2.2.3.1. General description

Biodentine™ (Septodont, St Maur-Des-Fosses, France) has been developed and produced (through active biosilicate technology), with the aim of combining biocompatibility and bioactive behavior with enhanced mechanical properties (Arora, 2013; Fouad & Youssef, 2012; Malkondu et al., 2014). This hydraulic silicate cement has the same clinical applications of MTA and has gained attention in the endodontic field due to its superior physicochemical properties (high compressive strength, excellent sealing ability, ease of handling, versatility, increased density, decreased porosity and as well as fast setting time), micromechanical anchorage, absence of tooth discoloration and ease of handling (Camilleri et al., 2013; Cuadros-Fernández et al., 2016; Mestieri et al., 2015; Niranjani et al., 2015; Rajasekharan et al., 2017).

2.2.3.2. Chemistry: manufacture / setting / reactions / constitution

BD is a new tricalcium silicate (Ca_3SIO_5) based inorganic nonmetallic restorative cement, marketed commercially as a 'bioactive dentine substitute' (Rajasekharan et al., 2017). According to the manufacturer, the powder contains tricalcium silicate as the main component, which makes BD similar to MTA (Zanini et al., 2012), but the particle size has been manufactured to provide a denser, less porous structure (Shen, 2015), dicalcium silicate, calcium carbonate, and zirconium oxide (radiopacifier) (Camilleri et al., 2013; Rajasekharan et al., 2017). The aqueous constituent is mainly water, calcium chloride (responsible for the shorter setting time, which also accelerates the rate of early strength development (Septodont's Research Group, 2020; Shen, 2015), and a modified polycarboxylate (as a superplasticizer) (Bachoo et al., 2013; Camilleri et al., 2013; Rajasekharan et al., 2014).

These components give BD biocompatibility, bioactivity and enhanced properties, including rapid setting time (from the calcium chloride) (Arora, 2013), and high strength (provided by the low water-to-cement ratio, which is possible because of the water-soluble superplasticizing agent) (Laurent et al., 2008).

Setting reactions

BD is presented as a modified powder, in a pre-dosed capsule formulation, for use in a mixing device, and liquid in a pipette, which enhances the physical properties and makes it more user-friendly with a shorter setting time (Grech et al., 2013b). Grech *et al.* described an initial setting time of less than 20 minutes and a final setting time of over 45 minutes (Grech et al., 2013a; Wang et al., 2008; Wongkornchaowalit & Lertchirakarn, 2011). According to the manufacturer the setting time is between 9-12 min (Septodont's Research Group, 2020) (Gandolfi et al., 2013).

Compared to other calcium silicate cements, the reduction in setting time is described to have been achieved by higher specific surface particle size, the addition of calcium chloride accelerator to the liquid phase and a decrease in the liquid content (Septodont's Research Group, 2020).

The literature states that the trituration methods to mix the powder phase with the liquid phase lead to higher calcium ion release and pH compared with manual mixing for all cements and don't influence the flow, setting time and volume, although this does have an impact on solubility (Grech et al., 2013b).

The sequence of steps occurs through the following order: tricalcium silicate is mixed with the water component and results in the formation of a hydrated calcium silicate gel (C-S-H) structure and calcium hydroxide. The growth of the gel structure progresses through nucleation and growth on the tricalcium silicate surface, which progressively fills the spaces, interposed between the tricalcium silicate grains. The crystallization of the C-S-H gel structure occurs through continuous hydration, resulting in the formation of $CaCO_3$ crystals in between the unreacted grains, which gradually fill in the porosities between the unreacted grains of cement for approximately two weeks. The final structure of the set material is made up of a hydrated calcium silicate gel matrix with crystals of $CaCO_3$ in between grains of unreacted cement. The process of crystallization is responsible for structure impermeability and slows down the effects of further reactions (Duque et al., 2018).

2.2.3.3. Cellular responses and physiological effects

There is a lot of evidence in the literature that shows the positive effects of BD on vital pulp cells, how tertiary dentin formation is stimulated and the early formation of reparative dentin (Bachoo et al., 2013). BD seems to stimulate dentine regeneration by inducing early odontoblast-like cell differentiation from pulp progenitor cells (Laurent et al., 2012; Peng et al., 2011; Tran et al., 2012; Zanini et al., 2012). This may be due to a modulation of TGF-β1 secretion coming from the dental pulp cell (Raskin et al., 2012).

During the initial setting time BD is rich in calcium compounds and releases a substantial amount of Ca^{2+}, which get lessens throughout the long-term follow up. This is favorable for the mineralization process and pulp tissue repair (De Rossi et al., 2014). According to Han *et al.*, the amount of Ca^{2+} released and the depth of the incorporation of Ca^{2+} ions into root dentine are greater than those for MTA (Han & Okiji, 2011).

A several number of studies in human pulp fibroblast cultures, dental pulp in a whole human tooth culture models and animal models (rats, dogs, porcine) have reported effective dentinal mechanism repair by BD (Laurent et al., 2012; De Rossi et al., 2014; Shayegan et al., 2012).

2.2.3.4. Applications

BD is a base-restorative cement with dentin-like mechanical properties having a similar main component - tricalcium silicate - and applications as MTA (Laurent et al., 2012, 2008; Tran et al., 2012). Manufacturers claim that BD has some superior features, namely that it has greater consistency, making it more suitable for clinical use, that its formulation ensures better handling and safety, that the setting is faster with a lower risk of bacterial contamination (antimicrobial properties due to its very high pH = 12 (Nowicka et al., 2013), and its suitability as a dentine substitute (El Meligy et al., 2016). Furthermore, it is thought to provide a denser, less porous structure (Fouad & Youssef, 2012), with greater viscosity and less potential for discoloration (Camilleri et al., 2013; Rajasekharan et al., 2017). Finally, because aluminates and other impurities have been eliminated, it has increased final mechanical strength (Bachoo et al., 2013; Rajasekharan et al., 2014; Yoldas et al., 2016).

Applications in Pediatric Dentistry

BD is considered an important alternative material for vital pulp treatments in terms of its clinical, radiographical and histologic performance (Fouad & Youssef, 2012) (Annex II). It has been generally used in primary molar pulpotomies due to its favorable properties; these include high biocompatibility and bioactivity, excellent sealing ability, short setting time and ease of handling (Dawood et al., 2015; Rajasekharan et al., 2017). Furthermore, BD has been shown to have good marginal adaptation and strength and may be used as a restorative material, particularly in PD, due to its clinical setting as it shortens the procedure time, eliminating the need to place a separate restoration (Camilleri et al., 2005; Chng et al., 2005; Chong et al., 2009; Chong, 2012; Meligy et al., 2016).

2.2.3.5. Potential problems

Like other calcium silicate-based materials, displayed higher arsenic content than the level specified by ISO 9917–1 (requiring low Lead and Arsenic contents <100 and <2 ppm, respectively) (Prismus et al., 2019). However, levels of lead were considered acceptable (Kusum et al., 2015). This is a matter of debate between authors since the values and testing techniques for the MTA materials vary widely without indicating a severe health hazard (Camilleri et al., 2012; De-Deus et al., 2009; Grech et al., 2013a; Matsunaga et al., 2010).

BD is a potential substitute for other tricalcium silicate-based cements (Primus et al., 2019), but a relatively new material and long-term RCT are still lacking to evaluate its clinical performance. Most of the available data refers to 1-year follow-up, is limited to young patients and a few types of vital pulp treatments (pulpotomies and direct pulp cap treatments) (Rajasekharan et al., 2017) (Annex II). BD was the first tri/dicalcium silicate product to contain zirconia as a radiopacifier; although, its radiopacity is lower than the abovementioned products and similar to dentin and it may compromise diagnosis and follow-up evaluation (Awawdeh et al., 2018).

2.3. Adhesion

2.3.1. General description: definition of adhesion / history and evolution

In 1955, adhesive Dentistry experienced a groundbreaking contribution from Michael Buonocore in the Journal of Dental Research when he published his visionary findings on the use of 85% phosphoric acid to change enamel surfaces, making them immediately more suitable for mechanical adhesion and improved retention (Meerbeek et al., 2020).

After sixty-five years etching enamel with phosphoric acid is still considered the gold standard for bonding resin-based materials to tooth structure. Occurring mainly on a micromechanical level, it remains the most clinically reliable enamel bonding technique, (interlocking of resin tags within the microsized porosities left by enamel chemical etching) which enables an effective seal of the restoration margins (Buonocore, 1955; Swift et al., 1995).

In 1952 Kramer and McLean had already reported the concept currently known as "hybrid layer" in the British Dental Journal observing a narrow zone bordering the cavity composed of dentine and with a strong affinity for hematoxylin/eosin in the groups where sevriton-adhesive was used, which contains phosphate monomer, later identified by the research group of Dr. Buonocore as glycerol phosphoric acid dimethacrylate (Perdigão, 2020).

These were some of the important milestones that were responsible for a paradigm shift in Dentistry, which is the foundation of adhesive system knowledge. Since that time, the key challenge is still to find a parallel effective behavior on two dental substrates - enamel and dentin -, whose nature is entirely different: enamel contains a small amount of protein and can be dried without causing any collapse of the roughened surface; dentin contains 45 vol% mineral, 33 vol% organic matrix, the remainder being water (Brudevold et al., 1956), which guarantees a long-lasting, stress resistant interface between tooth structure and restorative material (Nanci, 2013). On the enamel the bonding interface is more reliably achieved with predictable sealing results by the interlocking of resin tags within the microporosities in acid-etched enamel, although the bonding on the dentin is more challenging with regard to its humid characteristics and organic structure (Perdigão, 2020).

2.3.2. Mechanism

Adhesion can be defined as the capacity of one substance to adhere to another (Perdigão, 2020). In the field of Dentistry the primary mechanisms responsible for the interface between adhesives, cements and self-adhesive restoratives and tooth tissue involve surface wetting, microretention and chemical interaction (Vinagre & Ramos, 2016).

In order to achieve a durable bond between the organic substrate and the restorative material it is clinically imperative to make optimal use of these bonding mechanisms (Meerbeek et al., 2020). Proper surface wetting is imperative to achieve good interfacial contact between the adhesive material and the substrate. It is a primary requirement for a liquid to spread uniformly through a solid surface that its surface tension be less than the free surface energy of the substrate.The contact between the substrate and the adhesive depends on the superficial wettability of the substrate by the adhesive, which is characterized by the contact angle measurements that ideally approach zero when a drop of adhesive disperses on the substrate surface; the smaller the angle, the greater the adhesive wettability (Meerbeek et al., 2020).

In terms of the above, substrates with very hard crystal structures, with strong intermolecular strengths display high surface energy and this is the case with enamel (Meerbeek et al., 2020; Vinagre & Ramos, 2016). Conversely, substrates with a more organic phase in their structure, as in dentin, have less surface energy. (Meerbeek et al., 2020; Vinagre & Ramos, 2016). An ultramorphological study of human dentine exposed to adhesive systems and concluded that adhesive wettability is potentially easier on enamel than it is on dentin (Meerbeek et al., 2020).

Microretention or micromechanical interlocking is considered to be the primary mechanism of bonding to mineralized tissues, such as enamel and dentin. It can be achieved, either by micromechanical roughening, or by chemical (self-) etching. Cavity preparation by means of a bur plays two different and important roles that contribute to better adhesion (Vinagre & Ramos, 2016)

Generally, and prior to adhesive procedures, it is important to roughen and remove surface contamination of the cavity. In particular, substrates that are marginally receptive to bonding, i.e. aprismatic and fluorotic enamel and glassy sclerotic dentin, are supposed to be coarsened, or even partially or totally removed (Meerbeek et al., 2020).

When considering enamel and dentin separately, there is a consensus in the literature that enamel requires phosphoric-acid etching and sufficient microretention to achieve a durable bond (Ermis et al., 2007; Pashley & Tay, 2001; Tay & Pashley, 2004; Meerbeek et al., 1994). These become micromechanically

interlocked as a result of removing any smear-layer barriers creating deep pits in which resin tags are filled by capillary flow (Pashley et al., 2011).

Today phosphoric-acid etching dentin has become less preferred since it leads to the exposure of a microporous collagen-fibril network that is hardly ever fully hybridized through resin interdiffusion. Consequently, a thick mineral-free and collagen-rich etch and rinse (ER) hybrid layer is produced, which is not as tight and resistant to hydrolytic degradation and enzymatic biodegradation (Meerbeek et al., 2020).

Alternatively, the mild self-etching (mild-SE) approach uses acidic functional monomers that provide microretention to dentin by mild (self-) etching and thus partial demineralization of the surface layer. There is also a primary chemical (ionic) interaction (the closest contact possible between atoms and molecules) of the functional monomers with hidroxyapatite. Although it does not convert into higher bond strengths, it will prevent bond strength reduction in aging (Meerbeek et al., 2020).

Like other functional components present in mild-SE, the monomer 10-MDP is by far the most extensively investigated due to its chemical bonding potential, which ionically interacts with hydroxyapatite Ca^{2+} through their phosphate group (Inoue et al., 2005). 10-MDP also etches to this chemical bond and thus releases substantial Ca^{2+} ions from the Hydroxyapatite-based substrate, causing 10-MDP to self-assemble into about 4-nm nanolayers, a process driven by stable 10-MDP-Ca salt formation. This stable structure is expected to contribute to durable nanolayering of 10-MDP-Ca salts in the hybrid and adhesive layer and consequently improve clinical longevity of the adhesively bonded restoration (Fukuda et al., 2014; Yoshihara et al., 2019; Yoshihara et al., 2010).

2.3.3. Classification of dental adhesive systems

Since the first report of acrylic resin bonding to tooth structure by the Swiss Chemist Hagger in 1951 dental adhesive technology research continues to progress at a rapid pace. Based on the retrospective chronological introduction in the market and temporal-based criteria the adhesive systems are classified into "generations". As a result, the categories aren't distinguishable in terms of scientific-based criteria and, therefore, there is a lack of knowledge concerning its clinical performance, which may imply that the last generations are more advanced with regard to its clinical behavior (Meerbeek et al., 2020).

First-generation adhesives contained a functional monomer - glycero-phosphate dimethacrylate (GPDM) - which has ionic bonding potential in relation to hydroxyapatite. However, and according to recent reports in the literature, it is incapable of creating a stable chemical bond (Sezinando, 2014; Vinagre & Ramos, 2016).

One of the first examples of a type of dentin bonding agent was commercialized as Cervident (SS White® Dental) in the 1960s (Yoshihara et al., 2018); these initial adhesives showed a propensity for being unstable with very low 2-3 MPa bond strengths to dentin (Bowen, 1965).

Second-generation adhesives included functional monomers designed to chemically interact with both inorganic (hydroxyapatite) or organic (collagen) dentinal components, corresponding to calcium- and collagen- bonding types (Meerbeek et al., 2020). These were marketed as dentin bonding agents' and were actually bonded to the smear layer, although the smear layer was loosely attached to the underlying dentin (Asmussen & Bowen, 1987). As a result, the bond strength was also poor, less than 5-6 MPa, with suboptimal clinical outcomes (Meerbeek et al., 2020).

From the chemical interaction concept with second-generation adhesives the research community understood that the principal mechanism of adhesion is sustained by micromechanical interlocking with tooth surfaces. The concept of a hybrid layer as being the structure created at the surface of dentin by previous (partial/full) demineralization followed by infiltration and polymerization of monomers, as introduced by Nakabayashi in 1982, was an important milestone, as described above (Heymann et al., 1991; Tyas et al., 1989).

A third generation of adhesives was developed in the 1980s and attempted to deal with the smear layer by either modification or removal (by etching dentin) of the layer to permit resin penetration into the underlying dentin (Nakabayashi, Kojima, & Masuhara, 1982). Prominent examples of this generation were Scotchbond™ 2 (3M™ ESPE), Gluma® (Bayer), Prisma Universal Bond 2 and 3 (Dentsply Sirona) (Edward J. Swift, 2002).

The fourth-generation adhesives and the subsequent current era of resin-dentin bonding were sustained by the total-etch concept, which was introduced based on the work of the Fusayama research group (Edward J. Swift, 2002). The technique involves phosphoric acid-etching on enamel, as well as dentin (Fusayama et al., 1979; Iwaku et al., 1981; Edward J. Swift, 2002), aiming to completely remove the smear-layer by demineralizing the underlying dentin, followed by water rinsing and exposure of the microporous network of hydroxyapatite poor collagen fibrils. A multi-step system was put forward and in fact was a typical three-step application procedure with a conditioner and primer, prior to the application of the actual adhesive resin (Meerbeek et al., 2020).

Primer serves as an adhesion promoter, which contains hydrophilic monomers dissolved in different ethanol, acetone and/or water solvent combinations, acting as carriers, such as the mono-functional monomer HEMA in particular. Today manufacturers try to substantially reduce, or even to replace, HEMA, with alternative monomers, like methacrylamide monomer variants because of some major disadvantages such as low ability to polymerize and thus contribute to mechanical strength, high water sorption, unfavorable biocompatibility and documented allergic potential (Bertolotti, 1991; Fusayama, 1992).

The main purpose of these total-etch primers is to make the moist collagen fibril network more receptive following application of monomers, which are relatively hydrophilic, present in the actual adhesive resin (Van Landuyt et al., 2007).

Indeed, the completion of the third step with the infiltration of the resin into the open dentin tubules results in the formation of abundant resin tags which, along with intertubular hybridization, constitute the primarily micromechanical interlocking bonding mechanism of total-etch adhesives (Bertolotti, 1991).

The total-etch technique was considered quite controversial at first because of the allegedly harmful effect of the phosphoric-acid etchants on the underlying pulp, even with a dentin barrier in between (Meerbeek et al., 2020), until the discovery that etching dentin with 30-40% phosphoric acid etchants was no longer regarded as harmful to the pulp. Three-step adhesives are regarded as the first adhesive class for arriving at a favorable clinical outcome (Retief, Austin, & Fatti, 1974).

Currently, all adhesives are applied simultaneously to enamel and dentin and more recent classifications favor the term 'etch&rinse' (ER) instead of total-etch. This highlights the clinical importance of the rinse step, in particular the risk of collagen-fibril collapse due to post-etching drying, instead of keeping the dentin visibly moist using the wet-bonding technique (Meerbeek et al., 2003).

Thus, the fourth-generation adhesives are at present referred to as three-step ER adhesives, as they involve the successive application of a conditioner, primer and adhesive resin in three application steps (Meerbeek et al., 2003).

The major shortcomings of 2-step ER adhesives (the fifth-generation adhesives) compared to 3-step ER is that they are more user friendly because they combine the primer and bonding agent in a "one-bottle" adhesive (Meerbeek et al., 2003). This incorporates the lower resin content along with the higher solvent content (Van Landuyt et al., 2007), with a reduced thickness in the adhesive film (Munck et al., 2012, 2005; Peumans et al., 2014; Peumans et al., 2005; Meerbeek et al., 2010; Meerbeek et al., 1994). However, lower mechanical strength has also been reported (Meerbeek et al., 1993), as well as higher hydrophilicity, permeability and water sorption (Ikeda et al., 2005b, 2005a), lower laboratory bond-strength (Hashimoto et al., 2004; Malacarne et al., 2006; Margunato et al., 2015; Reis et al., 2007; Tay et al., 2004; Tay & Pashley, 2003) and inferior clinical behavior (De Munck et al., 2012; Meerbeek et al., 2003). It is possible to improve the bonding performance through the application of multiple layers of one-step adhesives, with light-curing done separately or an extra bonding layer, but mainly by transforming the simplified one-bottle adhesive process back into a multi-step process (Peumans et al., 2014, 2005; Meerbeek et al., 2010).

Sixth-generation adhesives are also designated as 2-step self-etch adhesives and contain an acidic self-etch primer that results from the combination of acid etchant with the primer and adhesive resin. Since these don't require the rinse phase, they are sometimes also referred to as 'etch&dry' adhesives (Lorenzo Breschi, personal communication) (Meerbeek et al., 2003).

Seventh-generation adhesives are the true 1-step self-etch adhesives or "all-in-one" adhesives and combine etching, priming and bonding functions in one single application step without the water rinse phase (Meerbeek et al., 2003). Consequently, a thick 3 to 4 μm hybrid layer with full collagen exposure is produced at the dentin and the dissolved calcium phosphates are not removed (rinsed off) but embedded within the hybrid layer, in contrast to what happens with E&R adhesives (Van Landuyt, et al., 2006).

Both 1- and 2-step SE adhesives can be divided into three types according to their acidity and how aggressively they self-etch by taking into account their pH, measured as 'strong' (pH<1), 'intermediate strong' (pH=1-2), 'mild' (pH≈2) and 'ultra-mild' (pH>2.5) (Meerbeek et al., 2020).

In the literature mild SE and particularly ultra-mild SE adhesives seem to be considered to be the most reliable approaches for durable bonding to dentin (Meerbeek et al., 2020) by combining micromechanical interlocking with chemical bonding. An important disadvantage is still present and is related to unfavorable bonding performance to enamel due to a combination of factors, i.e., the lower etching effect of the functional acidic monomers present in (ultra)mild self-etch adhesives and lower chemical reactivity of functional monomers with enamel hydroxyapatite crystals. Nevertheless, these drawbacks can be clinically compensated for by means of a clinically popular combined ER/SE bonding routine. This involves selectively pre-etching enamel with phosphoric acid, followed by application of the SE adhesive onto the pre-etched enamel and unetched dentin (Meerbeek et al., 2011; Meerbeek & Yoshihar, 2014).

The latest generation of 1-step adhesives have achieved enhanced clinical and laboratory results approaching the superior performance of multi-step adhesives (Meerbeek et al., 2020).

Groundbreaking research is being undertaken on eighth-generation adhesives or universal adhesives, and can be applied according to the dentist's preference: full ER or SE bonding modes or a combined mode involving selective enamel ER with a 1-SE bonding mode (Meerbeek et al., 2011).

2.3.4. Applications in Pediatric Dentistry

Much of the research and development of dental adhesives has evolved towards more user-friendly clinical procedures by reducing the number of bottles and/or steps. This is particularly relevant in PD where, due to the particularities of patient behavior management and sensitivity, less complicated, time efficient and more versatile adhesive materials are needed (Meerbeek et al., 2020).

2.4. Restorative treatments

2.4.1. Generalities

Conventional restorative materials, such as amalgam, have long been associated with several limitations, including more destructive cavity preparation, anesthetic for visible restorations and environmental pollution considerations. With the development of resin-based restorative materials, since the discovery in the early 1960s of Bowen's Bis-GMA (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane) with inorganic particle formulations (Primus et al., 2019), some of these disadvantages have generally been addressed. Today, modern dental composite restorative materials are mostly considered the first choice in anterior and posterior teeth, due to their enhanced physical properties and clinical handling combined with the development of adhesive systems (Bowen, 1965; Chen, 2010). At the same time, new concerns arose, such as the release of pulp-damaging toxic monomers, polymerisation shrinkage and microleakage (Bachoo et al., 2013).

2.4.2. Constituents

Dental composites are made up of synthetic polymers, inorganic fillers, initiators, activators (that promote light-activated polymerization of the organic matrix) and silane coupling agents, which bond the reinforcing fillers to the polymer matrix (Vinagre, 2014). The three main components (inorganic fillers, the organic resin matrix and silane coupling agents) can be modified in order to improve the development of the composites (Chen, 2010).

Matrix

In most dental composites the organic matrix is made up of Bis-GMA monomer, which is a bulky monomer with methacrylate groups at both ends (dimethacrylate). The double-bonded carbons of the methacrylate groups, at each end of the active site on the monomer cross-link during the polymerization process, produces an initial linear polymer followed by a reaction with the second site, and a highly cross-linked polymer. The hydrogen bonding interactions that occur between the hydroxyl groups and the monomer molecules result in an extremely high Bis-GMA viscosity. To overcome this, it should be diluted with more fluid diacrylate monomers (e.g. Thyleneglycol dimethacrylate - EGDMA and triethyleneglycol dimethacrylate - TEGDMA) to acquire the correct viscosity for dental use (Chen, 2010). Urethane dimethacrylate (UDMA) was approved for dental use in the 1970s and is used on its own or in combination with other diacrylate monomers (Ferracane, 1995). Besides Bis-GMA and urethane dimethacrylate, none of the other base monomers have been shown to be clinically superior with regard to shrinkage, aging, and the negative effects of environmental factors such as moisture, acidity, and temperature changes (Yap, A U Low & Ong, 2000).

Fillers

Inorganic filler particle content in the organic matrix is either crystalline silica (quartz) amorphous silica (colloidal or fumed silica) or silica with metals (silica glass containing barium, strontium, and zirconium). Fillers range in size, with a distribution from less than 0.1 μm to averages between 10-100 μm (Burgess et al., 2002). These fillers increase strength and modulus of elasticity and reduce polymerization shrinkage, the coefficient of thermal expansion and water absorption (Peutzfeldt, 1997).

Modern composite systems contain fillers such as quartz, colloidal silica and silica glass containing barium, strontium, and zirconium. An increase in the percentage volume of filler (filler loading) improves the physical and mechanical properties of the resin composites (Chen, 2010) and increases strength and modulus of elasticity, providing resistance to wear, improving fracture toughness, reducing polymerization shrinkage, thermal expansion coefficient and water absorption (Burgess et al., 2002).

Of some interest is the fact that most changes in composite resin technology are related to filler particle size and distribution rather than the resin matrix, which is still based on Bis-GMA, also known as Bowen's resin (Chen, 2010; Miletic, 2018).

2.4.3. Classification

The size of filler particles incorporated in the resin matrix and volumetric percentage are important parameters influencing the physical-mechanical properties mentioned above, enhancing the esthetic and handling characteristics of commercial dental composites (Perdigão, 2020). In general, and over the years, the size has systematically decreased from traditional to nano-composite materials and the percentage volume has increased (Vinagre, 2014).

The resin composites can be described in terms of numerous intrinsic characteristics relating to the fillers, including filler size distribution, geometry and composition. Even though, they have usually been classified according to the size of their filler particles (Ferracane, 1995; Ilie & Hickel, 2009). Macrofilled particles range from 10-50 μm (one size regime), also called traditional or conventional; midifilled particles range from 1.0-10 μm, also called midifil, fine or small particle, provide higher strength than a microfilled and better polishability than a macrofill; minifilled particles range from 0.1-1.0 μm, still have relatively high strength and better polishability than midifilled; microfilled particles range from 0.01-0,1 μm (homogeneous or heterogeneous) (Miletic, 2018). Unfortunately, this particle size increases the surface area of the fillers and only a relatively small amount of filler can be suspended in the monomer, limiting the amount of filler loading (25%-50% by volume) (Burgess et al., 2002; Miletic, 2018).

Most materials produced thereafter became "hybrids," incorporating both, so-called nano- and micron-sized particles (particles with 0.6 - 5 μm are combined with particles with 0.04 μm) to improve the handling properties (flow) and control stickiness (Burgess et al., 2002). In particular, in the minifilled hybrid composites the size ranges between 0.6 a 0.7 μm with microfilled particules and volumetric percentage is 70% (Burgess et al., 2002) (50%-70% by volume) (Burgess et al., 2002). Today, minifilled hybrids became the most popular attending the overall applicability, with a variety of shades, translucencies and opacities, superior strength, but lower polishability, compared with microfilled (Vinagre, 2014).

Besides filler characteristics, additional properties that are intrinsic to the materials should be considered to guide a composite selection: these include the filler volume content, the thixotropy and the matting of the surface when handling (Burgess et al., 2002).

Silane coupling agents

To obtain good physical-mechanical properties in dental composites, a strong covalent bond between inorganic fillers and the organic matrix is necessary. This is reached by coating the fillers with a silane coupling agent that has functional groups to link chemically both the filler and the matrix; an usual example is 3-methacryloxypropyltrimethoxysilane (MPTS) (Miletic, 2018).

In summary, an appropriate composite resin selection requires a balance between mechanical properties (high strength, fracture toughness, surface hardness, optimized modulus of elasticity, low wear, low water sorption and solubility, low polymerization shrinkage, low fatigue and degradation, high radiopacity) biological properties (good biocompatibility - systemic and local-, no postoperative pain or hypersensitivity, preservation of tooth integrity, as well as caries-inhibiting ability) and esthetic considerations (good color matching and color stability - translucency, shades), optimum polishability, long-term surface gloss, absence of marginal or surface staining) (Chen, 2010).

2.4.4. Applications in Pediatric Dentistry

Modern Dentistry shifted to a more conservative approach and increased attention to composite resin and GIC due to adhesion and greater preservation of remaining tooth structure, limiting the cavity preparation mostly to decayed tissue removal (Ilie & Hickel, 2011). Despite favorable esthetic outcome and mechanical properties of composite resin, the restorative technique is more sensitive, time-consuming procedure and bigger risk of moisture contamination. So that, in PD, when patient compliance is limited because of the age or behavior, the GIC may be an alternative since they are adhesive materials, with a faster insertion technique – one increment (Dias et al., 2018) and fluoride release to the oral environment (Casagrande et al., 2013).

The self-adhesive restorative composites released several years ago were considered a major simplification procedure without leading to a wide use because of its inferior clinical and laboratory performance. However, a new era of self-adhesive restorative materials seems to be really breakthrough, with new restorative materials developed and marketed by the companies that may guarantee a further procedure simplification with reliable, predictable and durable bonding results (Almuhaiza, 2016).

3. Dissertation topic choice

One of the most common reasons for dental emergencies in Pediatric Dentistry (PD) is the pain caused by irreversible pulpitis (Shqair et al., 2012). Particularly in the permanent dentition, root canal treatment has traditionally been recommended as a treatment option (American Association of Endodontist, 2013). However, the evidence has proven that in patients between 6 to18-year-old, root canal treatment was performed in only 20% of teeth with signs and symptoms indicative of irreversible pulpitis, while 24% and 59% of teeth were extracted or received temporary restorative treatments, respectively (Al-Madi et al. 2018). The difficulties inherent to the treatment – time-consuming and costs; the characteristics of young permanent dentition – thin dentine walls and open apices; the particularities of the child – lack of cooperation for long-time procedures and also related with pain experiences, may justify the low number of root canal treatments effectively performed. Only 36% of these endodontic treatments in children aged 8 to16 years were associated with complete healing after long-term follow-up (Peretz et al 1997). In PD, the VPT has been a controversial topic (Mahmoud Torabinejad, 2014) and it is a treatment option recommended in teeth with high proximity to the pulp, or with variable pulp exposure or even with signs and symptoms indicative of irreversible pulpitis. In this last scenario, the VPT had a comparable rate success to endodontic treatment, as it was reported in a 5-year randomized clinical trial performed in vital mature permanent molars clinically diagnosed with irreversible pulpitis (Asgary et al., 2015).

Nowadays, the most important challenge in Dentistry is to design new strategies or agents, in order to preserve the tooth structure and function, based on well-recognized clinical requirements and understanding of the related biological events. Particularly, the comprehension of the molecular mechanisms present in the pulp–dentin complex highlighted the similarities between developmental and regenerative tissue engineering (Tziafas, 2010). The development of new biomaterials should fulfill major requirements related with nature system, half-life of the molecules, dose-response effects, side-effects of the treatment and also outermost chemical surface, as several reactions occur at the interface between cells and the biomaterial which determine the host responses (Tziafas, 2010).

Contemporary Dentistry aims at performing minimally invasive treatments, such as VPT, in order to preserve pulp vitality, but the success of such treatments relies on a perfect adhesion between the tooth structure and the biomaterials used in the restorative procedure. The bond strength between the biomaterial used as a pulp capping and the restorative material, usually a resin-based composite, is crucial as a perfect adhesion between both materials will allow the distribution of the masticatory stress over the entire adhesion surface and will prevent the microleakage, which is essential for the long-term success of the restoration (Altunsoy et al., 2015a; Palma et al., 2020).

Despite the development of new adhesive strategies with improving sealing and bonding capabilities, the bond interface between tooth structure and biomaterials remains the weakest zone of a dental restoration. Because, the optimum technique for bonding composite to a base material is an important issue with high clinical impact, many studies investigating specific bonding techniques and material combinations have been published to date (Al-Ashou et al., 2014; Altunsoy et al., 2015a; Bayrak et al., 2009; Tunç et al., 2008). However, the wide variety of base materials, priming and bonding procedures, composite types and different experimental conditions do not allow universal conclusions (Anastasiadis et al., 2018). Therefore it is mandatory to develop a specific protocol, considering the bonding strategy and the appropriate time of restoration for each VPT, attending the characteristics of the HCSC selected, the proximity of the pulp in order to guarantee the pulp healing.

Chapter II. Experimental procedures

1. Aims

Besides the biocompatibility, bioactivity and remineralization abilities of calcium silicate-based cements, the bond strength between them and the restorative can be an important clinical factor affecting the longevity and predictability of the final restoration (Aksoy & Ünal, 2017; Hashem et al., 2014; Tunç et al., 2008). The characteristics of the adhesive interface obtained depend on the technique used, the type of materials (adhesive systems and calcium silicate cements) and its hybridization pattern, namely de micromechanical and chemical interaction. Therefore, this experimental work intends to provide data of the HCSC.

Therefore, the primary objectives of the present research were to study the interfaces between HCSC and adhesive restorations concerning shear bond strengths and ultramorphological patterns, evaluating the effect of:

- two different hydraulic calcium silicate cements – NuSmile® NeoMTA and Biodentine™;
- two different bonding systems - two-step self-etch adhesive (Clearfil™ SE Bond 2) and an universal adhesive (Clearfil™ Universal Bond Quick);
- an additional hydrophobic resin bonding layer application (HBL);
- the timing of the final definitive restoration - immediate or delayed.

For this part of the study the tested null hypotheses were:

- $H_0$1 - There is no difference between the two HCSC evaluated: NuSmile® NeoMTA and Biodentine™.
- $H_0$2 - There is no difference between the two adhesive systems tested: Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick.
- $H_0$3 - There is no difference between groups with or without an additional hydrophobic resin bonding layer.
- $H_0$4 - There is no difference between groups with different times of completion of the final restoration: immediate or delayed (seven days).

2. Material and Methods

The methodology of this research included a quantitative and a qualitative analysis of the adhesive interfaces between HCSC and adhesive composite restorations, comprising shear bond strength tests and ultramorphological analysis by scanning electronic microscopy (SEM) and confocal laser scanning microscopy (CFLS).

2.1. Shear bond strength tests

For this study design the sample size was calculated using the software G*Power 3.1.9.2 (University of Düsseldorf) for SBS tests. Power calculation was conducted to determine the minimal number of teeth required for the SBS test, as the principal measure. The expected mean difference was 2.0 MPa and the standard deviation of difference was 4.5 MPa according to previous study findings with a similar design (Palma et al., 2020). The t-Student test bilateral for two categories of main effects with a ratio 1:1 was set at 95% with a margin of error of 5%. This yielded, at least, 133 samples for each group of main effects. In the present work it was decided to perform 160 samples for each main effect comparison, making a total of 320 specimens and SBS tests.

Specimen preparation

Metallic blocks (30 mm height x 15 mm diameter) were produced containing a central cavity measuring 4 mm diameter and 2 mm height. The retentive design was accomplished by using an insert cutting tool (CoroTurn® XS Profiling size 05, Sandvik Coromant, Sandviken, Sweden) to create a 360° groove at the bottom of the cavity. These tubes were previously and specifically designed and fabricated for another research work by the Laboratory of Applied Biomechanics, Coimbra Institute of Engineering – Polytechnic Institute of Coimbra (Department of Mechanical Engineering) (Palma et al., 2020) (Figure 2.1).

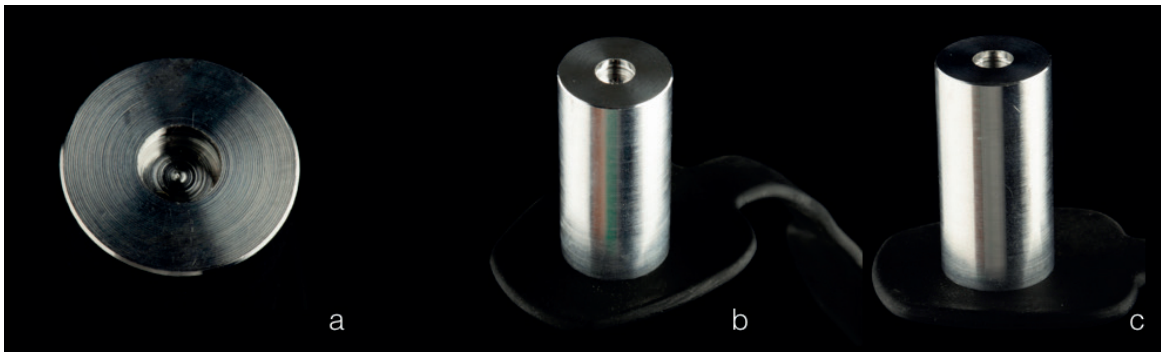


Figure 2.1. a) A detail of 360° groove at the bottom of the central cavity; b) and c) Aluminum blocks specifically fabricated within the scope of this kind of studies (photographs courtesy of Professor Paulo J. Palma).

Biomaterial preparation

Each HCSC was prepared and homogeneously mixed according to the manufacturer's instructions (Table 2.2). The central hole of the metallic block was filled with the HCSC using a spatula, digital compressed with a humid cotton pellet and allowed to set. Samples from the immediate restoration groups were left for 3 min for NuSmile® NeoMTA or 12 min for Biodentine™ prior to adhesive application, while samples from the delayed restoration groups were stored in an incubator (Thermo Scientific Heraeus® BK 6160) at 37 °C with 100% of humidity for seven days (Figure 2.2b), (Table 2.1).

The 16 experimental groups (n=20) were randomly selected for specimen preparation. Each group was prepared separately and according the type of HCSC, the type of adhesive, the application of the additional hydrophobic resin bonding layer and the timing of that adhesive restoration was performed (Table 2.1).

Table 2.1. Experimental groups, composition and details.

Groups	HCSC	Adhesive system	Additional HBL (Clearfil™ SE Bond 2 – Bond)	Time	Restorative material
1	Biodentine™	Clearfil™ SE Bond 2	no	12 min	SDR™ Bulk-fill flowable composite
2		Clearfil™ SE Bond 2	no	7 days	
3		Clearfil™ SE Bond 2	yes	12 min	
4		Clearfil™ SE Bond 2	yes	7 days	
5		Clearfil™ Universal Bond Quick	no	12 min	SDR™ Bulk-fill flowable composite
6		Clearfil™ Universal Bond Quick	no	7 days	
7		Clearfil™ Universal Bond Quick	yes	12 min	
8		Clearfil™ Universal Bond Quick	yes	7 days	
9	NuSmile® NeoMTA	Clearfil™ SE Bond 2	no	3 min	SDR™ Bulk-fill flowable composite
10		Clearfil™ SE Bond 2	no	7 days	
11		Clearfil™ SE Bond 2	yes	3 min	
12		Clearfil™ SE Bond 2	yes	7 days	
13		Clearfil™ Universal Bond Quick	no	3 min	SDR™ Bulk-fill flowable composite
14		Clearfil™ Universal Bond Quick	no	7 days	
15		Clearfil™ Universal Bond Quick	yes	3 min	
16		Clearfil™ Universal Bond Quick	yes	7 days	

Restorative procedures

A – Immediate restorative procedure

After the initial setting time (12 min for Biodentine™ and 3 min for NuSmile® NeoMTA), the adhesive systems were applied over the biomaterials surface basically according to the manufacturer's instructions (Table 2.2).

The Clearfil™ Universal Bond Quick was applied to the entire surface of the material with the applicator brush and left in place for 20 s. After that, the surface was dried by blowing mild. Following, in groups with application of an additional hydrophobic resin layer, the adhesive was light-cured for 10 s at "High Power" mode (Bluephase® Style M, Ivoclar, Vivadent, Schaan, Liechtenstein), followed by an extra layer of Clearfil™ SE Bond 2 – Bond application, drying with a mild airflow and no immediate light curing. For both adhesives, in groups without this additional hydrophobic resin layer, the adhesive light-curing was not immediately performed until the restorative procedure begins, as will be explained below.

After the adhesive procedures were finished, and before its final light-curing step was performed, a #9 gelatin cylindrical capsule (Torpac® Fairfield, NJ, USA) was placed over the adhesive surface. Just after this step, the final 10 s adhesive light-curing was done (Bluephase® Style M). After that, the gelatin capsule was incrementally filled (first increment of 1 mm) with a flowable composite resin – SDR™ Bulk-fill flowable composite (Dentsply DeTrey; Konstanz, Germany) and light-cured for a total time of 60 s with a dental curing unit (Bluephase® Style M, Ivoclar,Vivadent, Schaan, Liechtenstein, in the "High Power" mode) (Figure 2.3).

From the two-step self-etch bonding system Clearfil™ SE Bond 2, the primer was applied to the entire surface with an applicator brush, left in place for 20 s and dried with mild airflow. Then, the bond was applied and distributed evenly with mild airflow and left for 20 s. The following steps were carried out as described previously for Clearfil™ Universal Bond Quick, namely with regard to photo-polymerization, placement or not of additional hydrophobic resin layer and restorative procedures.

B – Delayed restorative procedure: 7 days

The HCSC specimens were covered by glass ionomer bulk filling material (Ionostar® Molar - VOCO GmbH, Cuxhaven, Germany). For that, Ionostar® Molar capsules were previously activated and loaded into a high-frequency mixer with approximately 4000 oscillations/min (Softly Satelec Amalgamator), for 15 s. After this covering by a glass ionomer provisional restoration all the samples were stored in an incubator (Thermo Scientific Heraeus® BK 6160) at 37°C with 100% humidity for 7 days (Figure 2.2a). After this storage period, the GIC was removed with black coarse aluminum oxide abrasive discs – Sof-Lex™ (3M ESPE, St Paul, USA) until a flat surface of the HCSC was exposed. After that, the biomaterial surface was polished using #360 and #600 water sandpaper in a circular motion, 60 s each (WS-FLEX 18-C, HERMES, Hamburg, Germany). Following this, the same adhesive and restorative procedures were applied as previously described for the groups of immediate restorations (Figure 2.3).

No acid etching was performed prior to bonding system application in any of the study groups. A single operator carried out all the adhesive and restorative procedures. During all specimen preparation, the registered room temperature was 23°C, with 40% humidity.

Figure 2.2. a) Thermo Scientific Heraeus® BK 6160. b) Universal test machine (Model AG-I, Shimadzu Corporation, Kyoto, Japan).

Table 2.2. Materials, manufacturers, composition, application, lot number and expiration date

Material (batch)	Manufacturer	Classification	Composition	Mode/ steps of application
Biodentine™ (B25180) 09-2021	Septodont, Saint-Maur-des-Fosses Cedex, France	HCSC	Powder tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, iron oxide, and zirconium oxide Liquid calcium chloride and hydrosoluble polymer	1. Put 5 drops of liquid into the capsule. 2. Place the capsule on a mixing device. 3. Mix for 30 s.
NuSmile® NeoMTA (2019100803) 28-08-2022	NuSmile Ltd. Houston, TX, USA	HCSC	Powder tricalcium silicate, dicalcium silicate, tantalite, calcium sulfate, tricalcium aluminte Gel Water-based liquid	1. Dispense 1 scoop of powder. 2. Dispense one drop of gel. 3. Incorporate the gel by spatulating the powder/gel mixture firmly until a putty-like consistency is obtained.
Clearfil™ SE Bond 2 Primer (9J01010) 31-01-2022 Bond (9C0191) 28-02-2023	Kuraray Noritake Dental Inc.; Sakazu, Kurashiki, Okayama, Japan	Two-step self-etch adhesive system	Primer MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, water Bond MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, initiators, accelerators, silanated colloidal silica	1. Apply primer, leave it for 20 s and dry with mild airflow. 2. Apply bond and distribute evenly with mild airflow. 3. Light cure for 10 s.
Clearfil™ Universal Bond Quick (000018) 30-09-2022	Kuraray Noritake Dental Inc.; Sakazu, Kurashiki, Okayama, Japan	One-step self-etch adhesive system	Bond MDP, Bis-GMA, HEMA, hydrophilic amide monomers, colloidal silica, silane coupling agent, sodium fluoride, dl-Camphorquinone, ethanol, water	1. Apply bond and dry the entire cavity wall by blowing mild until the bond does not move. 2. Light cure for 10 s.
SDR™ Bulk-fill flowable composite (0217) 02-2020	Dentsply DeTrey GmbH, Konstanz, Germany	Bulk fill flowable composite	Barium-alumino-fluoroborosilicate glass, strontium alumino-fluoro-silicate glass, modified urethane dimethacrylate resin, EBPADMA, TEGDMA, CQ, photoaccelerator, BHT, UV stabilizer, titanium dioxide, iron oxide pigments, fluorescing agent	1. Dispense SDR™ material . 2. Light-curing for at least 20 s.
Ionostar® Molar (1933039) 06-2021	VOCO GmbH, Cuxhaven, Germany	Glass ionomer bulk filling material	Fluoro-aluminosilicate glass, polyacrylic acid, tartaric acid	1. Mix the activated application capsule for 10 - 15 s. 2. Apply the material directly into the cavity and it can be worked for at least 1.5 min. 3. Finish the restoration with a diamond bur or a polisher and apply a protective varnish.
K-Etchant syringe (6K0113) 30-04-2023	Kuraray Noritake Dental Inc.; Sakazu, Kurashiki, Okayama, Japan	35% phosphoric acid	Fluoro-aluminosilicate glass, polyacrylic acid, tartaric acid	1. Apply to the entire cavity surface (enamel and dentin. 2. After 30-60 s wash thoroughly and dry with air syringe.
ProBase® Cold (VT0556) 09-2020	Ivoclar Vivadent AG Schaan / Lichtenstein	auto-polymerized acrylic resin	Powder: Polymethyl methacrylate, softening agent, benzoyl peroxide, catalyst, pigments Liquid: Methyl methacylate, dimethacrylate, catalyst	1. Add 15 g polymer (powder) / 10 ml monomer (liquid). 2. Mix polymer and monomer with the spatula. Subsequently, allow the mixture to rest for 15 seconds to permit any trapped air to rise. 3. Fill with auto-polymerized acrylic resin the circular aluminum mold.

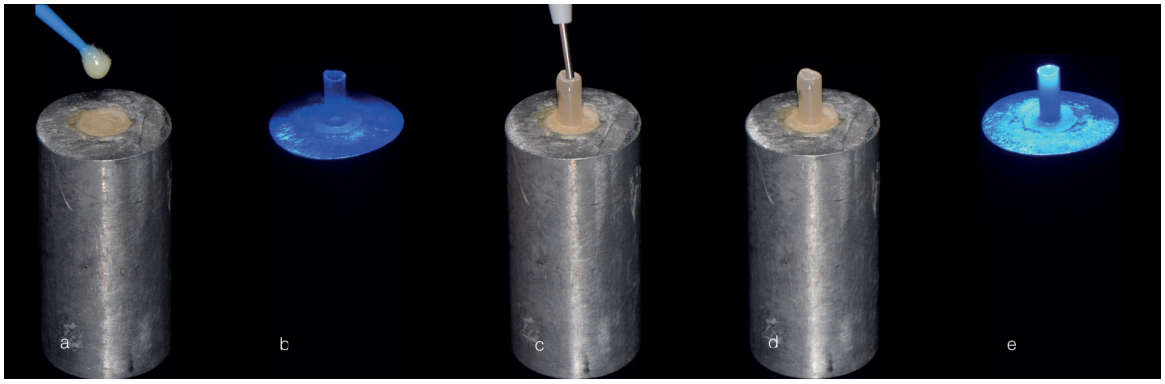


Figure 2.3. Adhesive and restorative protocol. a) Adhesive system placement; b) Light curing of the adhesive; c) and d) Flowable resin-based composite placement; e) Light curing of flowable resin-based composite.

Shear bond strength (SBS) tests

Before proceeding the SBS tests all samples were stored in an incubator (Thermo Scientific Heraeus® BK 6160) (Figure 2.2a) at 37°C 100% humidity, for 48 hours.

For SBS test, each block was fixed in an universal testing machine (Model AG-I, Shimadzu Corporation, Kyoto, Japan), in a shear mode (Figures 2.2b, 2.4) at a cross-head speed of 0.5 mm/min and 250 N, with a chisel-shaped rod, until failure occurred. The force registered, measured in Newton (N) was divided by the cross-sectional area of the bonded interface and expressed in MegaPascal (MPa).

To avoid bias, a single and blinded operator carried out bond strength measurement procedures.

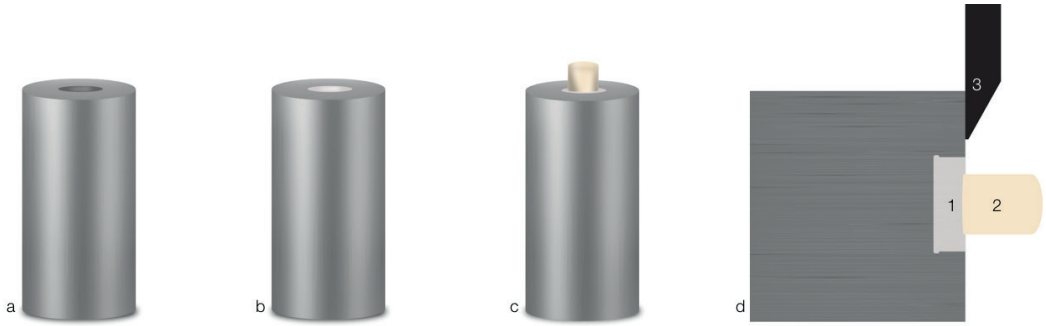


Figure 2.4. Schematic diagram of the experiment set-up showing how the samples were prepared for SBS strength testing. a) Cylindrical metallic blocks; b) The hole in the middle was filled with the HCSC; c) After adhesive procedures a soluble gelatin capsule was applied on the surface of the HCSC and filled with the flowable composite resin; d) A chisel-edge plunger was mounted into the testing machine and positioned, so that the leading edge was aimed at the HCSC / adhesive interface. The metallic tube with the groove detail: 1) the central hole filled with HCSC; 2) the composite resin; 3) loading jig of universal testing machine (SBS strength) [Adapted from (Altunsoy, Tanriver, et al., 2015a) and from (Palma et al., 2020).]

Fracture pattern analysis

Following the SBS test the fractured surfaces of each sample were examined under a stereomicroscope (Opmi Pico, Carl Zeiss Surgical, Oberkochen, Germany) equipped with a halogen light source and a global magnification of 21.3x.

The specimens were classified into 4 groups according to the failure modes (Atabek, Sillelioglu, & Ölmez, 2012; Meraji & Camilleri, 2017; Odabas, Bani, & Tirali, 2013): (1) adhesive fracture, (2) cohesive fracture exclusively in the silicate, (3) cohesive fracture exclusively within the restorative material, or (4) mixed fracture (comprises both adhesive and cohesive fracture).

A total of 32 specimens (10% of the sample) were randomly selected and reanalyzed the bond failure classification to determine the intra-examiner reproducibility. The same examiner repeated scoring 1 month after the initial one. Weighted kappa coefficients were calculated to determine agreement between observations.

Statistical analysis

The SBS test results were described using mean, median, standard deviation, interquartile range and minimum and maximum values. The normality of data distribution testing was carried out using the Shapiro–Wilk test.

A four-way ANOVA was conducted to compare the main effects (type of HCSC, type of adhesive system, presence or absence of additional hydrophobic resin layer - HBL and timing of restoration procedure). The interaction between different combinations of effects was evaluated with a dispersion graph and a descriptive table for each group generated by the conditions analyzed.

The association between the fracture type and the HCSC, adhesive system, presence of HBL and restoration time was assessed using Fisher's exact test.

The Bonferroni correction was used to adjust for multiple comparisons. Two-tailed p values were calculated with a significance level set at $\alpha = 0.05$.

Statistical analysis was performed using IBM SPSS® version 26 software (Chicago, IL, USA). The significance level was set at $\alpha = 0.05$.

2.2. Qualitative analysis of the bond interface

2.2.1. Bond interface evaluation by scanning electron microscopy

Specimen preparation

From a pooled biobank of extracted teeth, 32 primary molars with at least one third of the root and without furcation involvement were randomly selected. Before the extraction, the patients and their parents were informed about the use of their teeth for research or educational purposes and their informed consent was obtained. Because the samples used in this part of the research study were collected from a pooled biobank, they are categorized as "irreversibly anonymised" (approval was obtained by the Commission for Medical Ethics of Faculty of Medicine of the University of Coimbra Of. Refª 002-CE-2020-020) (Annex I).

The extracted teeth were stored in an aqueous chloramine solution 0.5%, at 4° C for up to 6 months, following the norm ISO/TS 11405:2015, and renewed every month before being used.

Occlusal cavities were made in each tooth using cylindrical and round diamond burs (Edenta AG, Switzerland, ISO-Nº 806 314 001 544 014) under a water-cooled high-speed handpiece. The remaining pulp tissue in the pulp chamber was removed with a large spoon excavator. The access cavities were then rinsed with sterile 0.9% saline solution and air-dried.

These teeth were mounted in auto-polymerized acrylic resin blocks, color clear (ProBase®, Ivoclar Vivadent, Schaan, Liechtenstein) using a circular aluminum mold in dimension such that the CEJ was flush with the resin surface (Table 2.2).

The teeth were randomly allocated into 16 groups (n=2), according to the same variables described for SBS tests.

HCSC placement

The HCSC were placed into the pulp chamber cavity allowed to set, adhesively treated, restored and stored as described previously for the same 16 groups evaluated by SBS tests.

In the delayed restoration groups, the rest of the occlusal cavity was provisional filled with a GIC color A2 (IonoStar® Plus, VOCO GmbH, Cuxhave Germany) and the teeth were stored in an incubator (Thermo Scientific Heraeus® BK 6160) at 37°C with 100% of humidity, for 7 days. A periodontal probe was used to measure the depth of the opening assuring that it could accommodate at least 3-4 mm of the temporary filling material (American Association of Endodontists (AAE, 2018).

Restorative procedure

When completed the 7-days storage period the GIC was then removed down to the level of the HCSC surface using a high-speed air turbine under water coolant with a round bur (Edenta AG, Switzerland, ISO-NO 806 314 001 534 014); the final cavity conformation was finished using a diamond round end taper bur (Edenta AG, Switzerland, ISO-NO 806 314 196 514 025). Then, the restorative procedure was performed as described previously (Figure 2.5).

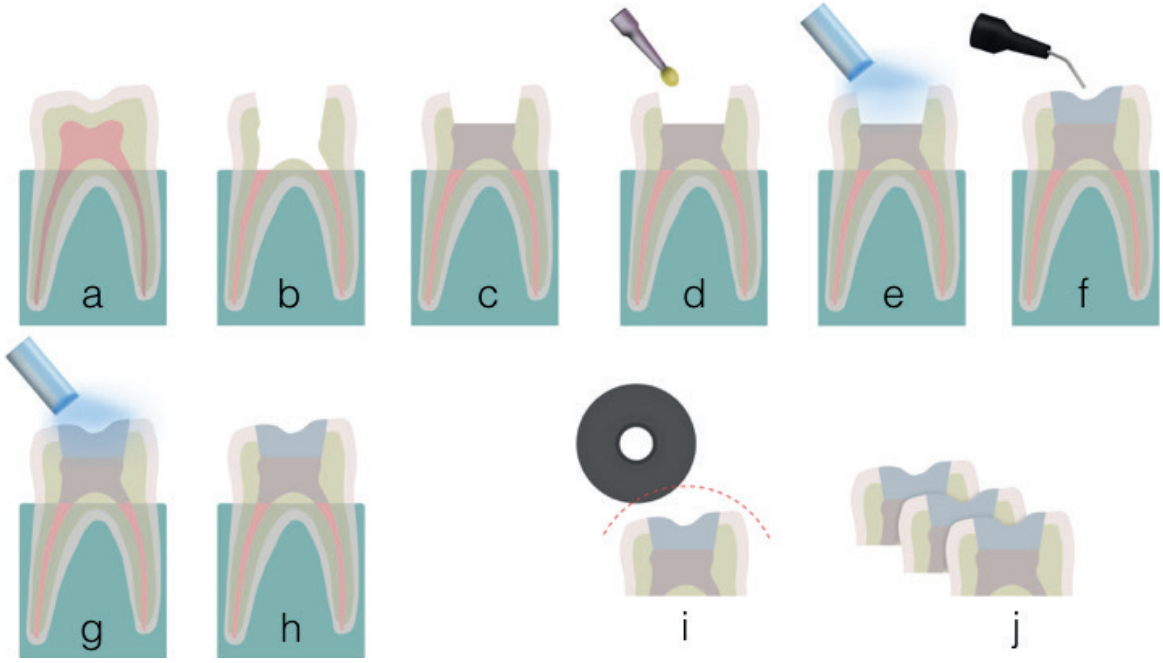


Figure 2.5. Schematic illustrating the tooth preparation, obturation, restorative procedures and subsequent sectioning for SEM evaluation [Adapted from (Pires, Lenzi, Soares, & Rocha, 2019)]. a,b) The cavity access was prepared and pulp removed; c) The pulp chamber was filled with HCSC; d-h) The bonding system was applied and restorative procedures were completed; i,j) The teeth slices were prepared using a water-cooled diamond disk.

After storage at 37 °C and 100% humidity for one week the restored teeth were multi-sectioned in a buccolingual direction along their longitudinal axis using a high precision diamond cut-off wheels from a high precision machine (Accutom 50 machine, Struers, Denmark) (Figure 2.6), under water refrigeration and 3000 rpm, with a feed of 0.05 mm/s, obtaining three slices by restoration with approximately 1000 µm thickness (Figure 2.7).

Figure 2.6. High precision cut-off machine (Accutom 50 machine, Struers, Denmark).

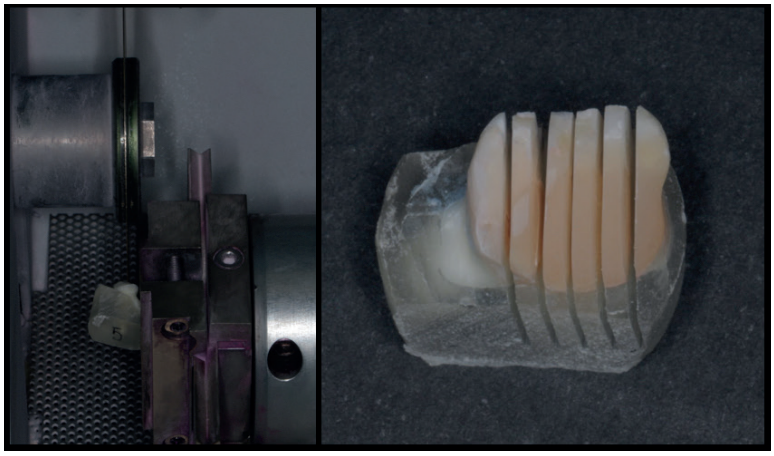


Figure 2.7. Each tooth was multisectioned in a buccolingual direction along their longitudinal axis followed the section on the JAC to achieve three cuts by restoration.

The specimens were soaked by 35% phosphoric acid gel on both sides for 15 s, followed by washing and drying. Then, they were sequentially dehydrated in increasing concentrations of ethanol (50% – 75% – 95% – 100%). Finally, the specimens were mounted on aluminum stubs, sputter-coated with gold-palladium and observed by field-emission scanning electron microscopy (FE-SEM) (Hitachi S-4100, Japan) at various magnifications.

2.2.2. Bond interface evaluation by confocal laser scanning microscopy

For this analysis 32 artificial maxillary first molars (DRSK RCT™, Hassleholm, SWEDEN) (Fig. 2.8) were divided into 16 groups (n=2) according to the same variables described for SBS tests and SEM evaluation. These model teeth are transparent and made from material with mechanical properties comparable to a natural dentin in its hardness e-modulus (DRSK Group AB, 2019).

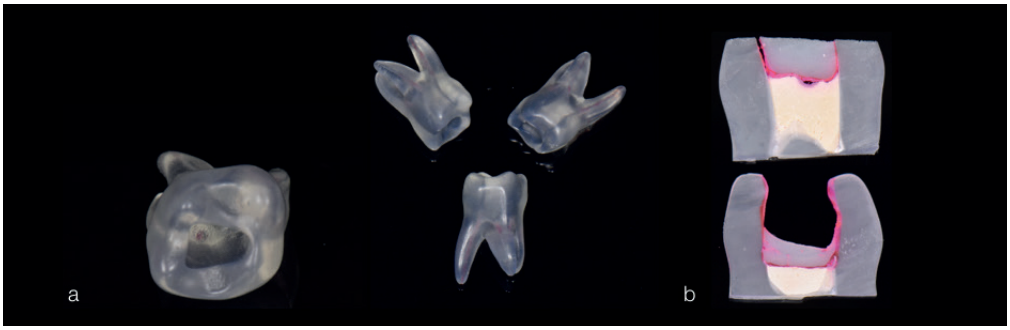


Figure 2.8. a) First artifical molars DRSK RCT™ (Hassleholm, SWEDEN). b) Teeth slices.

The experimental groups were the same as abovementioned in Table 2.1 and the materials used are listed in Table 2.2.

Excepting for the kind of the teeth, cavity preparation and adhesive dye-labeling, all of the HCSC preparation, adhesive and restorative procedures, timings and storage were performed as previously described for the 16 groups.

The HCSC materials were prepared according to the manufacturer's instructions (Table 2.2), as previously described, but placed inside a standardized access cavity, already present in the teeth, involving the pulp chamber. The amount of each HCSC placed was standardized by the use of an amalgam carrier.

Adhesive preparation and application

Prior to adhesive application, Rhodamine B (Sigma, St. Louis, MO, USA) was weighed on an analytical balance (AE 200 Mettle Toledo, US) (0.32 mg) wrapped in aluminum foil and the adhesive component was then transferred to the corresponding eppendorf tube. Following this, each eppendorf tube was carefully adapted to a dental mixer and vigorously mixed for 40 s, in order to homogenously dissolve the Rhodamine in the resin. After mixing, no Rhodamine clusters should be detected in the labeled adhesives with the naked-eye.

After this adhesive dye-labeling, the adhesive and restorative procedures were performed as previously described for the 16 groups (Figure 2.9).

A single operator carried out all the procedures at room temperature.

All teeth were stored under the same conditions (humidity and temperature) in the incubator for at least one week. Subsequently, samples were multi-sectioned in parallel slices in a buccolingual direction along their longitudinal axis using a high precision diamond cut-off wheels from a high precision machine (Accutom 50 machine, Struers, Denmark) (Figure 2.6), under water refrigeration, at 3000 rpm and feed of 0.05 mm/s. Four sections of approximately 900 μm thickness were obtained per tooth.

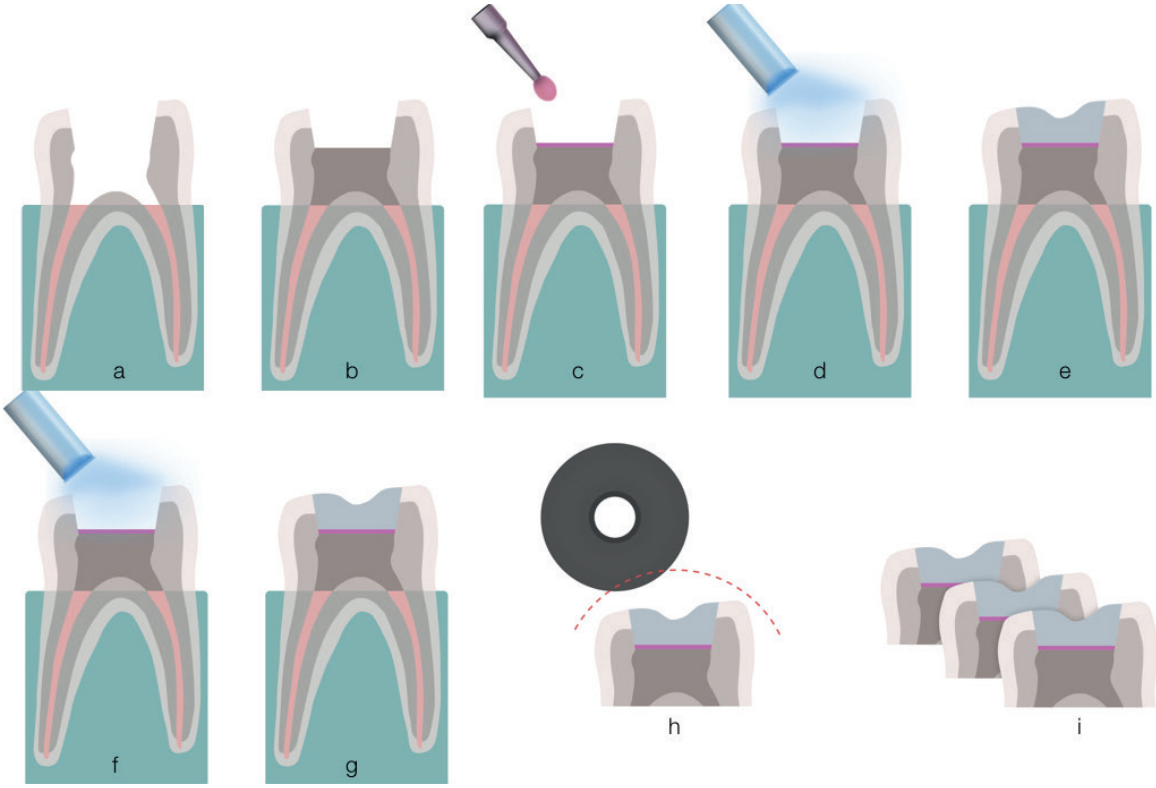


Figure 2.9. Schematic diagram of cavity obturation with HCSC, adhesive aplication, restorative procedures and subsequent sectioning [Adapted from (Pires et al., 2019)]. a) The first artificial molars teeth (DRSK Group AB, 2019) with a standardized cavity access; b) The pulp chamber was filled with HCSC to the entrance to the root channel; c-g) The bonding system with Rhodamine B was applied and the restorative procedures were completed; h,i) The teeth slices were prepared using a water-cooled diamond saw.

Confocal laser scanning microscopy analysis

The sliced samples were observed using a laser scanning confocal inverted microscope (LSM 710 configured to a Axio Observer Z1 microscope, QUASAR detection unit), equipped with a EC Plan-Neofluar 10x/0.3 objective and Zen Black 2012 Software, all from Carl Zeiss, Germany. Images were acquired using the following laser lines: diode 405 nm (Autofluorescence) and DPSS (Diode-Pumped Solid-State) 561 nm (excitation of Rhodamine). Imaging settings (laser power, pinhole and PMT gain) photomultiplier tube were conserved within all conditions. Between 6 to 9 images were captured and registered for each sample.

Photos of each section were evaluated using ImageJ software version: 2.1.0/1.53c., (U. S. National Institutes of Health, Bethesda, Maryland, USA), using a calibrated measuring tool. The measurements were taken from the adhesive – HCSC interface to the deepest level at which the Rhodamine was visualized – corresponding to the maximum thickness of the dye penetration. However, this maximum was not necessarily uniform in all the thickness. The lateral margins and areas close to adhesive failures or HCSC defects were excluded.

Two examiners evaluated the measurement of dye penetration separately for each specimen at two separate times. If there were differences in evaluation, the surface was evaluated once more by the two examiners simultaneously to decide on the final score.

Chapter III. Results

1. Shear bond strength tests

1.1. Main effects of independent variables

Concerning de main effects of independent variables on shear bond strength, the normality of data distribution testing was carried out using the Shapiro–Wilk test and the normality assumption was violated. Since the number of samples between groups for main effect analysis was similar (n=160) and ANOVA is considered a robust test against normality violation, a four-way ANOVA analysis was conducted to establish statistically significant differences between the main effects (HCSC, adhesive system, additional HBL and restoration time) as well as their interaction effects on the SBS test results. The Mann-Whitney test was also performed, but results are not presented because they were similar to the ANOVA test.

Overall, a statistical significant difference was found in the ANOVA test for the mean SBS among the tested groups for the main effects of four independent factors: $F(4, 315) = 13.112$, $p<0.001$.

1.1.1. Main effect "type of HCSC"

For the main effect "type of HCSC", Biodentine™ versus NuSmile® NeoMTA, no statistically significant differences were found in the mean SBS values between the two materials ($p = 0.897$). Table 3.1 shows the composite SBS obtained over the two tested HCSC, including the mean, standard deviation, median, interquartile range, minimum and maximum values.

Table 3.1. SBS results (in MPa) of composite resin over the two HCSC (Biodentine™ and NuSmile® NeoMTA).

Groups	n	Mean (SD)	Median (IQR)	Min/Max	
Biodentine™	160	7.10 (3.91)	6.19 (5.26)	0.84/ 18.48	0.897 [a]
NuSmile® NeoMTA	160	7.16 (4.50)	6.18 (5.66)	0.85/ 21.50	

(a) Main effect ANOVA

As the SBS results were not statistically different between Biodentine™ and NuSmile® NeoMTA, the respective null hypothesis ($H_0 1$) was not rejected.

The comparison of the two HCSC SBS datasets was also performed by Weibull analysis to evaluate the tension to failure for the two tested HCSC. As seen in Figure 3.1, there is a complete overlap between the two curves at the 95% confidence level confirming that the SBS results for the two tested cements were not statistically different. The Biodentine™ has a 99% probability of survival is response to SBS values up to 690 kPa and NuSmile® NeoMTA up to 545 kPa.

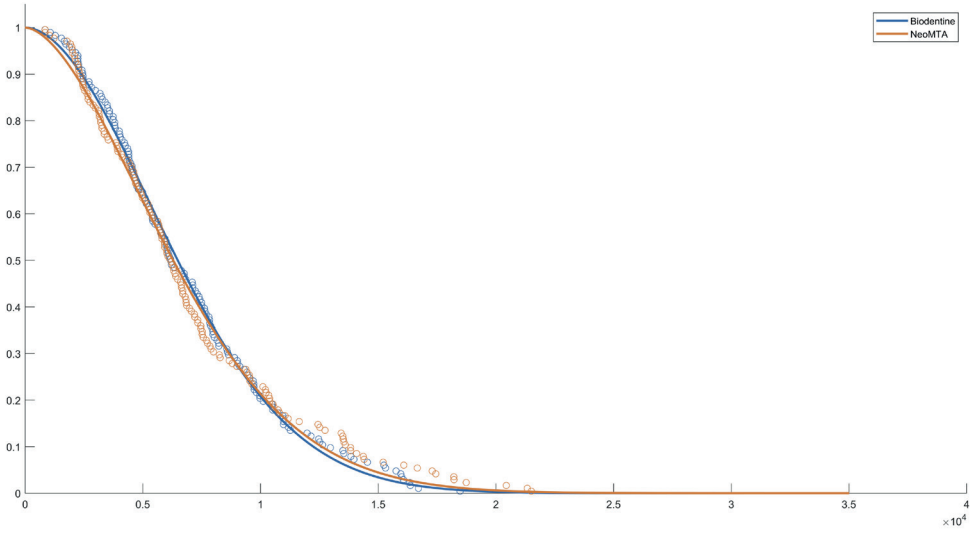


Figure 3.1. Comparison of the two HCSC by Weibull analysis. The horizontal axis indicates SBS (kPa) whereas the vertical axis indicates the probability of survival, from near 0 (zero) to 1 (indicating chance of survival from near 0 to 100%). The Weibull modulus (IC95 %) for Biodentine™ was 1.89 (1.86; 1.92) and for NuSmile® NeoMTA 1.73 (1.69; 1.77) (Table 3.2).

Table 3.2. The Weibull analysis for Biodentine™ and NuSmile® NeoMTA

Group	Weibull modulus (IC95%)	R2	Predict kPa for 99% survival
Biodentine™	1.89 (1.86; 1.92)	1.00	690
NuSmile® NeoMTA	1.73 (1.69; 1.77)	0.99	545

The comparison of the two HCSC SBS datasets was performed by Weibull analysis to evaluate the tension to failure for the two tested HCSC. As seen in Figure 3.1, there is a complete overlap between the two curves at the 95% confidence level confirming that the SBS results for the two tested cements are not statistically different. The Biodentine™ has a 99% probability of survival is response to SBS values up to 690 kPa and NuSmile® NeoMTA up to 545 kPa.

Furthermore, to test the interaction effects of the other independent variables on the SBS results the comparison between all the groups with Biodentine™ and NuSmile® NeoMTA was done keeping the same variable combination (restoration timing, adhesive system and number of HBL) (Table 3.3).

Table 3.3. Comparison of SBS results between the two HCSC, overlaid by the same adhesive system, the time of definitive restoration (immediate or delayed – 7 days) and the presence of additional HBL.

	Clearfil™ SE Bond 2				Clearfil™ Universal Bond Quick			
	No additional HBL		Additional HBL		No additional HBL		Additional HBL	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
Biodentine™	3.62[a] (2.78)	5.85[b] (2.83)	9.19[c] (4.52)	7.90[e] (4.63)	6.01[f] (3.31)	9.44[g] (4.58)	6.93[h] (1.94)	7.87[i] (2.68)
NuSmile® NeoMTA	4.77[a] (2.01)	5.10[b] (2.17)	4.69[d] (2.29)	7.65[e] (5.06)	6.49[f] (4.27)	11.36[g] (5.72)	6.75[h] (3.11)	10.44[i] (4.65)

Mean shear bond strength value (standard deviation) (MPa).

n = 20 specimens per group combination.

In each column, mean values with same letter were not significantly different ($p < 0.05$).

Only the combination of Clearfil™ SE Bond 2 with an extra HBL and immediate restoration was statistically different between the two groups, Biodentine™ or NuSmile® NeoMTA. There were no statistically significant differences in the mean SBS values between the other tested combinations.

1.1.2. Main effect "type of adhesive system"

Regarding the two adhesive systems tested Clearfil™ Universal Bond Quick showed statistical higher SBS values than Clearfil™ SE Bond 2 (p-value < 0.001). Therefore, the respective null hypothesis ($H_0 2$) was rejected (Table 3.4).

Table 3.4. SBS results (in MPa) for the two adhesive systems evaluated.

Groups	n	Mean (SD)	Median (IQR)	Min/Max	p-value
Clearfil™ SE Bond 2	160	6.09 (3.86)	5.05 (4.84)	0.84/ 18.22	< 0.001 [(a)]
Clearfil™ Universal Bond Quick	160	8.16 (4.30)	7.15 (5.22)	1.78/ 21.50	

(a) Main effect ANOVA

Thus the Weibull analysis confirmed a statistically significant difference between Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick with a high rate of survival for Clearfil™ Universal Bond Quick (Figure 3.2).

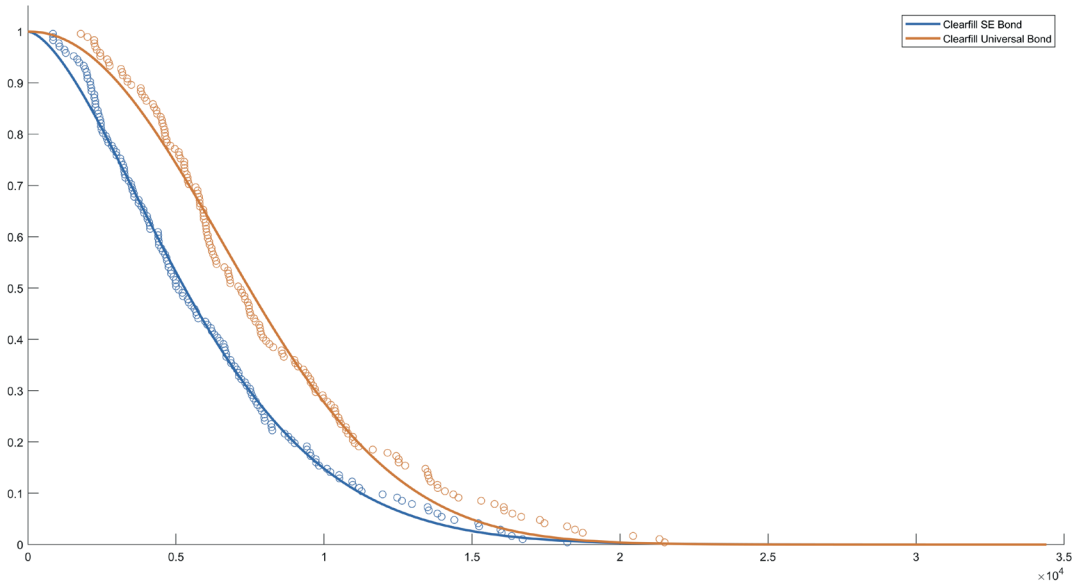


Figure 3.2. The comparison of Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick results was performed by Weibull analysis. The horizontal axis indicates SBS (kPa) whereas the vertical axis indicates the probability of survival, from near 0 (zero) to 1 (indicating chance of survival from near 0 to 100%). The Weibull modulus (IC95%) for Clearfil™ SE Bond 2 was 1.59 (1.57; 1.62) and for Clearfil™ Universal Bond Quick 2.11 (2.05; 2.17) (Table 3.5).

Table 3.5. The Weibull analyses for Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick

Group	Weibull modulus (IC95%)	R^2	Predict kPa for 99% survival
Clearfil™ SE Bond 2	1.59 (1.57; 1.62)	1.00	369
Clearfil™ Universal Bond Quick	2.11 (2.05; 2.17)	0.99	1004

The Weibull analyses confirmed improved mechanical stress of Clearfil™ Universal Bond Quick compared to Clearfil™ SE Bond 2; the Clearfil™ Universal Bond Quick has 99% probability of survival in response to SBS values up to 1004 kPa whereas Clearfil™ SE Bond will survive to 369 kPa only.

We further compared the interaction effect of the other independent variables (HCSC type, presence of additional HBL and restoration time) on the SBS results of these two adhesive systems (Table 3.6).

Table 3.6. Comparison of SBS results between two adhesive systems (Clearfil™ SE Bond 2 or Clearfil™ Universal Bond Quick), keeping the same HCSC, number of HBL and timing restoration.

	Biodentine™				NuSmile® NeoMTA			
	No additional HBL		Additional HBL		No additional HBL		Additional HBL	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
Clearfil™ SE Bond 2	3.62[a] (2.78)	5.85[b] (2.83)	9.19[c] (4.52)	7.90[d] (4.63)	4.77[e] (2.01)	*5.10[f] (2.17)	4.69[h] (2.29)	7.65[i] (5.06)
Clearfil™ UB Quick	6.01[a] (3.31)	9.44[b] (4.58)	6.93[c] (1.94)	7.87[d] (2.68)	6.49[e] (4.27)	11.36[g] (5.72)	6.75[h] (3.11)	10.44[i] (4.65)

Mean shear bond strength value (standard deviation) (MPa).
n = 20 specimens per group combination.
In each column, mean values with same letter were not statistically different. ($p < 0.05$).

Only the combination of NuSmile® NeoMTA with no extra HBL and delayed restoration was statistically different between the two adhesives.

1.1.3. Main effect "additional hydrophobic resin layer"

The application of an additional hydrophobic resin layer resulted in a significant higher mean SBS value compared to no additional HBL application (*p*-value = 0.014). Therefore, the respective null hypothesis ($H_0 3$) was rejected (Table 3.7).

Table 3.7. SBS results (in MPa) of composite resin over the HCSC, with or without an additional HBL.

Groups	n	Mean (SD)	Median (IQR)	Min/Max	p-value
No additional HBL	160	6.58 (4.32)	5.40 (4.51)	0.84/ 21.33	0.014 [(a)]
Additional HBL	160	7.52 (4.03)	6.95 (4.79)	0.25/ 21.50	

(a) Main effect ANOVA

These results were corroborated by the Weibull analysis indicating that the application of an additional HBL exhibited stronger bond strength. As seen in Figure 3.3 the group with extra HBL has a 50% chance of survival higher compared to the group without an extra HBL.

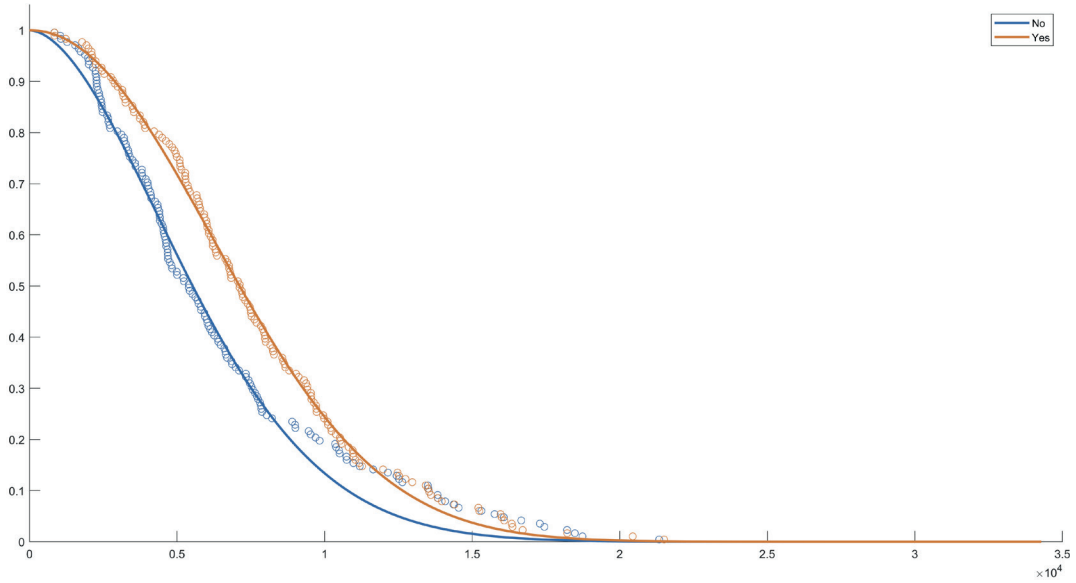


Figure 3.3. The comparison of SBS results with or without an extra HBL was also performed by Weibull analysis. The horizontal axis indicates SBS (kPa) whereas the vertical axis indicates the probability of survival, from near 0 (zero) to 1 (indicating chance of survival from near 0 to 100%). The Weibull modulus (IC95%) for no extra HBL was 1.80 (1.74; 1.85) and for no extra HBL was 2.09 (2.06; 2.12) (Table 3.8).

Table 3.8. The Weibull analyses for the application of an extra HBL.

Group	Weibull modulus (IC95%)	R^2	Predict kPa for 99% survival
No extra HBL	1.80 (1.74; 1.85)	1.00	526
Extra HBL	2.09 (2.06; 2.12)	0.99	939

These results indicate that the group with no extra HBL has 99% probability of survival in response to SBS values up to 526 kPa whereas the group with an extra HBL will survive to 939 kPa.

Table 3.9. Comparison of SBS results between the groups with or without an additional HBL concerning the other remaining independent variables (HCSC, adhesive systems and restoration timing).

	Biodentine™				NuSmile® NeoMTA			
	Clearfil™ SE Bond 2		Clearfil™ UB Quick		Clearfil™ SE Bond 2		Clearfil™ UB Quick	
	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed	Immediate	Delayed
No additional HBL	3.62[a] (2.78)	5.85[c] (2.83)	6.01[d] (3.31)	9.44[e] (4.58)	4.77[f] (2.01)	5.10[g] (2.17)	6.49[h] (4.27)	11.36[i] (5.72)
Additiional HBL	9.19[b] (4.52)	7.90[c] (4.63)	6.93[d] (1.94)	7.87[e] (2.68)	4.69[f] (2.29)	7.65[g] (5.06)	6.75[h] (3.11)	10.44[i] (4.65)

Mean shear bond strength value (standard deviation) in MPa.
n = 20 specimens per group combination.
In each column, mean values with the same letter were not significantly different ($p < 0.05$).

Although in 5 of the 8 comparisons the values were higher with the presence of an HBL, only the combination Biodentine™ Clearfil™ SE Bond 2 without HBL and immediate restoration was statistically different from the combination Biodentine Clearfil™ SE Bond 2 with HBL and immediate restoration (Table 3.9).

1.1.4. Main effect "timing of the definitive restoration"

Concerning the different restoration times, delayed definitive restorations (after seven days) revealed statistical higher mean SBS values than immediate restorations (p-value < 0.001). Thus, the null hypothesis ($H_0$4) was rejected (Table 3.10).

Table 3.10. SBS strength results (in MPa) of composite resin over the HCSC, after immediate or delayed definitive adhesive restoration.

Groups	n	Mean (SD)	Median (IQR)	Min/Max	p-value
Immediate	160	6.05 (3.49)	5.41 (4.50)	0.84/ 16.66	< 0.001 [(a)]
Delayed	160	8.20 (4.59)	7.20 (6.09)	0.85/ 21.50	

(a) Main effect ANOVA

The comparison of immediate versus delayed restorations was also performed by Weibull analysis showing better probability of survival for delayed restorations (Figure 3.4).

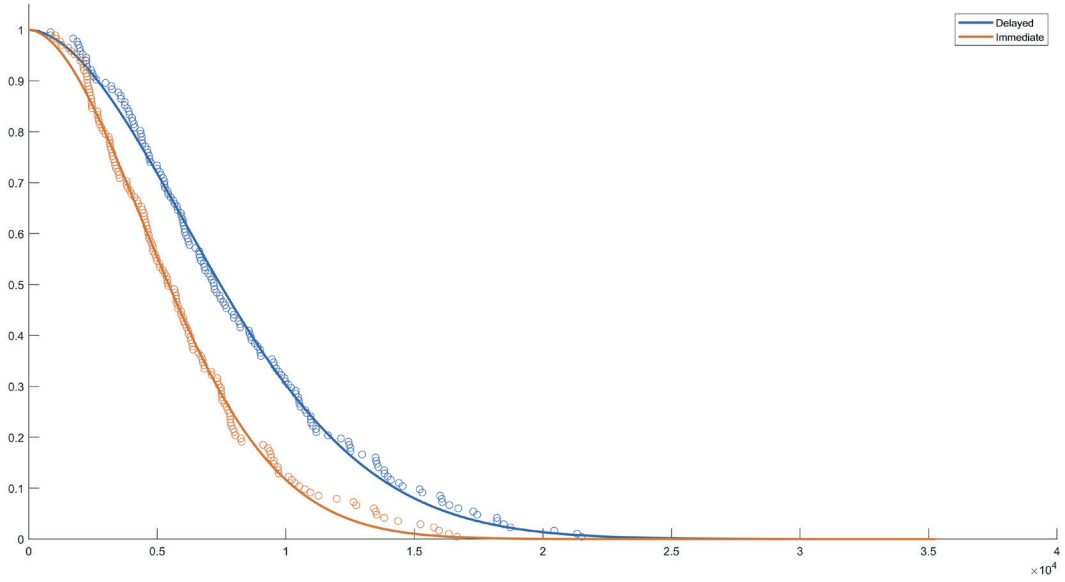


Figure 3.4. Comparison of time to survival for immediate versus delayed restorations by Weibull analysis. The horizontal axis indicates SBS (kPa) whereas the vertical axis indicates the probability of survival, from near 0 (zero) to 1 (indicating chance of survival from near 0 to 100%). The Weibull modulus (IC95%) for immediate restorations was 1.86 (1.83; 1.89) and for delayed restorations was 1.85 (1.82; 1.88) (Table 3.11).

Table 3.11. The Weibull analyses for the delayed and immediate definitive adhesive restorations.

Group	Weibull modulus (IC95%)	R^2	Predict kPa for 99% survival
Immediate	1.86 (1.83; 1.89)	1.00	559
Delayed	1.85 (1.82; 1.88)	1.00	756

The Weibull analysis indicated that the group with immediate resin composite restorations has 99% probability of survival in response to SBS values up to 559 kPa whereas the group with delayed restorations will survive to 756 kPa (Table 3.11).

Table 3.12. Comparison of SBS (in MPa) results between the groups, considering the individual effect of the other independent variables combined with the time of restoration.

	Biodentine™				NuSmile® NeoMTA			
	Clearfil™ SE Bond 2		Clearfil™ UB Quick		Clearfil™ SE Bond 2		Clearfil™ UB Quick	
	No HBL	HBL	No HBL	HBL	No HBL	HBL	No HBL	HBL
Immediate	3.62[a] (2.78)	9.19[b] (4.52)	6.01[c] (3.31)	6.93[d] (1.94)	4.77[e] (2.01)	4.69[f] (2.29)	6.49[g] (4.27)	6.75[i] (3.11)
Delayed	5.85[a] (2.83)	7.90[b] (4.63)	9.44[c] (4.58)	7.87[d] (2.68)	5.10[e] (2.17)	7.65[f] (5.06)	11.36[h] (5.72)	10.44[i] (4.65)

Mean shear bond strength value standard deviation (MPa).

n = 20 specimens per group combination.

In each column, mean values that share a superscript lowercase letter were not significantly different between the groups, considering the timing restoration, immediate (I) or delayed for 7 days (D) ($p < 0.05$).

The restoration timing was only significant for the combination NuSmile® NeoMTA, Clearfil™ Universal Bond Quick without an additional HBL, showing better results for delayed restoration (Table 3.12).

General distribution of shear bond strength results between all groups

A descriptive analysis and dispersion graph were done to overview all the groups. The table includes the mean, standard deviation, median, interquartile range, minimum and maximum values (Table 3.13, Figure 3.5).

Table 3.13. Global results of the tested groups regarding SBS values (MPa).

	Groups	n	Mean (SD)	Median (IQR)	Min/Max
1	Biodentine SE 0 I	20	3.62 (2.78)	2.59 (2.75)	0.84/ 12.64
2	Biodentine SE 0 7	20	5.85 (2.83)	4.71 (4.64)	1.73/ 11.19
3	Biodentine SE 1 I	20	9.19 (4.52)	9.57 (6.99)	2.44/ 16.34
4	Biodentine SE 1 7	20	7.90 (4.63)	7.55 (7.98)	2.24/ 16.71
5	Biodentine U 0 I	20	6.01 (3.31)	4.75 (3.94)	2.01/ 15.76
6	Biodentine U 0 7	20	9.44 (4.58)	8.55 (7.87)	3.49/ 18.48
7	Biodentine U 1 I	20	6.93 (1.94)	6.76 (3.15)	3.18/ 10.12
8	Biodentine U 1 7	20	7.87 (2.68)	7.50 (3.05)	5.10/ 16.36
9	NeoMTA SE 0 I	20	4.77 (2.01)	4.76 (3.45)	1.04/ 7.75
10	NeoMTA SE 0 7	20	5.10 (2.17)	5.43 (3.85)	1.98/ 9.53
11	NeoMTA SE 1 I	20	4.69 (2.29)	4.67 (3.23)	1.23/ 9.42
12	NeoMTA SE 1 7	20	7.65 (5.06)	7.56 (6.94)	0.85/ 18.22
13	NeoMTA U 0 I	20	6.49 (4.27)	5.81 (4.39)	2.27/ 16.66
14	NeoMTA U 0 7	20	11.36 (5.72)	12.04 (9.86)	2.24/ 21.33
15	NeoMTA U 1 I	20	6.75 (3.11)	6.08 (4.04)	1.78/ 13.43
16	NeoMTA U 1 7	20	10.44 (4.65)	10.46 (5.13)	5.28/ 21.50

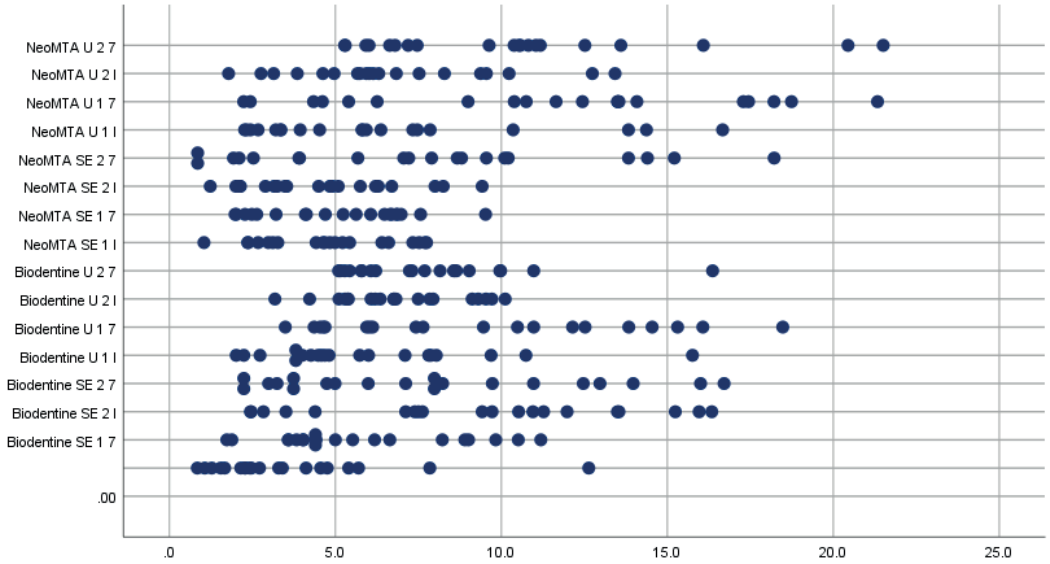


Figure 3.5. Dispersion graph presenting the SBS values distribution in the tested groups. The horizontal axis indicates SBS (MPa) whereas the vertical axis indicates all the groups. SE: Clearfil™ SE Bond; U: Clearfil™ Universal Bond Quick; 0: No extra HBL; 1: Extra HBL; I: Immediate restoration; 7: Delayed restoration (7 days).

Table 3.14. Direct comparison between all the 16 groups (*p* value).

G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		1.000	<0.001	0.039	1.000	<0.001	0.635	0.044	1.000	1.000	1.000	0.086	1.000	<0.001	1.000	<0.001
2			0.588	1.000	1.000	0.302	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.001	1.000	0.014
3				1.000	0.890	1.000	1.000	1.000	0.025	0.073	0.020	1.000	1.000	1.000	1.000	1.000
4					1.000	1.000	1.000	1.000	0.983	1.000	0.812	1.000	1.000	0.434	1.000	1.000
5						0.469	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.001	1.000	0.024
6							1.000	1.000	0.011	0.033	0.009	1.000	1.000	1.000	1.000	1.000
7								1.000	1.000	1.000	1.000	1.000	1.000	0.025	1.000	0.371
8									1.000	1.000	0.891	1.000	1.000	0.393	1.000	1.000
9										1.000	1.000	1.000	1.000	<0.001	1.000	<0.001
10											1.000	1.000	1.000	<0.001	1.000	0.001
11												1.000	1.000	<0.001	1.000	<0.001
12													1.000	0.215	1.000	1.000
13														0.006	1.000	0.108
14															0.013	1.000
15																0.223

Group 1: Biodentine SE 0 I; Group 2: Biodentine SE 0 7; Group 3: Biodentine SE 1 I; Group 4: Biodentine SE 1 7; Group 5: Biodentine U 0 I; Group 6: Biodentine U 0 7; Group 7: Biodentine U 1 I; Group 8: Biodentine U 1 7; Group 9: NeoMTA SE 0 I; Group 10: NeoMTA SE 0 7; Group 11: NeoMTA SE 1 I; Group 12: NeoMTA SE 1 7; Group 13: NeoMTA U 0 I; Group 14: NeoMTA U 0 7; Group 15: NeoMTA U 1 I; Group 16: NeoMTA U 1 7.

From all the tested groups, the NeoMTA U 0 7 showed the highest mean SBS value (11.36±5.72), followed by the NeoMTA U 1 7 (10.44±4.65), with no statistically significant difference between them ($p > 0.05$). The highest mean SBS value in the Biodentine™ group was Biodentine U 0 7 (9.44±4.58) and with no statistically significant difference between this group and NeoMTA U 0 7 (Table 3.14).

The Clearfil™ Universal Bond Quick revealed better bond performance in the NuSmile® NeoMTA™ group ($p < 0.05$), compared to Clearfil™ SE Bond. No application of an extra HBL, independently of

the timing restoration (immediate or after seven days), resulted in a weaker bond for Biodentine™ and NuSmile® NeoMTA combined with the Clearfil™ SE Bond 2.

No application of an extra HBL, independently of the timing of the restoration (immediate or after seven days), resulted in a weaker bond for Biodentine™ and NuSmile® NeoMTA combined with the Clearfil™ SE Bond 2, with statistically significant difference between NeoMTA Clearfil SE Bond 2, delayed restoration with and without HBL.

The group Biodentine U 0 7 (9.44±4.58) revealed the best performance within the Biodentine™ group. The Biodentine SE 0 1 revealed the weakest performance.

1.2. Fracture pattern analysis

The same examiner repeated the evaluation of fracture pattern one month after the initial, re-scoring 10% of the total sample, corresponding to 32 specimens. A Kappa coefficient of 0.808 ($p<0.001$) was found representing a strong agreement between the two analyses.

General comparison of fracture pattern between all groups

In order to compare the facture pattern, considering the HCSC, adhesive procedure (type of adhesive system and application of HBL) and restoration timing, a summary of fracture patterns is given in Table 3.15 and Figure 3.6.

Table 3.15. Distribution of Failure Modes within Groups (n = 20).

Groups	Biodentine™			NuSmile® NeoMTA		
	Adhesive failure	Cohesive failure HCSC	Mixed failure	Adhesive failure	Cohesive failure HCSC	Mixed failure
SE 0 1	1	15	4	0	14	6
SE 0 7	10	7	3	9	2	9
SE 1 1	3	10	7	0	18	2
SE 1 7	17	0	3	17	0	3
U 0 1	8	5	7	0	15	5
U 0 7	2	8	10	0	11	9
U 1 1	4	5	11	2	14	4
U 1 7	0	9	11	10	3	7

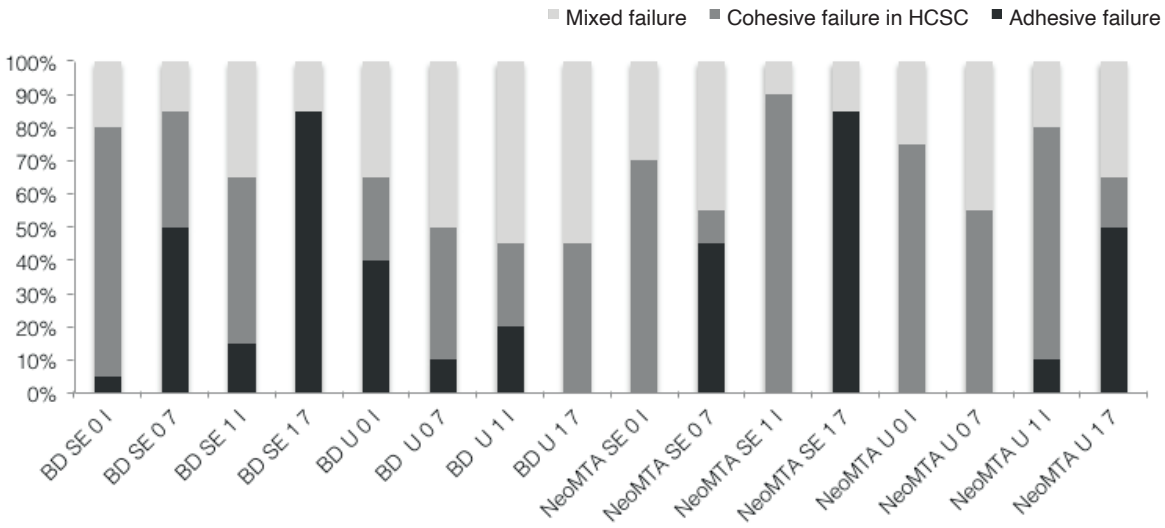


Figure 3.6. Failure mode in 16 different experimental groups. SE (Clearfil™ SE Bond); U (Clearfil™ Universal Bond Quick); 0 (No extra HBL); I (Extra HBL); I (Immediate restoration); 7 (Delayed restoration -7 days).

The fracture pattern was compared between the HCSC groups: Biodentine™ and NuSmile® NeoMTA (Table 3.16 and Figure 3.7).

Table 3.16. The fracture pattern related with the HCSC.

		HCSC	
		Biodentine™	NuSmile® NeoMTA
Fracture pattern	Adhesive	45	38
	Cohesive in HCSC	59	77
	Cohesive in RC	0	0
	Mixed	56	45

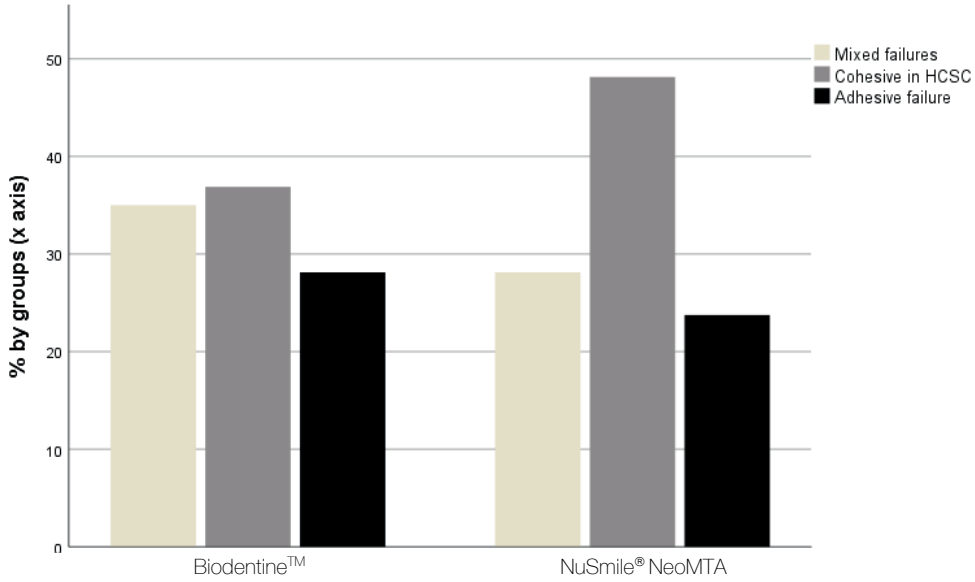


Figure 3.7. The fracture pattern related with the HCSC.

According to Fisher's exact test there was no statistically significant association between the fracture type and the HCSC used (p= 0.127), with more cohesive fractures in both groups.

The association of fracture pattern and the adhesive system was verified (Table 3.17 and Figure 3.8).

Table 3.17. The fracture pattern related with the adhesive system (Clearfil™ SE Bond 2 or Clearfil™ Universal Bond Quick).

		Adhesive system	
		Clearfil™ SE Bond 2	Clearfil™ Universal Bond Quick
Fracture pattern	Adhesive	57	26
	Cohesive in HCSC	66	70
	Cohesive in RC	0	0
	Mixed	37	64

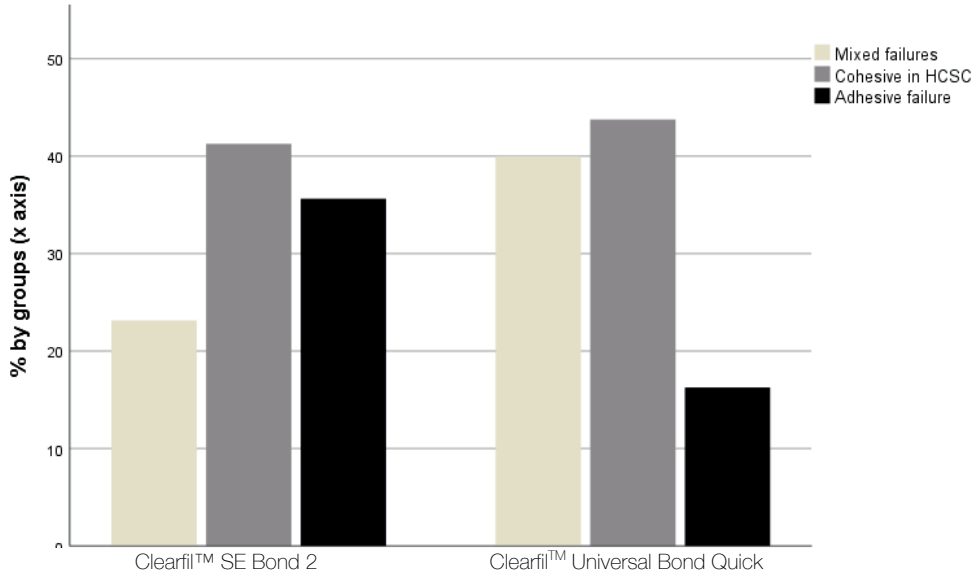


Figure 3.8. The fracture pattern related with the adhesive system applied

There was a statistically significant difference between the adhesive systems concerning fracture pattern (p-value < 0.001).

Although both adhesive systems have presented more cohesive fractures, Clearfil™ SE Bond 2 showed more adhesive fractures than Clearfil™ Universal Bond Quick. Conversely, Clearfil™ Universal Bond Quick showed more mixed failures than Clearfil™ SE Bond 2.

Additionally, the association of fracture pattern and the application of an additional hydrophobic bonding layer was analyzed. (Table 3.18 and Figure 3.9).

Table 3.18. The fracture pattern related to the presence of an additional HBL

		Application of HBL	
		No additional HBL	Additional HBL
Fracture pattern	Adhesive	30	53
	Cohesive in HCSC	77	59
	Cohesive in RC	0	0
	Mixed	53	48

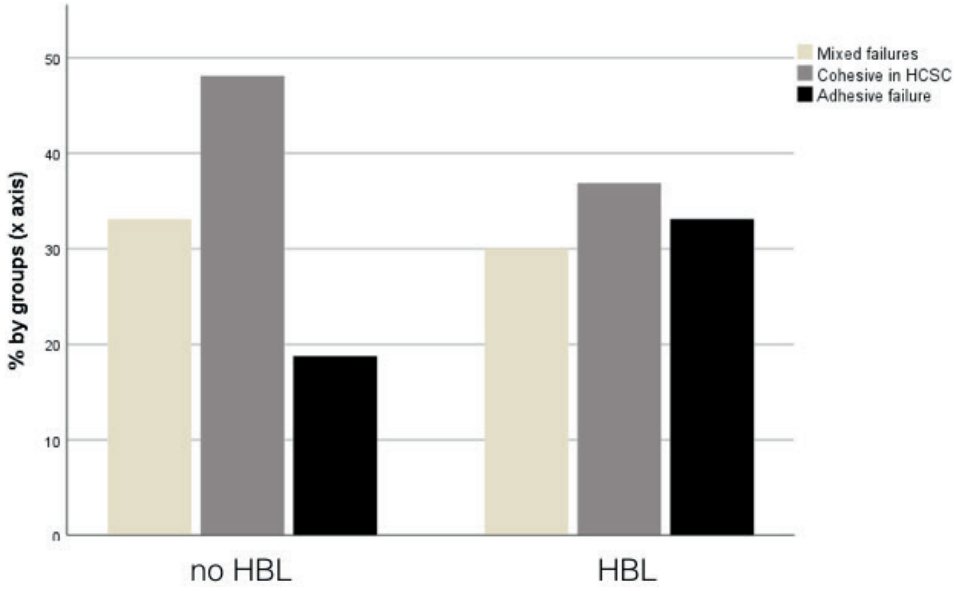


Figure 3.9. The fracture pattern related with the application of additional HBL.

We found a statistically significant association between the fracture pattern and the application of an extra HBL (p = 0.011).

The more prevalent fracture was cohesive in HCSC, followed by adhesive fracture in the group with an additional HBL and mixed in the group without an additional HBL.

The association of fracture pattern and the restoration timing (immediate or delayed, after 7 days) was also evaluated (Table 3.19 and Figure 3.10).

Table 3.19. The fracture pattern related with restoration timing (immediate or delayed after 7 days).

		Restoration timing	
		Immediate	Delayed
Fracture pattern	Adhesive	18	65
	Cohesive in HCSC	96	40
	Cohesive in RC	0	0
	Mixed	46	55

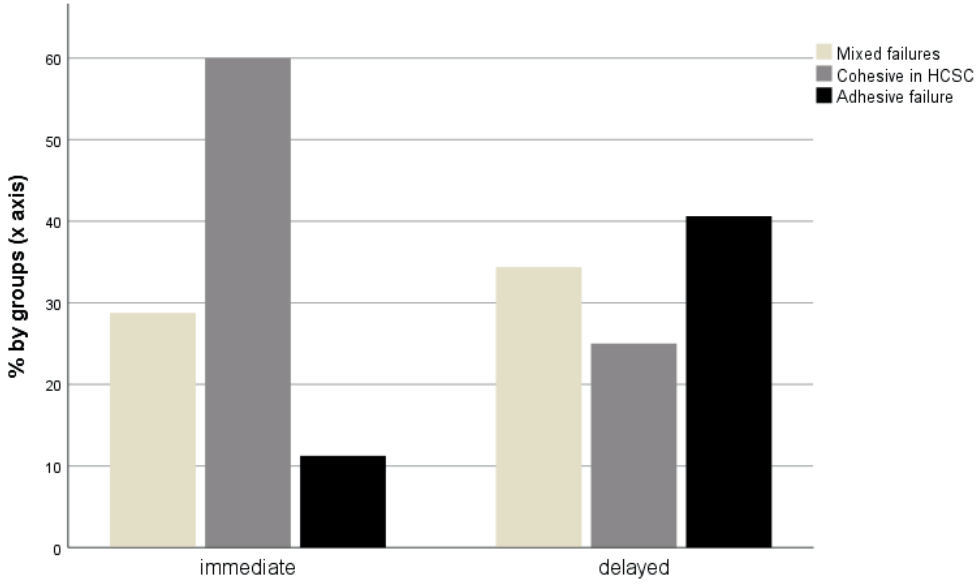


Figure 3.10. The fracture pattern related with restoration timing

A statistically significant association was verified between the fracture pattern and the timing of restoration ($p < 0.001$).

The delayed restoration group had more adhesive failures compared with the immediate group. Conversely, the immediate restoration had more cohesive failures in the HCSC.

Representative SEM images of specimens with three different patterns of failure between resin composite and HCSC are presented (Figures 1.11, 1.12, 1.13).

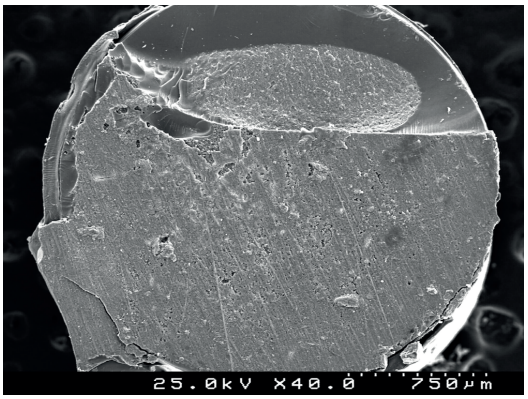


Figure 3.11. Scanning electron microscope image of mixed fracture (original magnification x40).

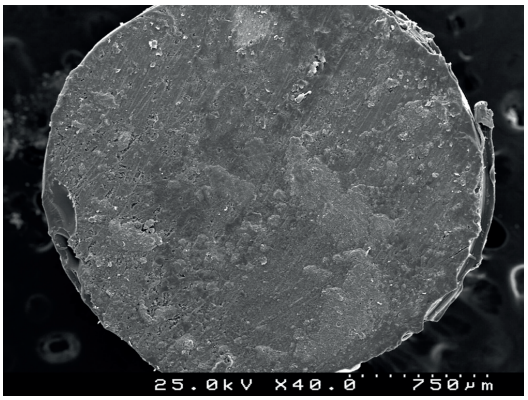


Figure 3.12. Scanning electron microscope image of adhesive fracture (original magnification x40).

Figure 3.13. Scanning electron microscope image of cohesive fracture (original magnification x40)

2. Qualitative analysis of the bond interface

2.1. Bond interface evaluation by scanning electronic microscopy

The scanning electron micrographs exhibiting the material interfaces of each HCSC (Biodentine™ and NuSmile® NeoMTA) with two adhesive procedures (type of adhesive system and application of an additional HBL) and restoration timing are shown in Figures below.

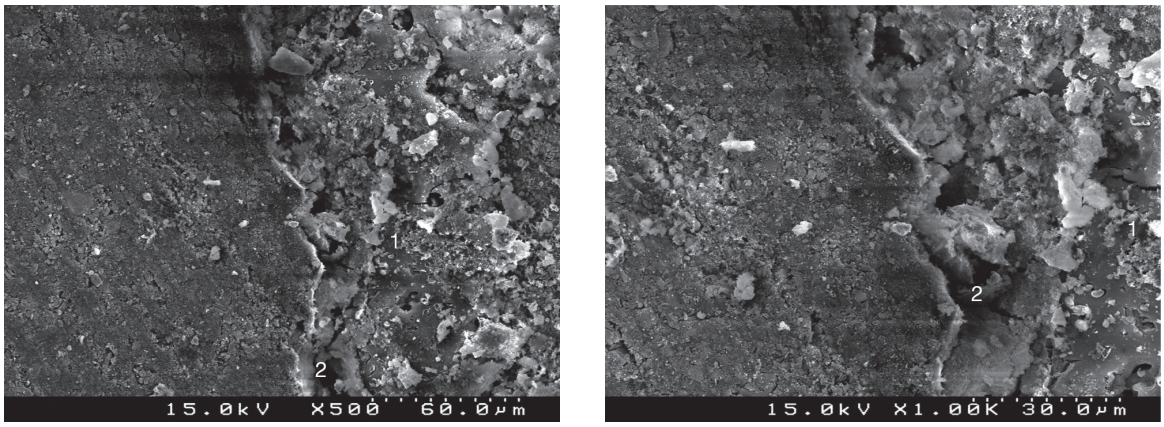


Figure 3.14 - A and B: A scanning electron micrograph of the interface of group 1, showing a straight interdiffusion of the adhesive material protruding into the HCSC. Cement particles are involved by the adhesive. A HCSC – adhesive hybrid layer is observed (1) with some empty spaces on the top of the hybrid layer (2) (original magnification, x500; x1000).

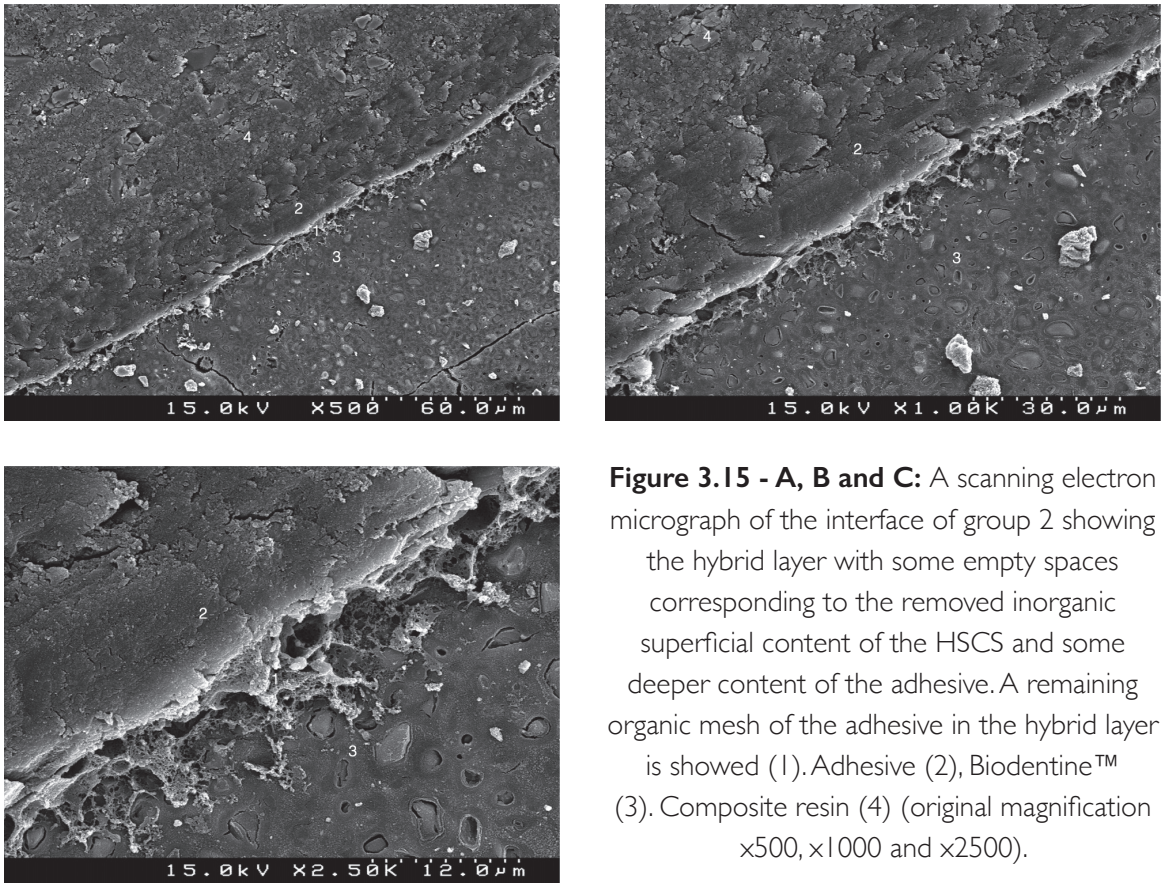


Figure 3.15 - A, B and C: A scanning electron micrograph of the interface of group 2 showing the hybrid layer with some empty spaces corresponding to the removed inorganic superficial content of the HSCS and some deeper content of the adhesive. A remaining organic mesh of the adhesive in the hybrid layer is showed (1). Adhesive (2), Biodentine™ (3). Composite resin (4) (original magnification x500, x1000 and x2500).

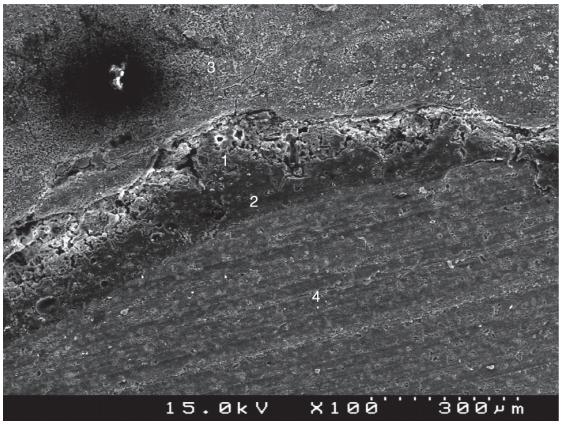


Figure 3.16. A scanning electron micrograph of the interface of group 3 showing a deep interdiffusion between the adhesive system and HCSC with a thick hybrid layer (1) between the adhesive (2) and Biodentine™ (3). Composite resin (4). Empty spaces visible in the deeper part of the hybrid layer may be due to the samples preparation process in which part of the superficial inorganic layer of HCSC was removed as well as a part of the adhesive organic mesh (original magnification x100).

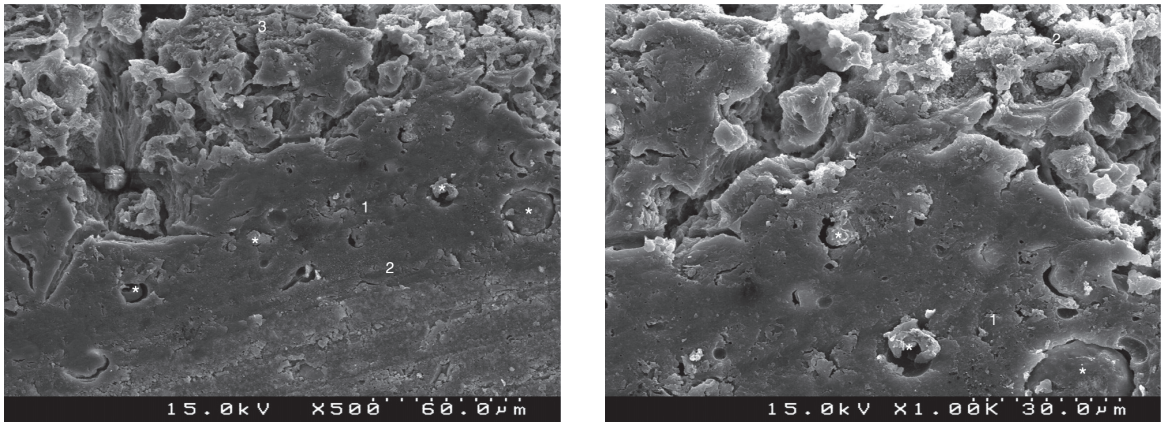


Figure 3.17 - A and B: A scanning electron micrograph of the interface of group 3 showing a considerable interdigitation between the adhesive system and HCSC. A thick hybrid layer is presented (1). Particles of cement involved by the adhesive (*). Adhesive (2) and Biodentine™ (3) (original magnification x500 and x1000).

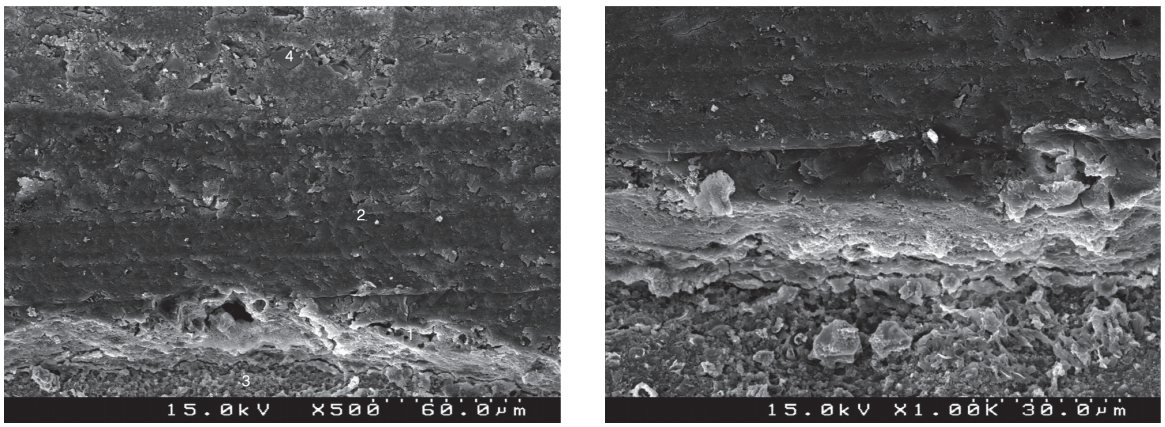


Figure 3.18 - A and B: A scanning electron micrograph of the interface of group 4 showing some interpenetration between the adhesive system and HCSC. A less deep hybrid layer is observed (1) between the adhesive with a thick layer (2) and the Biodentine™ (3). Composite resin (4) (original magnification x500 and x1000).

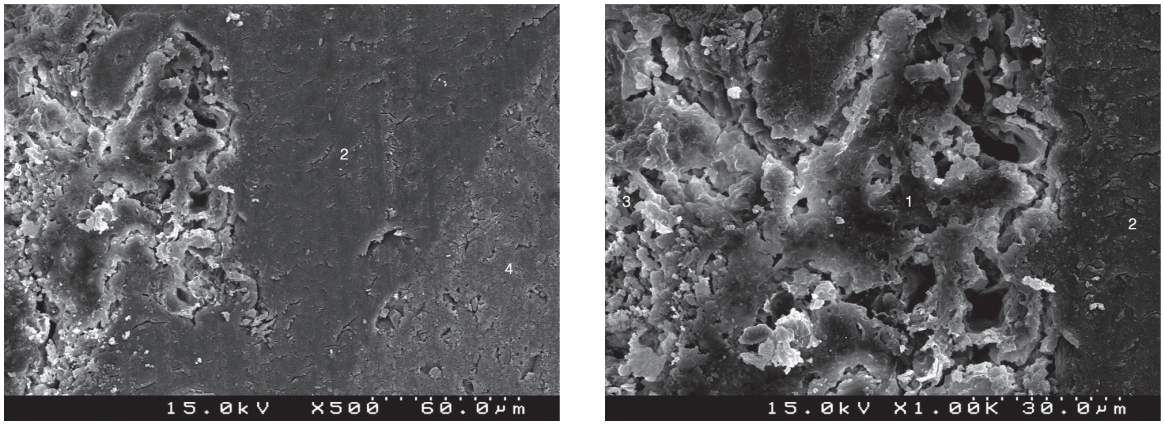


Figure 3.19 - A and B: A scanning electron micrograph of the interface of group 5 showing a deep interpenetration between the adhesive and the cement, with particles of cement involved by the adhesive. A thick hybrid layer is presented (1) between the adhesive (2) and the Biodentine™ (3). Composite resin (4) (original magnification x500 and x1000).

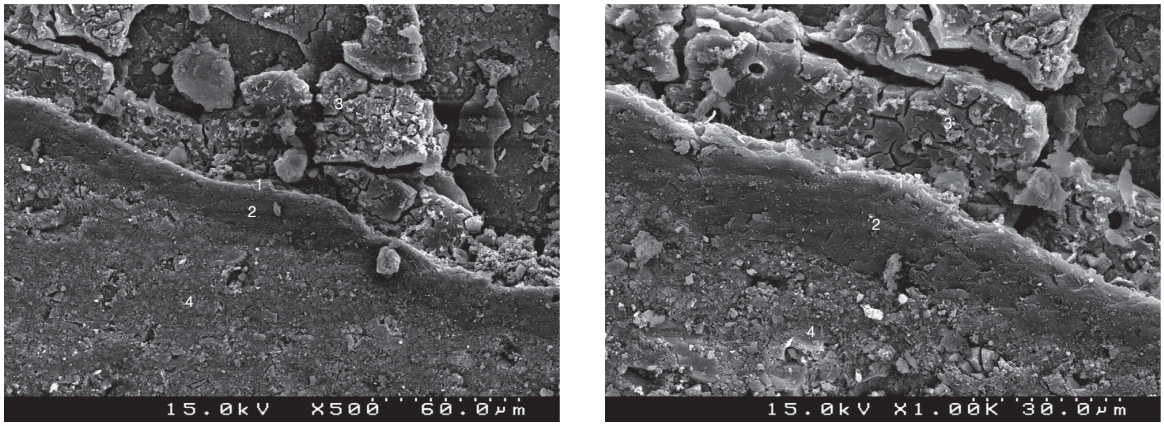


Figure 3.20 - A and B: A scanning electron micrograph of the interface of group 6 showing some interdigitation between the adhesive and cement. A less deep hybrid layer is observed (1) between the adhesive (2) and the Biodentine™ (3). Composite resin (4) (original magnification x500 and x1000).

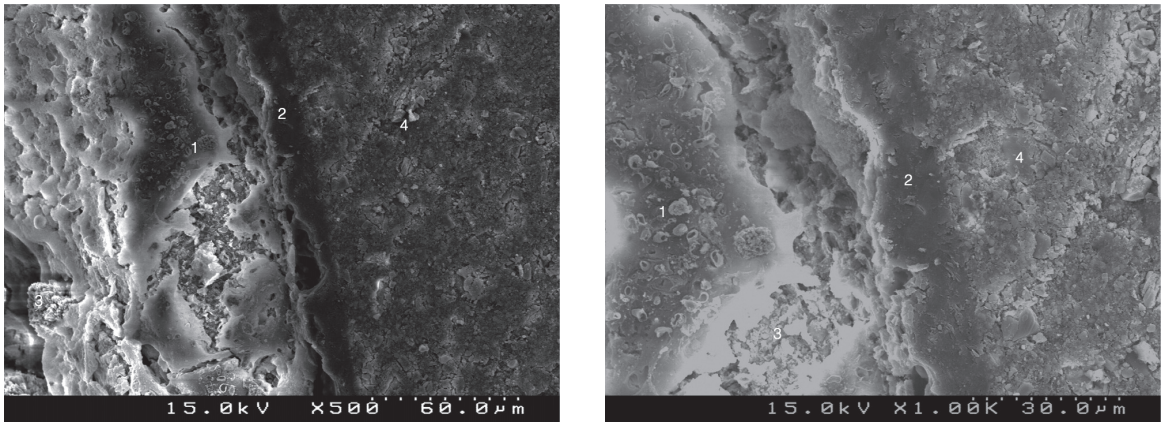
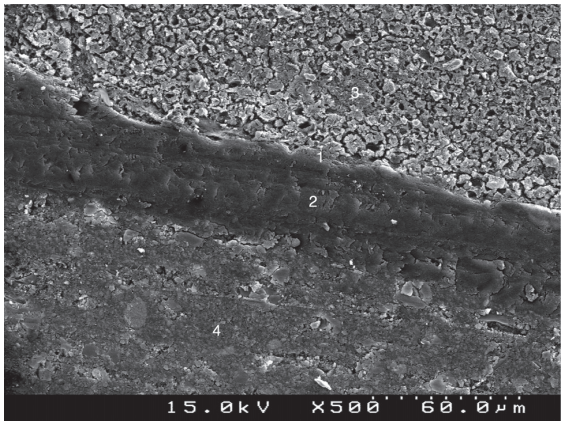


Figure 3.21 - A and B: A scanning electron micrograph of the interface of group 7 showing a deep interdigitation between the adhesive and the cement, with particles of cement involved by the adhesive. A thick hybrid layer is presented (1) between the adhesive (2) and the Biodentine™ (3). Composite resin (4) (original magnification x500 and x1000).



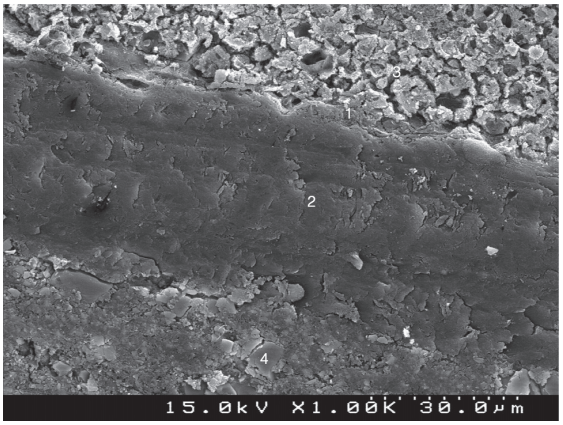
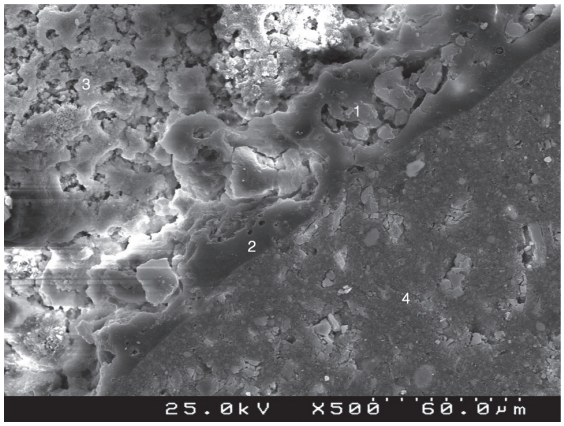
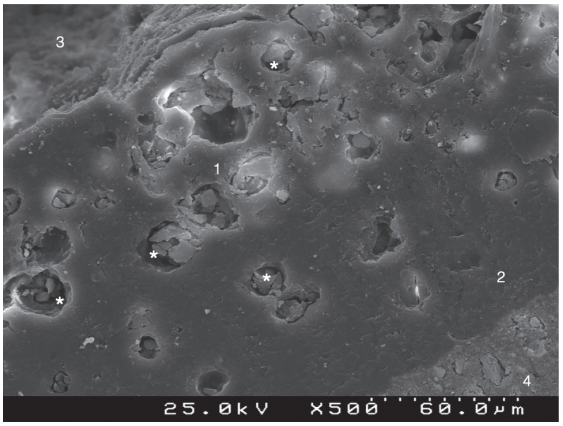


Figure 3.22 - A and B: A scanning electron micrograph of the interface of group 8 showing less interpenetration between the adhesive and the cement. A less deep hybrid layer is presented (1) between the adhesive in a thick layer (2) and the Biodentine™ (3). Composite resin (4) (original magnification x500 and x1000).





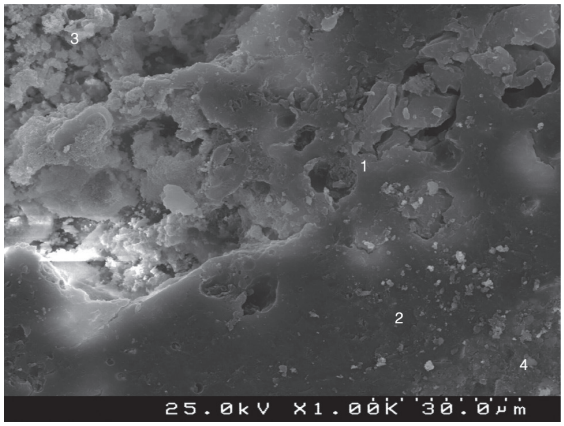


Figure 3.23 - A, B and C: A scanning electron micrograph of the interface of group 9 showing a deep interdiffusion between the adhesive and the cement, with particles of cement involved by the adhesive (*). A thick hybrid layer is observed (1) between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x500 and x1000).

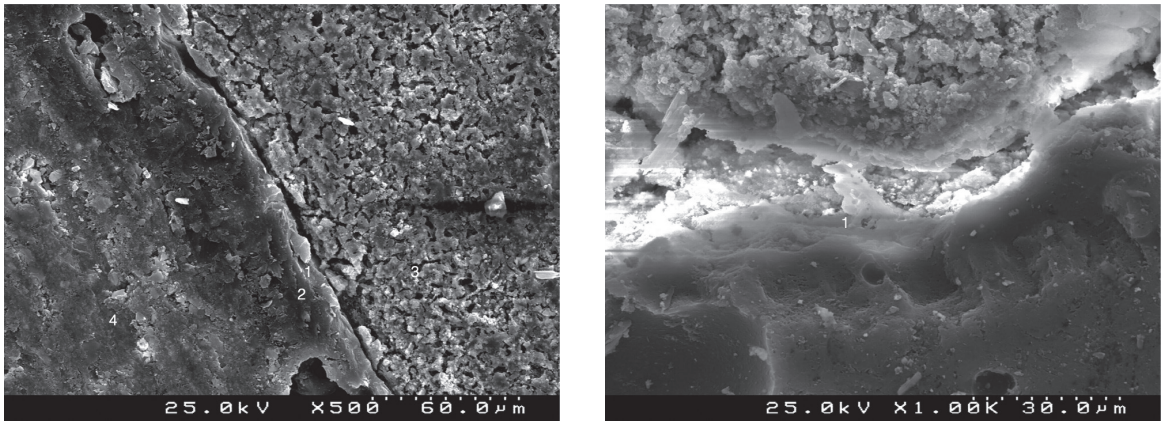


Figure 3.24 - A and B: A scanning electron micrograph of the interface of group 10 showing a less deep interdigitation between the adhesive and the cement. A less thick hybrid layer is observed (1) between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x500 and x1000).

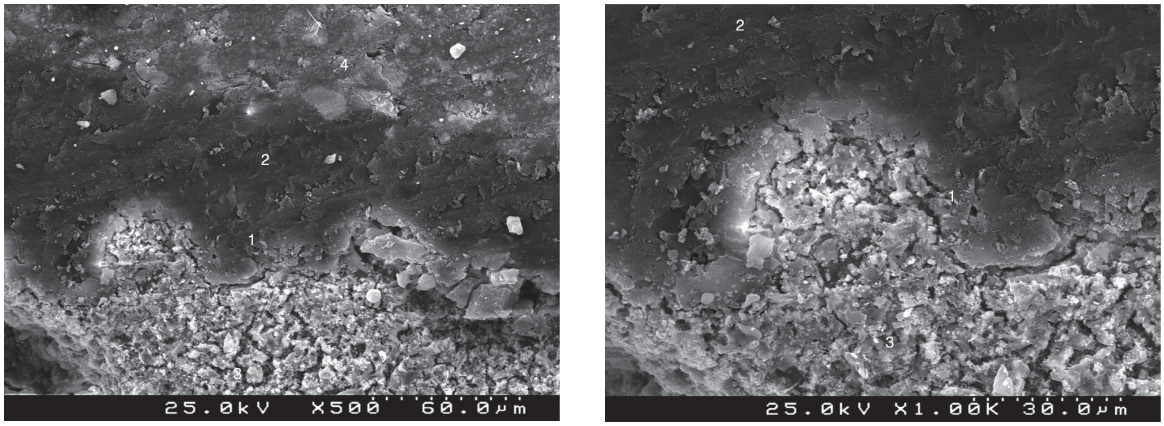


Figure 3.25 - A and B: A scanning electron micrograph of the interface of group 11 showing the hybrid layer (1) and the interpenetration between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x500 and x1000).

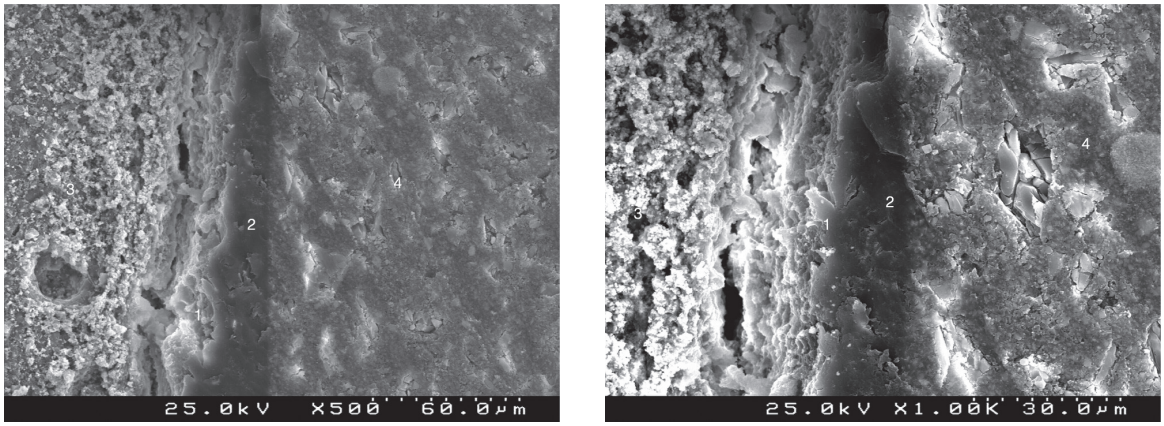


Figure 3.26 - A and B: A scanning electron micrograph of the interface of group 12 showing some interdigitation between the adhesive system and HCSC. A less deep hybrid layer is observed (1) between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x500 and x1000).

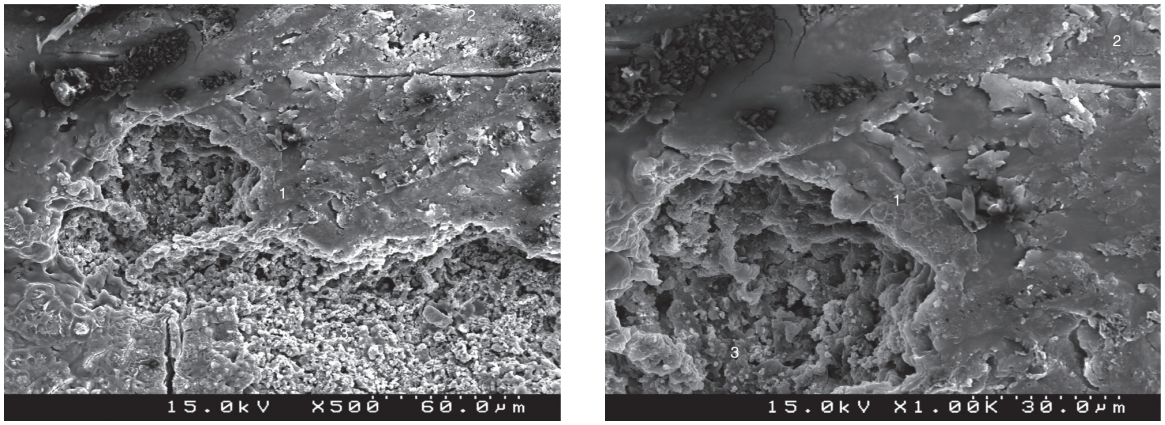


Figure 3.27 - A and B: A scanning electron micrograph of the interface of group 13 showing a interpenetration between the adhesive and the cement, with particles of cement involved by the adhesive. A thick hybrid layer is presented (1) between the adhesive (2) and the NuSmile® NeoMTA (3) (original magnification x500 and x1000).

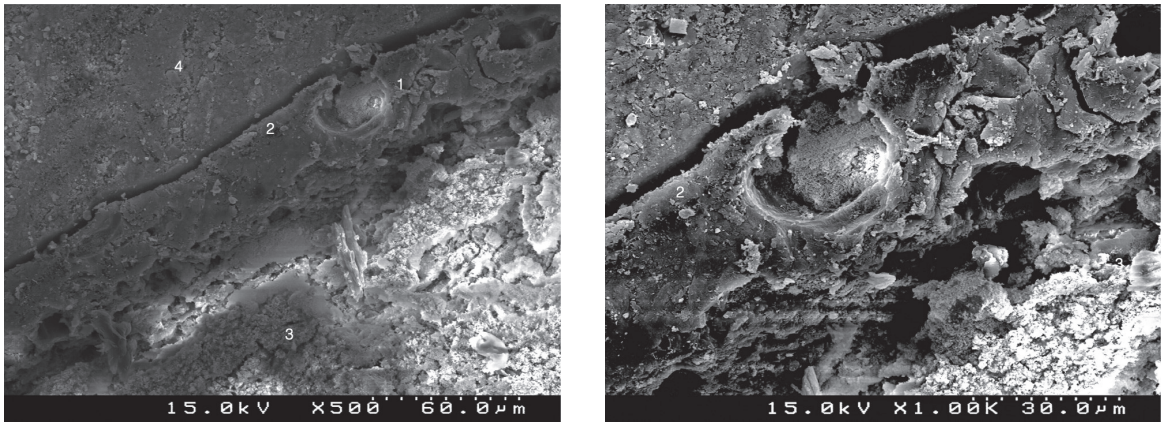
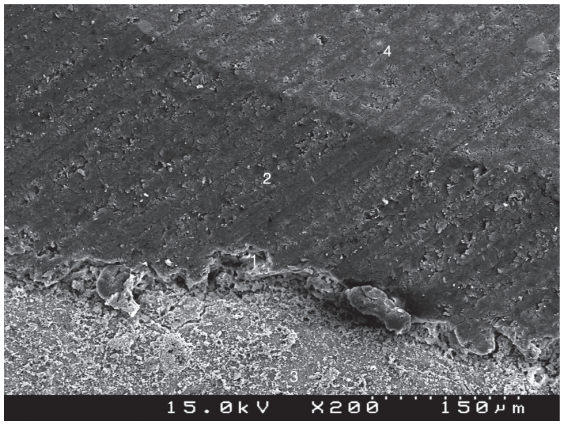
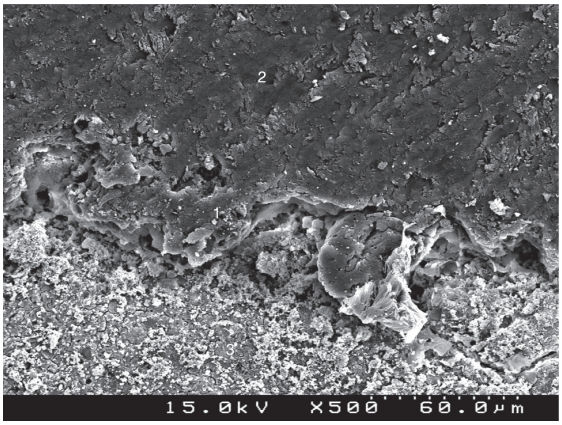


Figure 3.28 - A and B: A scanning electron micrograph of the interface of group 14 showing some interdigitation between the adhesive and cement. An interfacial gap and a less deep hybrid layer is observed (1) between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x500 and x1000).





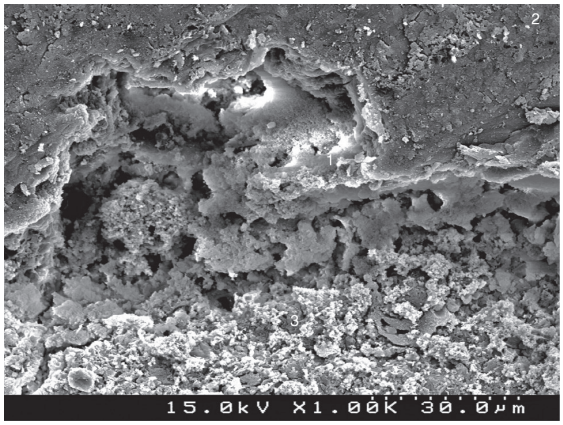
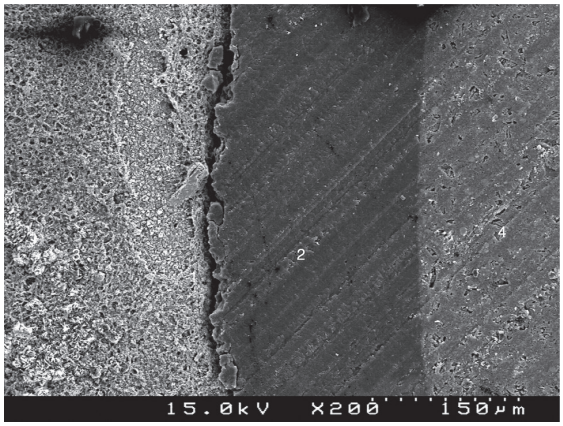
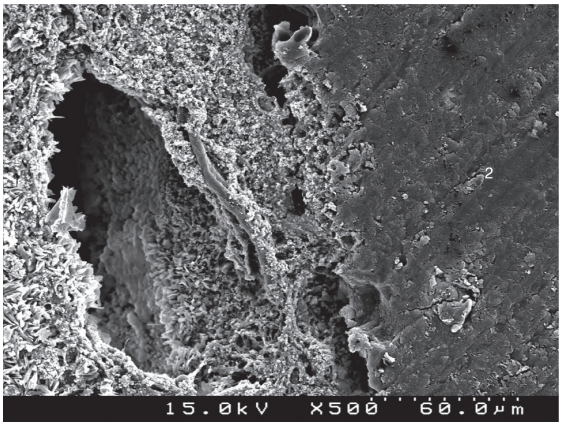


Figure 3.29 - A, B and C: A scanning electron micrograph of the interface of group 15 showing a deep interdigitation between the adhesive and the cement. A thick hybrid layer is presented (1) between the adhesive (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x200, 500 and x1000).





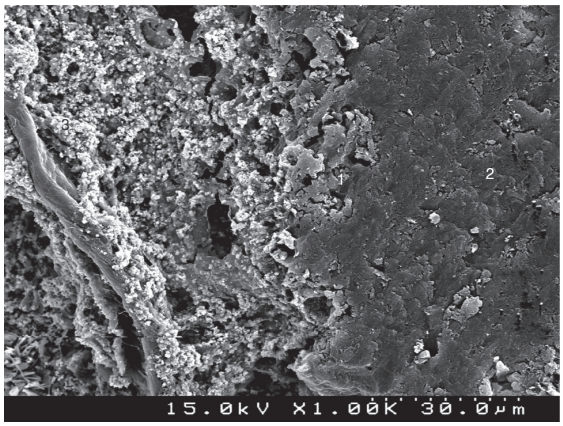


Figure 3.30 - A, B and C: A scanning electron micrograph of the interface of group 16 showing a less interdigitation between the adhesive and the cement and an interfacial gap on the hybrid layer. A less deep hybrid layer is presented (1) between the adhesive layer (2) and the NuSmile® NeoMTA (3). Composite resin (4) (original magnification x200, x500 and x1000).

Generally, in all the specimens the interpenetration between the HCSC and the adhesive systems were present, forming a hybrid layer or interdiffusion zone between adhesive and HCSC. The thickness and deepness of this layer varies essentially in accordance with the timing of restoration and adhesive procedure. In the delayed restoration group (7 days) this interpenetration was less deep than in the immediate groups.

Also, the pattern of the morphological interaction between the adhesive and the HCSC varies depending on the adhesive procedure and the time of restoration. In the Clearfil™ SE Bond 2 and in the groups with immediate restoration, the superficial "dissolution" of the HCSC and incorporation of particles into the adhesive layer was generally greater, as well as the adhesive filling of spaces between the inorganic content of the HSCS.

The thickness of the adhesive layer also varies according to the adhesive procedure. In general, it was thicker in groups with an additional layer of hydrophobic resin.

Some cracks and interfacial gaps observed can be related with artefacts due to technical preparation of the samples for SEM observation, primarily to the cutting and dehydration process.

2.2. Bond interface evaluation by confocal laser scanning microscopy

CLSM images allowed visualization the adhesive interfaces, enhancing the location of the adhesive labeled with Rhodamine B, by emitting a red fluorescence.

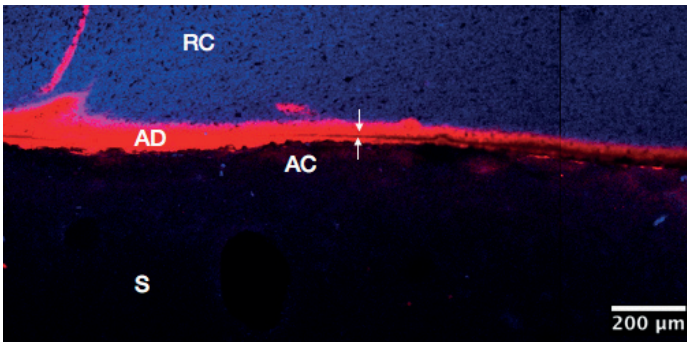


Figure 3.31. CLSM image of the interface of group 1 showing the penetration of the adhesive system into the HCSC. A debonded surface between the adhesive system and the HCSC is presented between the two arrows. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ SE Bond 2; AC - adhesive / HCSC hybrid layer; S - Biodentine™.

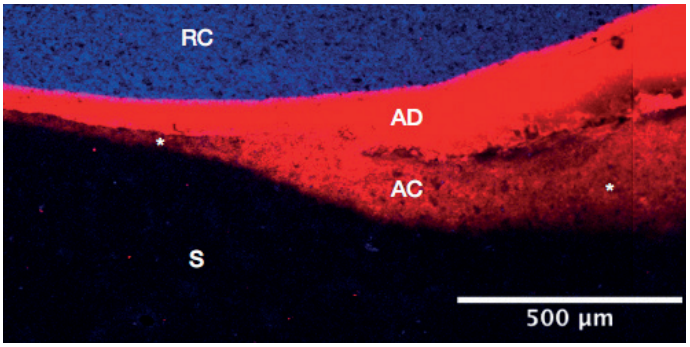


Figure 3.32. CLSM image of the interface of group 2 showing a non homogenenous thickness of the hybrid adhesive/HCSC layer along the whole extension of the HCSC (asterisk). RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ SE Bond 2; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

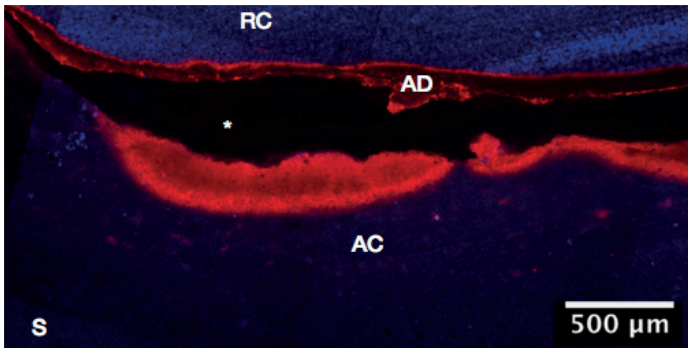


Figure 3.33. CLSM image of the interface of group 3 showing a considerable debonding surface within the adhesive layer (asterisk). RC - SDR™ Bulk-fill flowable composite; AD - Clearfil™ SE Bond 2; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

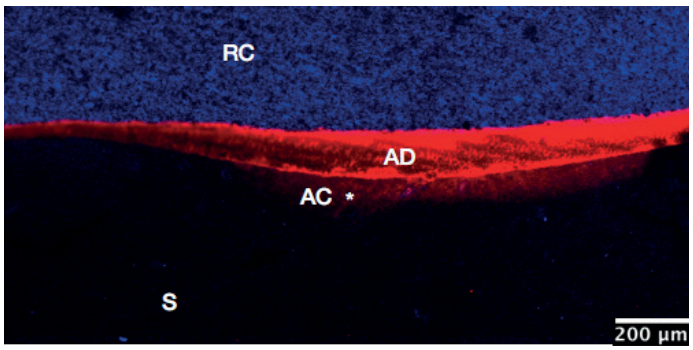


Figure 3.34. CLSM image of the interface of group 4 showing a thin layer of adhesive penetration (asterisk). The hybrid and adhesive layers thicknesses (intense red) are clearly discernible. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ SE Bond 2; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

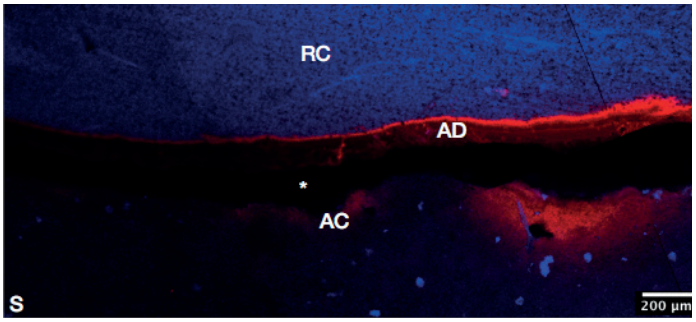


Figure 3.35. CLSM image of the interface of group 5 showing a thick non-uniform adhesive system/ Biodentine™ hybrid layer. A considerable debonding surface between the adhesive system and HCSC (asterisk) is present. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

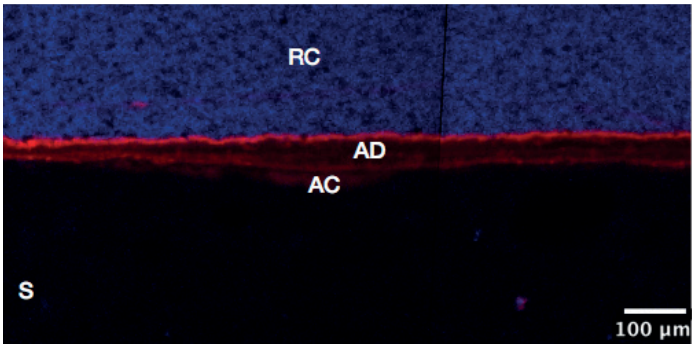


Figure 3.36. CLSM image of the interface of group 6 showing a regular adhesive layer, with a small penetration into the HCSC. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

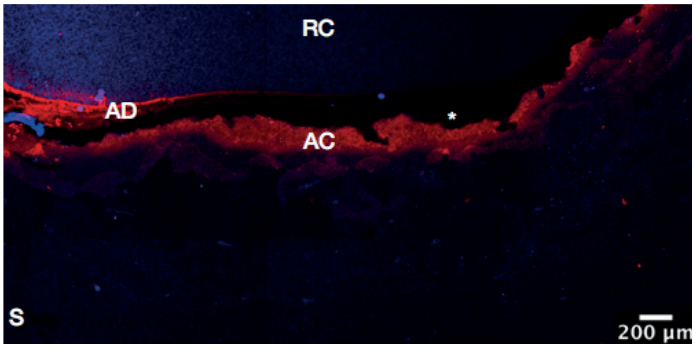


Figure 3.37. CLSM image of the interface of group 7 showing a deeper and irregular penetration of the adhesive system into the HCSC. The asterisk indicates a interfacial gap. RC - SDR™ Bulk-fill flowable composite resin; AD - Adhesive Clearfil Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

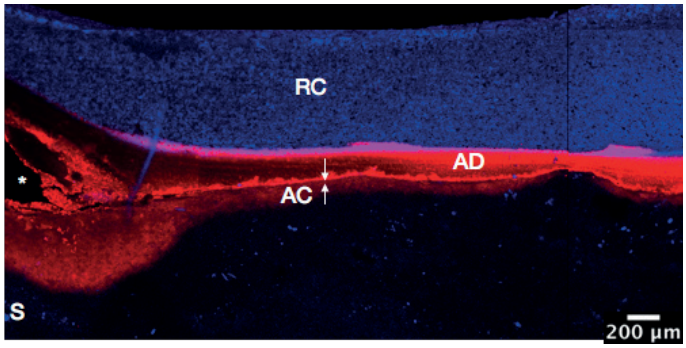


Figure 3.38. CLSM image of the interface of group 8 showing a regular and thick adhesive layer, but with superficial penetration into the HCSC surface. A lateral interfacial gap (asterisk) is present. The line between two arrows corresponds to the interface between the adhesive system and the top of the hybrid layer. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - Biodentine™.

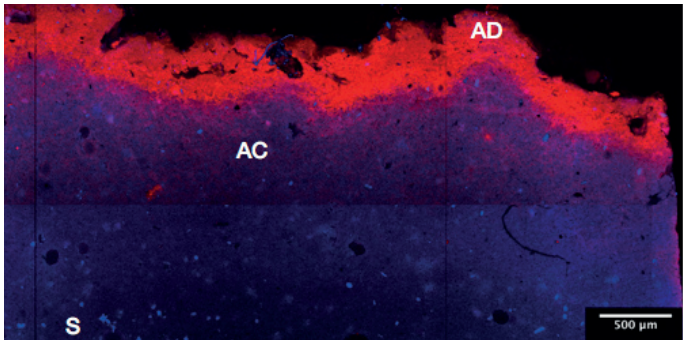


Figure 3.39. CLSM image of the interface of group 9 showing an irregular and very deep penetration of the adhesive into the HCSC. AD - Adhesive Clearfil™ SE Bond 2; AC - adhesive/ HCSC hybrid layer; S - NuSmile® NeoMTA.

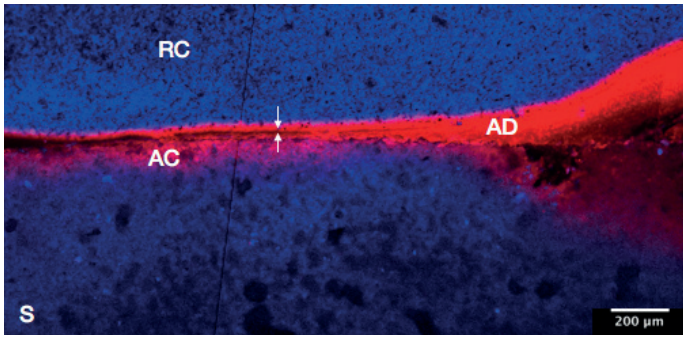


Figure 3.40. CLSM image of the interface of group 10 showing a more regular and superficial penetration of the Clearfil™ SE Bond 2 into the HCSC. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ SE Bond 2; AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

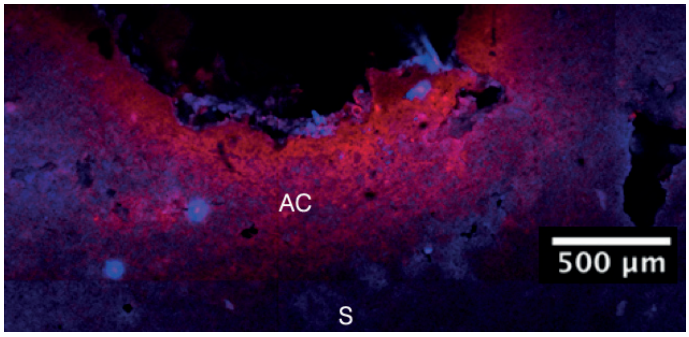


Figure 3.41. CLSM image of the interface of group 11 showing an irregular and very deep penetration of the adhesive into the HCSC and a detachment of the adhesive layer and composite resin from the top of the hybrid layer. AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

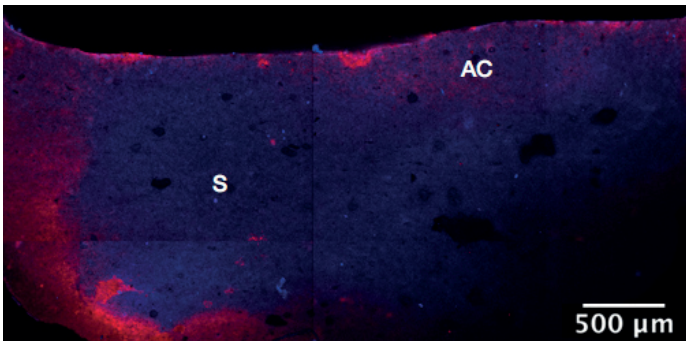


Figure 3.42. CLSM image of the interface of group 12 showing a more superficial penetration of the Clearfil™ SE Bond 2 into the HCSC. This picture results from the completely detachment of adhesive layer from the top of the hybrid layer. AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

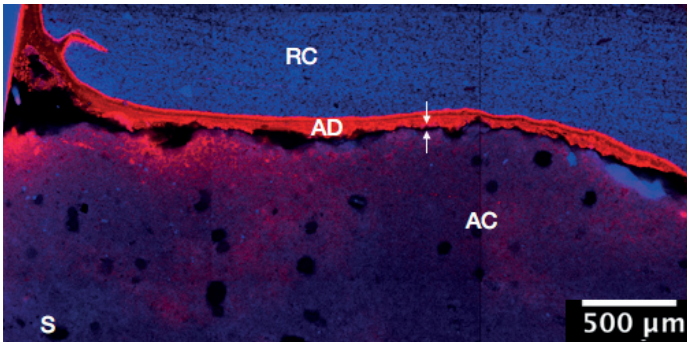


Figure 3.43. CLSM image of the interface of group 13 showing an irregular and very deep penetration of the adhesive into the HCSC. RC - SDR™ Bulk-fill flowable composite resin; AD - Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

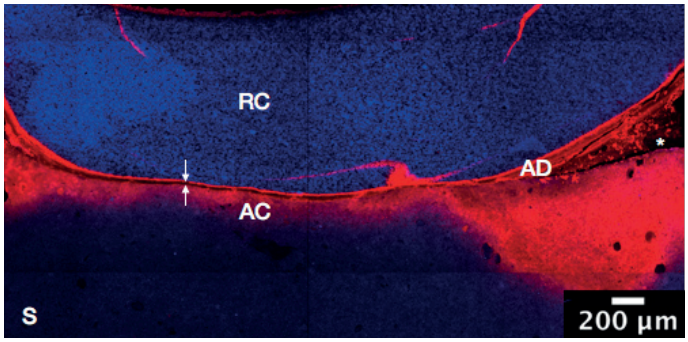


Figure 3.44. CLSM image of the interface of group 14 showing a more regular and superficial penetration of the Clearfil™ Universal Bond Quick into the HCSC. RC - SDR™ Bulk-fill flowable composite resin; AD – Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

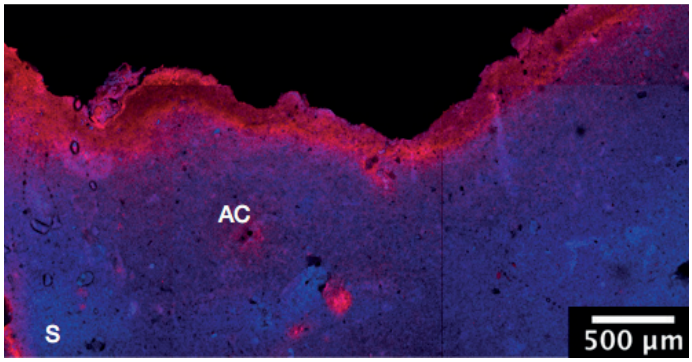


Figure 3.45. CLSM image of the interface of group 15 showing an irregular and very deep penetration of the adhesive into the HCSC and a detachment of the adhesive layer and composite resin. AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

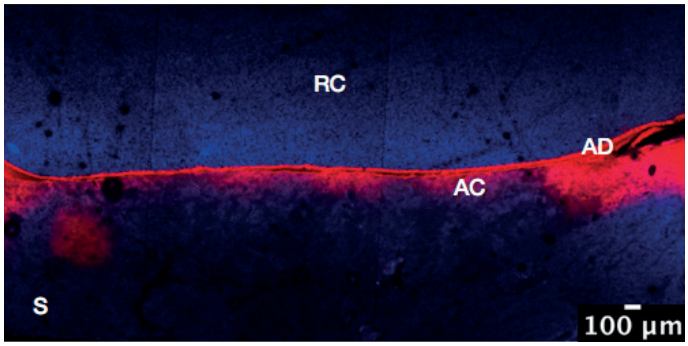


Figure 3.46. CLSM image of the interface of group 16 showing a more superficial penetration of the Clearfil™ Universal Bond Quick into the HCSC. RC - SDR™ Bulk-fill flowable composite resin; AD – Adhesive Clearfil™ Universal Bond Quick; AC - adhesive/HCSC hybrid layer; S - NuSmile® NeoMTA.

In a global way, it was possible to observe morphological differences in interaction pattern between the adhesives and the HCSCs across the different groups, both in terms of the regularity of the interface and the depth of penetration, resulting in hybrid zones with different patterns and dimensions. The restorations carried out immediately showed patterns of adhesive/HCSC interdiffusion zone that were more irregular and deeper than the groups in which the restorations were carried out after seven days. On the other hand, comparing both HCSC, NeoMTA® NuSmile® revealed a higher penetration rate than Biodentine™.

Chapter IV. Discussion

The regenerative vital pulp therapy (VPT) aims to seal the exposure site at the pulp–dentin interface in order to successfully prevent oral bacterial leakage, preserving pulp vitality and inducing dentin formation. The optimal end result of an adequate therapy is maintaining the long-term pulp vitality. Ideally, in cases of pulpal exposure, it includes the direct formation of a complete bridge of dentin by the pulp–dentin complex. The achievable therapeutic objective is to form a reparative dentin (tertiary dentin) by newly differentiated odontoblast-like cells, in direct continuum with reactionary dentin formed around the pulp exposure by the surviving primary odontoblasts (J. C. Ramos, 2007; Tziafas, 2010).

In the literature it is widely reported by clinical and experimental studies that a successful outcome of this treatment is mostly dependent on the type of injury, preoperative pulp status and the control of pre-operative and post-operative infection (Elmsmari et al., 2019). Additional clinical variables, such as the location of injury and the age of the tooth, may also affect the success rate. Hence, it is reasonable to suggest that the prognosis of pulp treatment depends on both the case selection, the existence of favorable conditions for pulp healing and the ability of a given therapeutic agent is capable to stimulate pulp–dentin regeneration (Tziafas, 2010).

The restoration over the VPT is a crucial parameter of success and remains one of the most challenging and unpredictable dental treatments for many decades (Bergenholtz, 2005). The risk of deficient bonding and sealing to the dentin substrate or loss of adhesion over time may result in potential leakage and open pathways for the penetration of infectious elements affecting the pulp tissue health and repair capacity (De Munck et al., 2005).

Hence, adhesives systems used in interface between HCSC and the final restorative material should have the ability to bond to the calcium-silicate cement after being applied, providing a proper seal, to be able to prevent leakage and remain in proper position under dislodging forces, such as chewing pressure (Schmidt et al., 2017).

The bonding characteristics of any lining material placed over HCSC are an important clinical factor, as well as the knowledge regarding the hybrid zone properties and characteristics between the restorative material and HCSC due to their implications on the VPT prognosis (Schmidt et al., 2017).

Thus, the optimum technique for bonding a composite resin to a HCSC is an important issue, with a potential high impact in clinical and histologic success. Many studies investigating specific bonding techniques and material combinations have been published to date (Altunsoy et al., 2015a; Bayrak et al., 2009; Odabas et al., 2013; Tunç et al., 2008). However, the wide variety of HCSC recently developed, bonding procedures, restoration times and different experimental conditions do not allow universal conclusions to be drawn (Atabek et al., 2012; Odabas et al., 2013).

The American Association of Endodontists recommends the use of a new material and/ or treatment protocol based on laboratory, biologic and clinical studies (American Association of Endodontics (AAE), 2017). The clinical trials are the most valid way to evaluate the quality and efficacy of materials and techniques and are the ultimate test for evaluating the success; however, long-term clinical trials are difficult to perform and they cannot identify the exact reason for failure due to the simultaneous impact of various factors within the oral cavity environment (Perdigão & Lopes, 1999; Meerbeek et al., 2003). The *in vitro* laboratorial tests, despite some limitations, have several advantages and applications, namely: the possibility to collect data quickly and easily in a more standard process; measuring one specific parameter, keeping all other variables constant; comparing the performance of a new and / or experimental material / technique with that of the current 'gold-standard', performing pre-screening

essays; ability to test various experimental groups simultaneously within one study set-up; the use of relatively unsophisticated and inexpensive test protocols / instruments. Even being impossible to have a single laboratory test or an assortment of tests capable to accurate predict the clinical performance of a specific material, they can expect the eventual clinical outcome in same conditions (Meerbeek et al., 2010).

A commonly used method to analyse an important part of the *in vitro* performance of an adhesive system to restorative material is the bond strength assessment. The bond strength evaluation tests include quantitative analysis, to predict the load capacity and longevity of the bonding and qualitative screening tests, to study bonding interfaces and bonding failures. Additionally, the laboratory tests may be also categorized into static or dynamic (depending whether the test specimen is stationary or dynamic) and in macro-tests (where the bond area is > 3 mm^2) and micro-tests (with < 3 mm^2 bond area) (Poitevin et al., 2010; Sirisha et al., 2014; Meerbeek et al., 2010).

Despite that bond strength tests are widely used to determine the interfacial strength between the bonded substrates, a consensus or standard approach is required and currently does not exist in Dentistry (Armstrong et al., 2010; De Munck et al., 2012). The strength-based testing does not quantify an inherent material property of the bond of restorative materials to the tooth structure (Van Noor et al., 1989). In fact, the bond strength measure and failure mode evaluation are influenced by various parameters related to the substrate (characteristics, size and geometry), to the composite and bonding area (material properties of each component, i.e. composite stiffness), operator skills and to the test design (i.e. crosshead speed, method of load application and different configurations employed to apply the shear force) (DeHoff et al, 1995; Leloup et al, 2001; Sudsangiam & van Noort, 1999). In particular, the limitation of the bonding area is an important factor that should be considered in the protocol test design and following the ISO/TS 11405; larger bonded area will produce lower SBS values due to the increased probability of the occurrence of critical sized defects (Sirisha et al., 2014b).

Academically, the SBS is defined as the interfacial adhesion between the substrate and the bonded material, intermediated by an adhesive layer. Therefore, testing involves two separate substrates and complicated interphases or zones of interdiffusion between these components because of the different materials properties (Schmidt et al., 2017).

In practice, the fracture may happen in the restorative material, in the bonding systems, in the substrate, or combined, and may extend beyond the initial bonded area (Schmidt et al., 2017), which means that the results cannot be analyzed as absolute test values and should cautiously be interpreted (De Munck et al., 2005). Even though, when gathered in a well-controlled design, shear bond strength tests can reveal valuable pre-clinical information (De Munck et al., 2005; Sirisha et al., 2014a).

The rationale behind these tests is that the stronger the adhesion between tooth and biomaterial, the better it will resist the stress imposed by resin polymerization and oral function. They are particularly valuable for the initial screening of new adhesive formulations on their bonding effectiveness and comparing experimental independent variables that are sometimes difficult to isolate and study *in vivo* (Armstrong et al., 2010; Braga et al., 2012; B. Meerbeek et al., 2010).

From the different bond strength tests developed in adhesive Dentistry, the shear and microtensile methods are the most currently used (Sirisha et al., 2014a), particularly the microtensile. However, attending the fact that HCSC are brittle in thin cross sections and must be used in bulk to avoid damage, it is not possible to subject them to this type of analysis (Neelakantan et al., 2012). On the other

hand, the shear tests allow simpler specimen preparation with a reduced risk of damage during sample preparation (Hashem et al., 2014). Considering all these characteristics, the shear bond strength test was chosen for the present study and the methodology followed the previous researches (Bayrak et al., 2009; Palma et al., 2020, 2018; Tunç et al., 2008).

The models used for sample standardized preparation were specifically developed by the Laboratory of Applied Biomechanics, Coimbra Institute of Engineering – Polytechnic Institute of Coimbra (Department of Mechanical Engineering) for this kind of studies and followed the characteristic previous described. In the published literature the dimensions of the central cavity of the mold from which the HCSC sample is produced differs between studies: in the study from Altunsoy e*t al.*, the hole had a diameter of 3 mm and a depth of 1.5 mm; Cantekin e*t al.* used samples with a central hole of 5 mm / 2 mm, coinciding with Palma e*t al.*; Odabas e*t al.* had a hole of 4 mm / 2 mm, according with Çolak e*t al.* (Altunsoy et al., 2015a; Cantekin & Avci, 2014; Çolak et al., 2016; Odabaş, et al, 2013; Palma et al., 2018).

Considering the studies abovementioned, in particular Palma e*t al.* which related the high frequency of cohesive fracture patterns within HCSC with the adhesive area, it was decided to use central holes of 4 mm / 2 mm, with a 360° deep groove allowing better retention of the filling material (Palma et al., 2018).

The use of gelatin capsules to build the composite resin blocks may contributed for the none occurrence of premature failure in our study. Besides the ease composite insertion, the procedure of capsule removal is very easy after storage in 100% humidity, not causing pressure or stress in the sample adhesive interface. Regarding the composite block dimension, in general many studies coincided (Carretero et al., 2019). In this study it had a diameter of 2.26 mm and a length of 3 mm approximately.

The MTA has been widely used in Endodontics and restorative Dentistry in deciduous and permanent dentition and is the most representative of the new class of HCSC. This is chiefly because of the following beneficial features: it causes less pulpal inflammation; its hard tissue formation is more predictable compared with calcium hydroxide containing materials; its ability to stimulate cytokine release from bone cells, thereby inducing hard-tissue formation; its dentinogenic effect on the pulp; its antimicrobial properties; its ability to maintain pulp integrity after pulp capping and pulpotomy without cytotoxic effects; high protection against microleakage and biocompatibility (Camilleri, 2014; Nair et al., 2008; Torabinejad, 2014).

Despite the desirable biological properties, the MTA have some drawbacks: low compressive and flexural strength and modulus of elasticity; long setting time, in particular the ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA), that can be up to 228 minutes; staining and poor handling characteristics (Camilleri et al., 2006; Kaup et al., 2015; Ramos et al., 2016). To overcome some of these shortcomings, fast-setting MTA-like calcium silicate cements, with improved mechanical properties and different radioopacifiers, have been developed (Camilleri, 2014; Shin et al., 2018; Torabinejad, 2014). In terms of setting time, the constant exposure to hydration from the dental tissues and the temperature of the mouth can be expected to drive the chemical reaction to completion. Several authors defend an additional appointment to apply resin-based restorative materials because they consider that more time between the placement of MTA and the final restoration is beneficial on the setting of MTA (Torabinejad, 2014).

MTA has been widely investigated resulting in more than 1000 articles published regarding this topic. However, no consensus exist for testing MTA or MTA-like cements, considering their unique properties and characteristics; all the testing methods for MTA products and experimental alternatives have been adapted from other dental materials and cements researches, beside the ISO 9917-1 2007 Dentistry-

Water-based cements. Part 1: Powder/liquid acid-based cement (Camilleri, 2014). There are several studies with variable results that evaluated SBS of resin composite to conventional MTA, compared to other pulp capping biomaterials bonded using different adhesive systems; nevertheless, to the best of our knowledge, none of them regarding SBS assessment of resin composite to NeoMTA or evaluated the combination of the independent variables analyzed in the present study (Altunsoy et al., 2015a; Atabek et al., 2012; Bayrak et al., 2009; Oskoee et al., 2014; Tunç et al., 2008).

Currently, dentin adhesives are based in one of two approaches, ER or SE bonding mode (Meerbeek et al., 2011; Wagner at al., 2014). The first approach needs the acid etch to create deep pits in the enamel hydroxyapatite and to demineralize dentin, exposing a hydroxyapatite poor or free collagen network (Pashley et al., 2011; Wagner et al., 2014). Conventional adhesive systems consist of a 3-step sequence, including phosphoric acid etching, priming and adhesive application (Shin et al., 2014). The SE system has been developed to simplify the bonding protocol, avoiding the etching by incorporating monomers with acidic functional groups that simultaneously bond to dentin and act as conditioners (Moszner et al., 2005; Van Landuyt et al., 2007).

The 6th and 7th generation dentin adhesives have been shown to be useful in PD, where behavior management of the patient is particularly important, by reducing procedure time, simplifying multi-step ER procedures and minimizing technical sensitivity (Ahmed et al., 2019).

The new multimode generation of adhesives has changed the traditional bonding adhesive protocol, using either an ER or SE systems and with an immediate clinical performance equivalent with that of gold-standard ER and SE reference adhesive systems, such as Optibond™ FL (Kerr, Orange, CA, USA) and Clearfil™ SE Bond 2, respectively. Recently, some manufacturers introduced the universal adhesives with a 'quick and flexible bonding' concept, claiming that some of them can be immediately light cured after its application, because the waiting time to guarantee its interaction with dentin and the solvent evaporation is no longer needed. Clearfil™ Universal Bond Quick is a 'no-wait' universal adhesive and it was used in this study, although the manufacturer's instructions were not followed since the long-term clinical performance still needs to be proven (Ahmed et al., 2019; Kuraray Noritake, 2017).

Clinically, dental adhesives and resin-based restorative materials were used for restoration of teeth with VPT, guaranteeing the conclusion of the conservative treatment. However, beside the SE adhesive systems and also some HCSC have been widely investigated, there is still a relevant lack of scientific information about its interface characteristics and implication in the treatment prognosis, namely concerning some clinical variables (Shin et al., 2018).

Most of the previous studies have evaluated the effect of various restorations materials and adhesives systems on the bond strength to MTA (Altunsoy et al., 2015a; Anastasiadis et al., 2018; Schmidt et al., 2017). It has been shown that the SBS to MTA was better with TE adhesive systems rather than with SE adhesive systems (Atabek et al., 2012). Further, composite resin with TE adhesive systems was suitable as a final restorative material over MTA (Tunç et al., 2008).

However, only a few studies have investigated the bond strength of composite resin to Biodentine™, and none regarding the SBS between NuSmile® NeoMTA and restorative materials. Hence, more studies are required to establish the effectiveness of restorations placed over Biodentine™ and NuSmile® NeoMTA.

According to our results the mean SBS varied between 11.36±5.72 and 3.62±2.78 MPa and the group NeoMTA U 0 7 exhibited the highest bond strength among all tested groups, followed by the NeoMTA

U I 7, with no statistically significant difference between them (p>0.05). The group Biodentine U 0 7 presented the highest mean SBS value compared to other Biodentine groups, and also with no statistically significant difference between this group and NeoMTA U I 7. The group Biodentine U 0 7 had a superior bond performance than NeoMTA SE 0 I, NeoMTA SE I I (p>0.05). The overall SBS results were not statically different between the two materials tested, Biodentine™ and NuSmile® NeoMTA.

The new HCSC formulation varies from traditional MTA, including finer particle sizes, which increase the surface area for faster hydration, shortening the setting time and improving handling characteristics (setting) (Primus et al., 2019). Also, through replacing the bismuth oxide as a radioopacifier by the stain-free tantalum oxide, which can bring relevant aesthetic advantages (Camilleri, 2015). Regarding particle size, NeoMTA Plus® dry powder has more regular structure, with smaller (10 μm or less) spherical particles (Siboni et al., 2017; Zeid et al., 2017). Biodentine™ has particles between 1–10 μm (Li et al., 2019). The literature has shown that the particle size affects the adhesion of cements to dentin by enhancing the interpenetration of these HCSC with it. Furthermore, it is believed that during setting, the small particles size leads to a significant decrease in the material's porosity and an increase in its compressive strength (About, 2016).

Since NeoMTA Plus® and Biodentine™ have similar particle's size and smaller compared to conventional MTA variants, this may be the reason for no statistically difference between the main effects of these two HCSC on SBS values (p-value 0.897).

Only the combination of Clearfil™ SE Bond 2 with an extra HBL and immediate restoration was statistically different between the two groups, Biodentine™ or NuSmile® NeoMTA.

It is important to consider that isolated SBS values cannot be used to draw absolute conclusions from, or be compared with data from other studies; only relative study outcomes are a valid basis for further interpretation of the results (De Munck et al., 2005). Furthermore, it is difficult to compare the SBS results obtained in other studies due to the variation of a several relevant parameters, such as restorative materials – conventional composite resins, flowable composite resins, glass-ionomer cements; adhesive systems and their technical application- ER systems, one-step SE and two-step SE systems; waiting and restoration time (Schmidt et al., 2017) and also differences in the experimental methods, i.e. the speed of load and the magnitude of maximum load when measuring SBS (Shin et al., 2018).

Even though, the results from the limited data concerning adhesion of restorative materials to set Biodentine™ that have been published to date reported that methacrylate-based composites and Biodentine™ can achieve optimal SBS values (17.7±6.2 MPa) (Cantekin & Avci, 2014). Also Odabas *et al.* evaluated the SBS of different adhesive systems to Biodentine™ and reported that the SBS of these materials varied between 15 and 19 MPa (Odabas et al., 2013).

On the other hand, Altunsoy *et al.* described the SBS of X-tra base (Voco GmbH, Cux- haven, Germany) or Vertise™ Flow (Kerr, Orange, CA) to Biodentine™ was 1.69 and 1.2 MPa, respectively. Deepa *et al.* referred that the SBS values for Biodentine™ overlaid by the composite resin (Filtek ™ Z-350 XT, 3M ESPE, St. Paul, MN, USA) with universal adhesive (Single Bond Universal ™, 3M ESPE, St. Paul, MN, USA) was 5.67±6.2 MPa, which are lower than the values of previous studies and the present study. Palma *et al.* assessed the SBS of the composite resin (SDR™ Bulk fill flowable composite) with adhesive system (Clearfil™ SE Bond) to Biodentine™ at two different times and results were 5.49±4.28 and 6.98±4.51 MPa, respectively (Altunsoy et al., 2015b; Deepa et al., 2016; Palma et al., 2020). Regarding the NeoMTA, to date, we are not aware of information regarding this type of tests that allows any type of comparison.

Currently, there is limited information about some important details on adhesion to HCSC. For instance, it is still unknown whether a chemical significant bond exists on the interface between them (Hashem et al., 2014). Since there is no resin structure in HCSC, such as NuSmile® NeoMTA or Biodentine™, it might be speculated that the bond is merely micromechanical and results from the interdiffusion and interlocking between the two materials, adhesive and HCSC (Oskoee et al., 2014). The hydrophilic characteristics of some monomers in adhesive systems can, in the first stage, facilitate this interdiffusion between the materials but in a second phase, it can also act as a negative factor with regard to excessive diffusion in depth and compromising the correct polymerization of the adhesive, and even of the cement setting reaction. On other side, it has to be kept in mind that the acidity of the adhesive or phosphoric acid may be buffered by the alkalinity of the calcium-silicate cement (Schmidt et al., 2017). The chemical composition of several current adhesive systems comprises a potential effect of chemical adhesion, not only to dentin, but also to HCSC, a subject that will be explored later.

According to Kayahan *et al.* the etching and rinsing procedures in the ER adhesive systems result in a selective loss of matrix from around the crystalline structures and produce a honeycomb etched appearance without penetrating deeply or removing substantial amounts of the cement. Moreover, these authors found that acid etch applied four hours after mixing MTA with water degrades the cement surface and reduces significantly its resultant compressive strength compared with the controls (Kayahan et al., 2009). Therefore, they only recommend performing restorative procedures with ER adhesives 96 hours after placing MTA (Kayahan et al., 2009). However, some authors also suggest that the changes promoted by acids over MTA surface might potentially improve the adhesion of resin composites. As an alternative, layering the MTA surface with GIC did not improve the microhardness and / or the sealing ability of MTA (Camargo et al., 2012).

When Biodentine™ is etched with 37% phosphoric acid for 20 s after 12 min of mixing, both structural and chemical changes can induce leakage (Camilleri, 2013); despite this fact, it was shown that etching time (5, 10, 15 s) does not affect the resin bond (Shafiei et al., 2019).

According to Shin *et al.*, the application of adhesive systems in either SE or TE modes was not seen to have statistically significant influence in bond strength to the composite. This in concordance with Hashem *et al.*, who reported that similar SBS of SE and TE were caused by the porous surface structure of Biodentine™, which may imply that there is no difference between the SE and TE techniques (Hashem et al., 2014). Finally, other authors have concluded that sufficient bonding performance may also be obtained without an acid etching procedure simplifying the adhesive step, since universal adhesive systems applied on Biodentine™ showed similar bond values in SE and ER modes (Shin et al., 2014).

On the other hand, Odabaş *et al.* revealed that two-step SE adhesive system (Clearfil™ SE Bond) exhibited higher shear bond strength, than one-step SE adhesive system (Clearfil™ S3 Bond) (Odabaş et al., 2013). This result was in agreement with those of previous studies, which found that the bond strengths of two-step SE adhesives were higher than those of one-step SE (De Munck et al., 2003).

Deepa *et al.* concluded that the universal adhesive - Single Bond Universal ™, 3M ESPE, St. Paul, MN, USA, used as a SE showed a SBS mean of 5.66 MPa (Deepa et al., 2016). This is in contrast with Carretero *et al.*, who used Scotchbond Universal® (3M ESPE, St. Paul, MN, USA) and found a value of 13.65 MPa, even both have respected the Biodentine™ setting time - 12 min (Carretero et al., 2019). These different results may be due to the different adhesive composition or inherent to the operator / technique variable.

There is controversy concerning the efficacy of SE systems applied over HCSC. Some investigations showed that they provide dentin bond strength comparable with that obtained with ER system (Borges et al., 2007; Cacciafesta et al., 2003), whereas others have observed significantly lower bond strengths (Bishara et al., 2001; Yamada et al., 2002).

In this research, the protocol design considered new adhesive strategies rather the bonding agents per se. The mean shear bond strength varied between 3.62 and 11.36 MPa. Concerning the overall main effect "adhesive system", the use of two-step self-etch adhesive Clearfil™ SE Bond 2 resulted in a weaker bond (p-value <0.001) compared with Clearfil™ Universal Bond Quick. However, concerning inter groups comparison, only the combination of NuSmile® NeoMTA with no extra HBL and delayed restoration was statistically different between the two adhesives.

Furthermore, the combination Biodentine SE 01 and NeoMTA SE 01 have presented the lowest bond strength in the Biodentine™ and NuSmile® NeoMTA groups, respectively. We hypothesized that these may be due to precocious application of the primer over HCSC. Although, Clearfil™ Universal Bond Quick had significantly higher values of bond strength than Clearfil™ SE Bond 2 in the present study, both contain similar functional monomers. The major difference between them is the thickness of the adhesive layer and primer application. Jang *et al.* reported that the thickness of the adhesive layer of the Clearfil™ SE Bond 2 was approximately 40 µm (Jang et al., 2016), but that of Clearfil™ Universal Bond Quick was approximately 5-10 µm (Kuraray Noritake, 2017). Although this characteristic does not adversely influence the bond strength, it may cause imperfect restorations in some clinical situations (Shin et al., 2014).

We also focused on the selection of adhesives according to their pH values, beside the functional monomers, HEMA and 10-MDP.

The functional monomers in different dentin adhesives are important to improve their clinical performance, by increasing the bond strength with teeth. The specific functional monomer 10-MDP has potential chemical bonding by reacting with calcium ions from dentin and from HCSC and, consequently, increasing adhesion efficacy (Yoshida et al., 2004).

In the present study, both adhesive systems contain the 10-MDP functional monomer and theoretically it could be presumed that the 10-MDP monomer may link chemically to the calcium in Biodentine™ and NuSmile® NeoMTA, hence promoting chemical adhesion in addition to micromechanical attachment (Hashem et al., 2014; Yoshida et al., 2004).

The HEMA monomer is hydrophilic and is similarly present in both adhesives systems. It forms a polymeric network able to stabilize the outer surface of the cement material after photopolymerization and absorbs moisture to aid hydration to the calcium silicate cement setting reaction (Gandolfi et al., 2011). However, the hydrophilic nature of many simplified adhesives is one of the most documented factors responsible for the hybrid layer degradation (Armstrong et al., 2017; De Munck et al., 2005; Tjäderhane et al., 2013). Furthermore, HEMA has a relatively high allergic potential, lower polymerization efficiency, high water uptake and reduced nanolayering by the 10-MDP. For the latter HEMA disadvantages, new adhesives have been marketed with lower HEMA content or even without any HEMA (Ahmed et al., 2019; Van Landuyt et al., 2008; Yoshida et al., 2012). The HEMA content in Clearfil™ Universal Bond Quick is 2.5 to 10%, compared to earlier Clearfil adhesive generations, in particular Clearfil™ SE Bond, which is 10 – 30 % (Ahmed et al., 2020; Altunsoy, et al., 2015). As a consequence of this reduction, water sorption is claimed to be reduced and polymerization conversion improved. This positive effect

seems to be reflected in the absence of its bond degradation upon 6 months aging when applied in both ER and SE bonding modes (Ahmed et al., 2019). Nevertheless, the water content of the cements themselves can remain a problem for the polymerization and stability of the adhesives.

The pH is an additionally important parameter. Previous findings by other investigators have established that a low pH can interfere in the setting of MTA-based materials and that an acidic environment enhances the release of Ca^{2+} ions in the bioactive cements (Lee et al., 2018; Rodríguez-Lozano et al., 2019). However, there is still limited information available regarding the changes in the biological properties of these materials after exposure to an acidic environment (Agrafioti et al., 2016; Tian et al., 2017). Moreover, previous reports demonstrated that low pH adhesives have low bond-strength values (Bayrak et al., 2009)

Concerning pH, the adhesive systems selected Clearfil™ SE Bond 2 and Clearfil™ Universal Bond Quick are classified, according to Tay and Pashley classification (Tay & Pashley, 2003), as mild self-etch adhesives (pH >2): Clearfil™ Universal Bond Quick pH ≈2.3; Clearfil™ SE Bond 2 pH ≈2.5 (Kuraray Noritake, 2016b, 2016a). Thus, the pH of 1-step SE adhesive is not likely to be the major contributor to its superior bond strength to the HCSC.

The organic solvents that act as carriers of the monomers into the collagen fibers network in dentin and as diluents to lower the resin viscosity, can also enhance the infiltration of resins into the micr-oporosities and spaces (Van Landuyt et al., 2007). In this aspect, other potential reason explaining the superior performance of Clearfil™ Universal Bond Quick is the better wettability of ethanol and water presented in its composition, in contrast to Clearfil™ SE Bond 2, which contains only water as a solvent (Neelakantan et al., 2012).

The degradation potential of resin–dentin interfaces present in the simplified one-step SE adhesives results, at least in part, from the water absorption from the environment through osmosis. This interferes with the cross-linked polymers formation and consequently a porous hybrid layer is produced because of the elution on unreacted monomers (Reis et al., 2013; Landuyt et al., 2007). One of the approaches used to bypass this drawback is the application of an additional layer of a hydrophobic resin over the polymerized adhesive (Reis et al., 2008, 2009). Previous reports have described its improved performance and degradation prevention of the resin-dentin bonds, as a consequence of the increasing thickness and uniformity of the adhesive layer, as well as to reduce the fluid flow across the adhesive interface (Andrade e Silva et al., 2009; Vinagre & Ramos, 2016).

However, this method has not been tested with SE adhesive systems applied over HCSC in order to evaluate the bond strength and interface structure between them. In the present study, we tested the null hypothesis that the application of an additional hydrophobic resin layer over the cured adhesive placed over Biodentine™ and NuSmile® NeoMTA will not influence the bond strength, which was rejected. Concerning main effect "application of an additional HBL" the overall analyses showed that this procedure significantly increases the SBS values. Although in 5 of the 8 inter-group comparisons the values were higher with the presence of an HBL, only the combination Biodentine Clearfil™ SE Bond 2 without HBL and immediate restoration was statistically different from the combination Biodentine Clearfil™ SE Bond 2 with HBL and immediate restoration.

In the literature there are many studies that evaluated the bond strength between restorative materials and MTA. According to our knowledge there are only eight studies published regarding the Biodentine™ (Altunsoy et al., 2015b; Cantekin & Avci, 2014; Nekoofar et al., 2018; Mustafa et al., 2020; Odabas et

al., 2013; Raina, et al., 2020; Schmidt et al., 2017; Tulumbaci et al., 2017). In all these studies the samples were stored in the same conditions: 100% humidity and 37 °C, but they differed in the storage time. In the data published it is presented that increasing the period of time did not have a significant effect on the mean SBS values (Carretero et al., 2019).

The Biodentine™ manufacturer indicates that after 12 min, the restorative treatment can be done (Altunsoy et al., 2015a). It begins with an initial setting reaction, which takes approximately 12 min following mixing the powder with the liquid, where a hydrated calcium silicate gel structure is formed, but with a weak structure. The surface set is achieved at this stage. The maturation of Biodentine™, where crystallization of the calcium silicate hydrated gel structure, continues for up to 2 weeks. Bulk set is achieved at this stage with improved physicomechanical properties (Hashem et al., 2014).

The NuSmile® NeoMTA, and according to the manufacturer information, allows composite application after 3 min of mixing and it takes 3 hours for the final setting (NuSmile, 2014). This is a fast-set material, very easily manipulated and remains in place without being washed out because of the gel properties. Unfortunately, scientific little information is available regarding this material and its hydration reaction is not yet well understood (Zeid et al., 2017).

For both HCSC evaluated in the present study, the setting time is shorter than for MTA and bonding the final restoration directly after mixing the calcium-silicate cement is worthwhile, as this would be easier and less time consuming. However, the quality and durability of the adhesive bond between HCSC and the filling material is clinically important in terms of the longevity and predictability of the final restoration. The adhesion between restorative material and HCSC influences the quality and durability of the final restoration (Hashem et al., 2014). Therefore, a higher level of HCSC setting is necessary before the restoration is done, since the durability of this bond may be affected by the state of the calcium-silicate cement (set or unset) and the curing shrinkage of the composite may stress the unset calcium-silicate cement. On the other hand, the composites and glass ionomer cement, when placed directly over freshly mixed calcium-silicate cement, may negatively affect the proper setting of the HCSC (Schmidt et al., 2017).

In this study the delayed restorations (after seven days) revealed a better bond performance compared to immediate restorations. However, the restoration timing was only significant for the combination NeoMTA, Clearfil™ Universal Bond Quick without an additional HBL, showing better results for delayed restoration.

For instance, and according to Hashem *et al.*, the importance of allowing Biodentine™ to set for a longer time period before application of a final restoration is because this HCSC has low initial cohesive strength in its early setting phase, that may result in its low shear bond strength (Hashem et al., 2014); so that, the Biodentine™ as a porous material needs at least two weeks for complete setting and crystallization of hydrated calcium silicate gel, to have bulk strength sufficient to achieve optimal physical properties (Deepa et al., 2016).

In agreement with this, Kaup *et al.* reported a significant increase in the shear bond values of Biodentine™ to permanent tooth dentin between 2 days and 1 week storage times and compared to that of MTA (Kaup, et al., 2015).

Concerning Biodentine™ failure pattern analysis, a cohesive fracture pattern within HCSC might reflect its low cohesive resistance compared to a high bond strength value (Palma et al., 2018). The literature

is scarce and with no consensus regarding fracture failure type between the HCSC and restorative composite (Carretero et al., 2019). Odabas *et al.* described more cohesive fractures (Odabaş et al., 2013). Deepa *et al.* showed both 60% cohesive fractures and 40% adhesive fractures (Deepa et al., 2016). Palma *et al.* reported approximately 50% of cohesive fracture patterns regardless of the restoration timing (Palma et al., 2018). Tulumbaci *et al.* found mostly adhesive failures (Tulumbaci et al., 2017). In opposite, Altunsoy *et al.* didn't have failures of the adhesion (Altunsoy et al., 2015a).

In accordance with the literature, the Biodentine™ and NuSmile® NeoMTA groups presented both similar rate of cohesive failures, but with no statistically significant association between the fracture type and the HCSC used. So, concerning fracture patterns, both materials, Biodentine™ and NuSmile® NeoMTA, presented a similar behavior.

To achieve a successful restorative treatment with two materials with different characteristics, there should be an appropriate bond on the interface of these two materials to guarantee the long-term success (Hinoura et al., 1991). In a simplistic analysis, the bond is considered acceptable when fracture occurs within the material, rather than in the bonded interface (i.e. cohesive fracture rather than adhesive) (Tate et al., 1996). However, regarding the interfacial adhesion between two substrates, if the adhesive procedures significantly interfere with the cohesive properties of the substrates, the assumption of satisfactory results based on cohesive fracture patterns is not applied.

Failure mode analysis showed a greater number of samples exhibiting more cohesive fractures in HCSC in both adhesive materials, followed by adhesive fractures in Clearfil™ SE Bond 2 and mixed fractures in Clearfil™ Universal Bond Quick, with a statistically significant difference between the two adhesives.

Regarding the application of an extra HBL, there was a statistically significant association between the fracture pattern and the application of an extra HBL. The more prevalent fracture pattern was cohesive in HCSC, followed by adhesive fracture in the group with an additional HBL and mixed in the group without an additional HBL.

Also, an association statistically significant was verified between the fracture pattern and timing restoration ($p<0.001$). The delayed restoration group had more adhesive failures compared with the immediate group. Moreover, the immediate restoration had more cohesive failures in the HCSC.

Theoretically, the stresses induced by composite shrinkage during polymerization can result in premature cohesive failure in the weak first stage of HCSC setting. In the present study, this did not happen in any group, at least causing the total sample fail before testing.

Similarly to our results, Palma *et al.* referred that the cohesive pattern was mostly present in the immediate group, whereas the adhesive failure mode had a higher rate in the delayed group (seven days) (Palma et al., 2018). Çolak *et al.* also described the cohesive pattern as the most prevalent, after the samples were stored in distilled water for a period of 24 hours (Çolak et al., 2016). These results are in contrast with Schmidt *et al.* that presented 70% of mixed fractures after 12 minutes (Schmidt et al., 2017). This difference may be due to fact that, in the last study, the specimens were stored for 28 days after application of the restorative material to guarantee a complete setting of the HCSC before SBS testing.

Altunsoy *et al.* applied the composite resin after 72 hours over the Biodentine™ and did not find any adhesive fractures, instead found cohesive or mixed (Altunsoy et al., 2015a). After 24 hours, Deepa *et al.* found 60% cohesive and 40% adhesive fractures, like Tulumbaci *et al.* had mainly adhesive fractures (Deepa et al., 2016; Tulumbaci et al., 2017).

Unlike most studies, which the tests were performed immediately after the restoration, in the present research and to avoid premature cohesive fractures within the incompletely set HCSC, the SBS tests were performed 48 hours after the restorative treatment procedure. Even though the cohesive pattern was the most prevalent in the immediate group.

There weren't cohesive fractures within the composite resin, as it happens in Palma *et al.* and Odabas e*t al.* (Odabaş et al., 2013; Palma et al., 2018).

Complementarily, in order to characterize the adhesive interfaces between HCSC and adhesive composite resin restorations an ultramorphological analysis, by SEM and CLSM, was performed.

Generally, in all the scanning electron micrographs from all the specimens, the interpenetration between the HCSC and the adhesive systems were presented, forming a hybrid layer or interdiffusion zone between adhesive and HCSC. The thickness and deepness of this layer varies essentially in accordance with the timing of restoration and adhesive procedure. In the delayed restoration group (7 days) this interpenetration was more regular and less deep than the immediate groups. The thickness of the adhesive layer was higher in groups with an additional layer of hydrophobic resin.

The pattern of morphological interaction of the adhesive with the HCSC was also affected by these two variables. In the Clearfil™ SE Bond 2 and in the groups with immediate restoration the superficial "dissolution" of the HCSC and incorporation of particles into the adhesive layer was commonly more evident, as well as the adhesive filling of spaces between the inorganic content of the HSCS. Some of these spaces were probably observed in SEM to be empty due to a possible wash-out effect of the adhesive, and even of HCSC particles during the preparation of the cuts for observation.

Some cracks and interfacial gaps observed in many samples can be related with artefacts due to technical preparation of the samples for SEM observation, primarily to the cutting and dehydration process.

CLSM has been widely used in adhesive Dentistry research since it is a simple method to evaluate the interfusion of dental materials to the dentin (Pioch et al., 1997; Watson, 1989, 1997). Particularly, it is ideally suited for investigation of the penetration, fit and thickness of adhesive bonding agents used in dental restorations (D'Alpino et al., 2006; Watson, 1989). This method has been used for investigating the distribution of primer and adhesive inside the hybrid layer and the dentinal tubules (Griffiths & Watson, 1995; Watson, 1989) and for nanoleakage analyses (D'Alpino et al., 2006; Pioch et al., 1997), as it offers a number of advantages over other techniques, that include: non-destructive examination of the samples; samples can be studied without vacuum in a humid environment; non-dehydrated samples can be used - drying of samples, which are indispensable for conventional SEM or TEM, are not necessary with CLSM leading to a decreased risk of shrinking or other drying artefacts; no specific sectioning technique is required; it can provides three dimensional images (Marciano et al. 2010; Pioch et al. 1997; Ordinola-Zapata et al. 2009).

It is of major importance the right selection of the dye for the fluorescence microscopy, since it might influence the adhesive polymerization and, therefore, its properties and performance (D'Alpino, et al., 2006). Depending on its concentration, the dye presence can absorb the polymerization light, reducing the monomer conversion of the bonding agent and reducing the polymer formation. The dyes can partially block the light from reaching the photoinitiators. The bonding resin not properly polymerized may affect the hybrid layer structure, change their mechanical properties and bond strengths of materials to their substrates (D'Alpino et al., 2006; Takahashi et al., 2002). Furthermore, the labelling of dental adhesives refers to a simple mixing process, in opposite to a covalent linkage between fluorescent molecules and the adhesive monomers. However, and even being inert, this may lead to the risk of

non-homogeneous dye distribution or dye leaching from cured adhesives, interfering with the analysis of microscope images (D'Alpino et al., 2006).

In the present research, Rhodamine B was the dye selected, in alternative to fluorescein or methylene blue, since it is routinely used for fluorescence microscopy (Diaspro et al., 2001; Pioch et al., 1997), particularly in adhesive Dentistry (Aguiar et al., 2012; Arrais et al., 2009). This dye is soluble in water, highly soluble in organic solutions and is stable under different pH conditions (Pioch et al., 1997). Nevertheless, its acidic pH may enhance its penetration (Wu et al. 1998, Souza et al. 2009). On the other hand, as a fluorescent dye, its presence can easily be made out since of the specific fluorescence. Therefore, there is no need to search for the dye since it shows by itself. It is also more sensitive than methylene blue and more important, doesn't change in the presence of materials rich in calcium oxide that leads to increased pH and may cause discoloration of the surfaces marked, like happen with methylene blue (Padey et al., 2018).

Although in the literature there are a few studies describing the application of this method of microscopy to analyze HCSC interfaces (Makkar et al., 2015; M. Torabinejad et al., 1993; Viapiana et al., 2016), as long as our best knowledge, none evaluated the penetration of the adhesive systems into HCSC, in order to analyze the interface between adhesive restorations placed over HCSC. As a consequence, there is a substantial lack in the literature regarding this methodology, materials and concentrations used, and subsequently study results to compare with.

One of the limitations found during the confocal analysis was that the bonding agent follows all the discontinuities or defects in HCSC and adhesive interfaces. Thereby, it would jeopardize the adhesive penetration measure into de HCSC, which happened in some groups; for this reason, when assessing the depth of penetration of the adhesive in the HCSC, areas close to the margins and defects of the restorations were disregarded.

The penetration of adhesive stained with Rhodamine B into the HCSC occurred in all groups; however, this infiltration was more evident in the NeoMTA NuSmile®, compared to Biodentine™, and in the immediate compared to delayed restorations. These findings are in accordance with SEM analysis.

Chapter V. Conclusions and future directions

Contemporary Dentistry is based on minimally invasive treatments; this assumes a major importance in Pediatric Dentistry field attending the characteristics of deciduous and young permanent dentition, the particularities of the pediatric patients and considering the treatments long-term follow-up.

In this work we assessed the interfacial adhesion and morphology between the regenerative and restorative materials used in vital pulp therapy. Concerning the objectives initially defined and considering the limitations of the methodologies employed, we can conclude that:

- The shear bond strength to Biodentine™ and NuSmile® NeoMTA was similar; however, both adhesives tested penetrated deeper in the NuSmile® NeoMTA, compared to Biodentine™.
- Clearfil™ Universal Bond Quick provided higher shear bond strength to calcium silicate-based cements evaluated compared to Clearfil™ SE Bond 2.
- The application of an additional hydrophobic resin layer over the adhesive improved the shear bond strength of composite adhesive restoration placed over calcium silicate-based cements.
- The delayed definitive composite restorations placed after seven days provided higher shear bond strength than immediate restorations.
- The SEM and CLSM morphological evaluation of adhesive/HCSC interfaces revealed some important aspects. Both techniques have identified the interdiffusion and inter-locking between the adhesives and calcium silicate-based cements, but with differences between the groups. Both adhesives penetrated deeper into the NuSmile® NeoMTA, compared to Biodentine™. Also, the penetration depth of the adhesives into the calcium silicate-based cements was higher in the group of immediate adhesive restorations, compared to those performed on the seventh day.

Overall, and within the limitations of an *in vitro* study, we believe that these relevant findings highlight the importance of an adequate choice of materials and techniques in order to optimize the clinical procedures. In addition, they reveal new problems and issues, with potential clinical implications, which can and should be evaluated by new and different studies.

The laboratory studies permit to predict the regenerative and restorative performance of HCSC and the adhesive systems. The influence of different variables suggests the necessity for additional research under thoroughly controlled experimental conditions.

In this study the total setting time of Biodentine™ and NuSmile® NeoMTA was not considered; future studies should observe the influence of the setting on the adhesion to the restorative material by evaluating the effect of allowing more time between the application of Biodentine™ and NuSmile® NeoMTA and the definitive adhesive restoration.

Further studies should include HCSC with different thickness, reproducing the different types of VPT, from the thin layers used in small direct pulp capping, to 2-3 mm applied in the pulpotomy. Also, since this is a radiopaque material, it would be interesting to evaluate the light penetration from the UV into cement and how deeply is the adhesive polymerized.

Complementarily to the knowledge from the underlying mechanisms of the adhesion to HCSC resulted from microscopy imaging, the molecular interactions at deeper layers should also be assessed, in order to understand how the interlocking relation and the deeper penetration of the adhesive monomers into HCSC may interfere with the biological properties of these materials, namely their biocompatibility and dentinogenic effect.

By carrying out *in vivo* studies, all possible micromechanical properties of HCSC and adhesive systems may be investigated accounting with their interaction and host conditions. Thereby, it would be possible to disclose which therapeutic strategy is truly reliable for the restoration of VPT.

Later, the clinical trials remain the ultimate way to collect scientific evidence on the clinical efficacy of these regenerative and restorative treatments attending the particularities of the PD clinical practice.

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Chapter II. Experimental procedures

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Annexes

Annexes

Annex I: Ethical approval from the FMUC ethics committee

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

COMISSÃO DE ÉTICA DA FMUC

Of. Refª **002-CE-2020**

Data 20/1/**2020**

C/conhecimento ao aluno

Exmo. Senhor

Presidente do Conselho Científico da FMUC

Assunto: Projeto de Investigação no âmbito do Programa de Doutoramento em Ciências da Saúde (refª CE-001/2020)

Candidato(a): Maria Teresa Antunes de Azevedo Xavier

Título do Projeto: ***"Avaliação das interfaces entre biomateriais regenerativos / restauradores usados em tratamentos pulpares conservadores".***

A Comissão de Ética da Faculdade de Medicina, após análise do projeto de investigação supra identificado, decidiu emitir o parecer que a seguir se transcreve:

"Parecer favorável".

Queira aceitar os meus melhores cumprimentos.

O Presidente,

Prof. Doutor João Manuel Pedroso de Lima

HC

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Annex II: Randomized controlled trials regarding the application of calcium silicate cements in permanent and deciduous teeth

Table A. Permanent Teeth

Study	Patients			Teeth		Clinical Information		Restorative treatment	
	n	Average [range]	Male/ Female	n	Teeth Groups	Diagnosis	Procedure	Material	Timing
ProRoot MTA vs Biodentine									
Abuelniel et al., 2020	33	7.5-9	n/a	50	I Central immature	RP	PP	GIC+C	I
Bakhtiar et al., 2017	27	[18-32]	n/a	27	3°M Mx 3°M Md	NP	PP	GIC	I
Brizuela et al., 2017	116	11.51 11.22	56/60	116	Mx: 1°M:33 2°M: 2 Md: 1°M:68 2°M:13	NP RP	DPC	C	I
Katge & Patil, 2017	29	n/a	n/a	58	Molars	RP	DPC	GIC / C C	3M
Nowicka et al., 2015	n/a	n/a	n/a	22	3°M Mx 3°M Md	NP	DPC	GIC / C C	1W
Parinyaprom et al., 2017	59	[6-18]	n/a	55	23Mx 32Md	NP RP IP	DPC	GIC+C/A/ SSC C/A/SSC	I
ProRoot MTA vs Portland									
Yildirim et al., 2016	n/a	n/a	n/a	70	1°M(33) 2°M(37)	RP	PP	GIC+SSC	I
Angelus MTA vs Biodentine									
Awawdeh et al., 2018	58	n/a [16-59]	23/35	68	I(5) PM(18) M(45)	RP	DPC (17) PP (51)	A,C	1W
ProRoot MTA vs 1 Root BP									
Azimi et al., 2014	12	14 [12-16]	n/a	24	1°PM	NP	PP	RMGIC+C	I
ProRoot MTA vs CEM									
Asgary et al., 2017	412	[9-65]	n/a	412	1°M 2°M	IP	PP	Cavit+A	1W
ProRoot MTA vs OrthoMTA									
Kang et al., 2017[2]	82	29.3	31/51	104	M PM	RP	PP	C,Cer	2-3D

Follow-up									
6 months		1 year		2 years		5 years		Other	
Clinical	x-ray	Clinical	x-ray	Clinical	x-ray	Clinical	x-ray	Clinical	x-ray
ProRoot MTA vs Biodentine									
100 25/25 100 25/25	88 23/25 96 24/25	84 22/25 88 21/25	88 22/25 96 24/25	n/a		n/a		18M 88 22/23 80 20/23	18M 92 21/23 100 23/23
n/a		n/a		n/a		n/a		2M 100 100	
91,9 34/37 100 38/38		100 22/22 100 25/25		n/a		n/a		n/a	
100 (21/21) 100 (21/21)		100 (21/21) 100 (21/21)		n/a		n/a		n/a	
n/a		n/a		n/a		n/a		1,5M 100 11/11 100 11/11	
91,9 (34/37) 100 (38/38)		100 (22/22) 100 (25/25)		n/a		n/a		n/a	
ProRoot MTA vs Portland									
100 34/34 93,9 31/33	n/a	100 33/33 93,5 29/31	n/a	100 33/33 93,3 28/30	93,9 31/33 86,7 26/30	n/a		n/a	
Angelus MTA vs Biodentine									
93.5 (29/31) 93.1 (27/29)		100 (28/28) 96,0 (24/25)		100 (27/27) 100 (24/24)		n/a		3Y 96,0 (24/25) 91,7 (22/24)	
ProRoot MTA vs I Root BP									
n/a		n/a		n/a		n/a		1,5M 100 12/12 100 12/12	
ProRoot MTA vs CEM									
n/a		n/a		98,9 176/178 96,4 163/169	98,9 176/178 84,6 143/169	98,1 151/154 98,0 147/150	84,7 116/137 78,1 107/137		
ProRoot MTA vs OrthoMTA									
96,4 (27/28) 93,8 (30/32)		96 (24/25) 92,8 (26/28)		n/a		n/a		n/a	

Study	Patients			Teeth		Clinical Information		Restorative treatment	
	n	Average [range]	Male/ Female	n	Teeth Groups	Diagnosis	Procedure	Material	Timing
ProRoot MTA vs RetroMTA									
Kang et al., 2017[2]	82	29.3	31/51	104	M PM	RP	PP	C,Cer	2-3D I
OrthoMTA vs RetroMTA									
Kang et al., 2017[2]	82	29.3	31/51	104	M PM	RP	PP	C,Cer	O:2-3D R:I
Angelus MTA vs CEM									
Zarrabi et al., 2010	n/a	[15-25]	n/a	32	1°PM	NP	DPC	GIC+C	I
ProRoot MTA vs Endocem									
Jang et al., 2015	35	72 [19-79]	n/a	46	I+PM(24) PM 22M(22)	RP	DPC	C, Cer	3M
ProRoot vs Theracal									
Bakhtiar et al., 2017	27	[18-32]	n/a	27	3°M	NP	PP	GIC	I
Biodentine vs Theracal									
Bakhtiar et al., 2017	27	[18-32]	n/a	27	3°M	NP	PP	GIC	I

Calcium silicates cements: ProRoot MTA (ProRoot® MTA - Dentsply Tulsa Dental, Johnson City, TN, USA); Biodentine (Biodentine™ - Septodont, Saint-Maur-des-Fossés Cedex, France); CEM (CEM - BioniquDent, Tehran, Iran); MTA-Plus (MTA Plus® - Avalon Biomed Inc., Houston, Texas): MTA Angelus (MTA Angelus® - Londrina, Paraná, Brazil); W MTA (White ProRoot® MTA - Dentsply Tulsa Dental, Johnson City, TN, USA); G MTA (Gray ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) Portland (Portland cement Votorantim-Cimentos, São Paulo, SP, Brazil); Theracal (TheraCal-LC® - Bisco Inc., Schamburg, IL, USA); OrthoMTA (OrthoMTA® - BioMTA, Seoul, Korea); Retro MTA (RetroMTA® - BioMTA, Seoul, Korea); iRoot BP (iRoot® BP - Innovative Bio Ceramix, Inc., Vancouver, BC, Canada); Endocem (Endocem – Maruchi Regenerative Endodontic materials)
Teeth groups: I (Incisor); PM (Premolar); M (Molars); Mx (Maxillary); Md (Mandibular).
Diagnosis: RP (Reversible pulpitis); NP (Normal pulp); IP (Irreversible pulpitis).
Procedure: DPC (Direct pulp capping); PP (Pulpotomy).
Coronal restoration: A (Amalgam); C (Composite); SSC (Stainless steel crown); GIC (Glass ionomer cement); RMGIC (Resin modified glass ionomer cement); Cavit (3M™ CAVIT™ Temporary Filling Material – 3M ESPE AG); Cer (Ceramic).
Timing: I (Immediate); H (Hours); D (Day); W (Week).
n/a: not available.
Inclusion Criteria: Clinical trials that compares at least two calcium silicates cements; Limited to humans; Randomized Controlled Trials (RCT); Vital pulp treatments; Each tooth is evaluated as a whole; CASP-RCT (Critical Appraisal Skills Programme) e evaluation was equal or superior to 50%.
Exclusion criteria: Root resorptions, pulpectomies and apical barriers; Articles with same sample was considered the longest follow-up; Indirect pulp treatment.

Follow-up									
6 months		1 year		2 years		5 years		Other	
Clinical	x-ray	Clinical	x-ray	Clinical	x-ray	Clinical	x-ray	Clinical	x-ray
ProRoot MTA vs RetroMTA									
96,4 (27/28) 96,8 (30/31)		96,0 (24/25) 96,2 (25/26)		n/a		n/a		n/a	
OrthoMTA vs RetroMTA									
93,8 (30/32) 96,8 (30/31)		92,9 (26/28) 96,2 (25/26)		n/a		n/a		n/a	
Angelus MTA vs CEM									
n/a		n/a		n/a		n/a		n/a (histology)	
ProRoot MTA vs Endocem									
n/a		87,0 (20/23) 83,3 15/18		n/a		n/a		n/a	
ProRoot vs Theracal									
n/a		n/a		n/a		n/a		2M 100 100	
Biodentine vs Theracal									
n/a		n/a		n/a		n/a		2M 100 100	

Table B. Deciduous teeth

Study	Patients			Teeth		Clinical Information		Restorative treatment	
	n	Average [range]	Male / Female	n	Teeth Groups	Diagnosis	Procedure	Material	Timing
ProRoot MTA vs Biodentine									
Bani et al., 2017	32	6.3 [4-9]	15/17	64	M Md	RP	PP	SSC	I
Carti, & Oznurhan, 2017	25	n/a [5-9]	13/12	50	1°M(27) 2°M(23)	RP	PP	SSC	I
Çelik et al., 2018	38	6.7 [5-9]	19/19	44	M Md	RP	PP	SSC	24H(M) 12M(B)
Cuadros-Fernández et al., 2015	n/a	n/a [4-9]	n/a	84	M	RP	PP	SSC	I
Guven et al., 2017	n/a	n/a	n/a	58	M	RP	PP	A	I(M) 12M(B)
Juneja, & kulkarni, 2017	n/a	n/a	n/a	34	M	RP	PP	GIC+SSC	n/a
Kusum et al., 2015	n/a	6.48 6.92	n/a	50	M	RP	PP	ZOE+GIC SSC	1D
Rajasekharan et al., 2017	38	4.65±1.1 5.18±1.2	11/43	54	Mx: 1°M(3) 2°M(15) Md: 1°M(12) 2°M(24)	RP	PP	GIC+SSC	I
ProRoot MTA vs CEM									
Ghajari et al., 2013	21	6.9±0.7 [5-8]	5/16	42	Mx: 2°M(19) Md: 2°M (23)	RP	DPC	A	I
Malekafzali et al., 2011	50	6 ± 0.75 [4-8]	23/17	80	Mx: 1°M(15) 2°M(6) Md: 1°M(28) 2°M(31)	RP	PP	A SSC	I
ProRoot MTA vs MTA Plus									
Guven et al., 2017	n/a	n/a	n/a	58	M	RP	PP	A	I
MTA Plus vs Biodentine									
Guven et al., 2017	n/a	n/a	n/a	58	M	RP	PP	A	I(M) 12(B)
ProRoot MTA vs Angelus MTA									
Celik et al., 2013	n/a	n/a	n/a	91	M	RP	PP	GIC+A	IW

Follow-up % (Total counts success/overall)										
6 months		1 year		2 years		5 years		Other	
Clinical	X-ray	Clinical	X-ray	Clinical	X-ray	Clinical	X-ray	Clinical	X-ray
ProRoot MTA vs Biodentine									
100 (32/32) 100 (32/32)		96,9 (31/32) 96,9 (31/32)		96,8 (30/31) 96,8 (30/31)	87,1 (27/31) 93,5 (29/31)	n/a		n/a	
100 (25/25) 100 (25/25)	100 (25/25) 100 (25/25)	96 (24/25) 88 (22/25)	80 (20/25) 60 (15/25)	n/a		n/a		n/a	
100 (24/24) 100 (19/19)		100 (23/23) 89,5 (17/19)		100 (22/22) 89,5 (17/19)		n/a			
95,3 (41/43) 97,6 (40/41)	100 (43/43) 100 (41/41)	97,4 (38/39) 100 (39/39)	97,4 (38/39) 94,9 (37/39)	n/a		n/a		n/a	
100 (29/29) 100 (29/29)	100 (29/29) 100 (29/29)	96,6 (28/29) 100 (29/29)	93,1 (27/29) 89,7 (26/29)	100 (28/28) 89,7 (26/29)	86,2 (25/29) 82,8 (24/29)	n/a		n/a	
100 15/15 100 15/15	100 15/15 93,3 14/15	100 15/15 100 15/15	100 15/15 93,3 14/15	n/a		n/a		n/a	
100 25/25 100 25/25	92,0 23/25 88,0 22/25	n/a		n/a		n/a		n/a	
100 29/29 96,0 24/25		100 (n/a) 96 (n/a)	92 (n/a) 96 (n/a)	n/a		n/a		n/a	
ProRoot MTA vs CEM									
n/a		n/a		n/a		n/a		20 M 94,7 18/19 89,5 17/19	
100 (36/36) 100 (36/36)		100 (33/33) 100 (33/33)	90.9 (30/33) 97 (32/33)	91,4* (32/35) 97,1* (34/35)	91,4* (32/35) 97,1* (34/35)	n/a		n/a	
ProRoot MTA vs MTA Plus									
100 (29/29) 100 (29/29)	100 (29/29) 100 (29/29)	96,6 (28/29) 100 (29/29)	93,1 (27/29) 96,6 (28/29)	96,6 (28/29) 100 (29/29)	93,1 (27/29) 86,2 (25/29)	n/a		n/a	
MTA Plus vs Biodentine									
100 (29/29) 100 (29/29)	100 (29/29) 100 (29/29)	100 (29/29) 100 (29/29)	96,6 (28/29) 89,7 (26/29)	100 (29/29) 100 (29/29)	86,2 (25/29) 82,8 (24/29)	n/a		n/a	
ProRoot MTA vs Angelus MTA									
n/a		n/a		97,3 97,4	95,3 90,8	n/a		n/a	

Study	Patients			Teeth		Clinical Information		Restorative treatment	
	n	Average [range]	Male / Female	n	Teeth Groups	Diagnosis	Procedure	Material	Timing
W MTA vs G MTA									
Agamy et al., 2004	n/a	n/a	n/a	60	M	RP	PP	IRM+SSC	I
Angelus MTA vs Portland									
Oliveira et al., 2013	n/a	n/a	n/a	30	M Md	RP	PP	ZOE+RMGIC	I
Sakai et al., 2009	30	6.9 [5-9]	19/11	30	M Md	RP	PP	IRM+RMGIC	I
ProRoot MTA vs Theracal									
Erfanparast et al., 2018	46	6.3 [5-7]	25/21	92	M	RP	DPC	IRM+A A	I
ProRoot MTA vs Ortho MTA									
Kang et al., 2015	143	n/a [3-10]	60/42	105	M	RP	PP	RMGIC	3W
ProRoot MTA vs Retro MTA									
Kang et al., 2015	143	n/a [3-10]	60/42	105	M	RP	PP	RMGIC SSC	3W I
Ortho MTA vs Retro MTA									
Kang et al., 2015	143	n/a [3-10]	60/42	105	M	RP	PP	RMGIC SSC	3W I

Calcium silicates cements: ProRoot MTA (ProRoot® MTA - Dentsply Tulsa Dental, Johnson City, TN, USA); Biodentine (Biodentine™ - Septodont, Saint-Maur-des-Fossés Cedex, France); CEM (CEM - BioniquDent, Tehran, Iran); MTA Plus® (Avalon Biome (Avalon Biomed Inc., Houston, Texas): MTA Angelus (MTA Angelus® - Londrina, Paraná, Brazil); W MTA (White ProRoot® MTA - Dentsply Tulsa Dental, Johnson City, TN, USA); G MTA (Gray ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) Portland (Portland cement Votorantim-Cimentos, São Paulo, SP, Brazil); Theracal (TheraCal-LC® - Bisco Inc., Schamburg, IL, USA); OrthoMTA (OrthoMTA® - BioMTA, Seoul, Korea); Retro MTA (RetroMTA® - BioMTA, Seoul, Korea);

Teeth groups: M (Molars); Mx (Maxillary); Md (Mandibular).

Diagnosis: RP (Reversible pulpitis).

Procedure: DPC (Direct pulp capping); PP (Pulpotomy).

Coronal restoration: A (Amalgam); C (Composite); SSC (Stainless steel crown); GIC (Glass ionomer cement); RMGIC (Resin modified glass ionomer cement); Cavit (3M™ CAVIT™ Temporary Filling Material – 3M ESPE AG); Cer (Ceramic).

Timing: I (Immediate); H (Hours); D (Day); W (Week).

n/a: not available.

* It was only considered the overall analyses.

Inclusion Criteria: Clinical trials that compares at least two calcium silicates cements; Limited to humans; Randomized Controlled Trials (RCT); Vital pulp treatments; Each tooth is evaluated as a whole; CASP-RCT (Critical Appraisal Skills Programme) e evaluation was equal or superior to 50%.

Exclusion criteria: Root resorptions, pulpectomies and apical barriers; Articles with same sample was considered the longest follow-up; Indirect pulp treatment.

Follow-up % (Total counts success/overall)									
6 months		1 year		2 years		5 years		Other	
Clinical	X-ray	Clinical	X-ray	Clinical	X-ray	Clinical	X-ray	Clinical	X-ray
W MTA vs G MTA									
95,0 19/20 96,0 24/25	n/a	80,0 16/20 100 19/19		n/a		n/a		n/a	
Angelus MTA vs Portland									
100 15/15 100 15/15		100 15/15 100 15/15		100 15/15 100 15/15		n/a		n/a	
100 14/14 100 15/15		100 13/13 100 15/15		100 9/9 100 9/9		n/a		n/a	
ProRoot MTA vs Theracal									
97,8 (45/46) 97,8 (45/46)		94,6 (35/37) 91,9 (34/37)	100 (37/37) 100 (37/37)	n/a		n/a		n/a	
ProRoot MTA vs Ortho MTA									
100 (38/38) 93,8 (30/32)	100 (38/38) 97,6 (40/41)	100 (38/38) 97,4 (37/38)	100 (33/33) 94,7 (36/38)	n/a		n/a		n/a	
ProRoot MTA vs Retro MTA									
100 (38/38) 100 (46/46)	100 (38/38) 93,5 (43/46)	100 (38/38) 100 (38/38)	100 (33/33) 94,7 (36/38)	n/a		n/a		n/a	
Ortho MTA vs Retro MTA									
100 (41/41) 100 (46/46)	97,6 (40/41) 93,5 (43/46)	97,4 (37/38) 100 (38/38)	94,7 (36/38) 94,7 (36/38)	n/a		n/a		n/a	

Table C. Histology

Study	Follow-up	Histology Results
		Bridge Formation
ProRoot MTA vs Biodentine		
Bakhtiar et al., 2017	8W	Dentinal bridge morphology and continuity – formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 5; Formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 4; no signs of dentin formation = 0. Dentinal bridge thickness – more than 0.25 mm = 1; between 0.1 and 0.25 mm = 8; less than 0.1 mm = 0.
		Dentinal bridge morphology and continuity – formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 9; formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 0; no signs of dentin formation(0). Dentinal bridge thickness – more than 0.25 mm(5); Between 0.1 and 0.25 mm(4); Less than 0.1 mm = 0.
Nowicka et al., 2015	6W	MTA, and Biodentine™ actively initiated the formation of reparative dentin in each tooth. Impossible to quantify from the graphic presented.
ProRoot MTA vs Theracal		
Bakhtiar et al., 2017	8W	Dentinal bridge morphology and continuity – formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 5; formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 4; no signs of dentin formation = 0. dentinal bridge thickness – more than 0.25 mm = 1; between 0.1 and 0.25 mm = 8; less than 0.1 mm = 0.
		Dentinal bridge morphology and continuity – Formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 1; formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 6; no signs of dentin formation = 2. Dentinal bridge thickness – more than 0.25 mm = 5; between 0.1 and 0.25 mm = 2; less than 0.1 mm = 2.
Biodentine vs Theracal		
Bakhtiar et al., 2017	8W	Dentinal bridge morphology and continuity – formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 9; formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 0; no signs of dentin formation = 0. Dentinal bridge thickness – more than 0.25 mm =5; between 0.1 and 0.25 mm =4; less than 0.1 mm = 0.
		Dentinal bridge morphology and continuity – formation of hard tissue beneath the cavity in the form of complete dentinal bridge = 1; formation of discontinuous bridge beneath the cavity (incomplete dentinal bridge) = 6; no signs of dentin formation = 2. Dentinal bridge thickness – nore than 0.25 mm = 5; between 0.1 and 0.25 mm = 2; less than 0.1 mm = 2.
ProRoot MTA vs I Root BP		
Azimi et al., 2014	6W	Hard tissue formation: none = 0; partial = 4; complete = 8. Appearance classified as resembling: tubular = 2; atubular = 8; presence of tunnel defects = 2.
		Hard tissue formation: none = 0; partial = 5; complete = 7. Appearance classified as resembling: tubular = 3; atubular = 8; presence of tunnel defects = 1.

Histology Results		Conclusions
Inflammation Degree	Other Characteristics	
ProRoot MTA vs Biodentine		
Intensity of pulp inflammation – absent = 9; type of pulp inflammation – absent = 9; extension of pulp inflammation – absent = 9.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 3; disorganization of pulp tissue beneath the cavity = 2; disorganization of entire pulp tissue = 4.	Dentin bridge at the site of injury was uniform and homogenous with Biodentine, followed by ProRoot MTA.
Intensity of pulp inflammation – absent =8, mild =1. Type of pulp inflammation – absent =8, mild = 1. Extension of pulp inflammation – absent = 8, mild = 1.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 6; disorganization of pulp tissue beneath the cavity = 3; disorganization of entire pulp tissue = 0.	
n/a	n/a	MTA and Biodentine™ actively initiated the formation of reparative dentin in each tooth (n = 11). The thickness of the dentin bridges were no significant different between the MTA and Biodentine groups.
ProRoot MTA vs Theracal		
Intensity of pulp inflammation – absent = 9. Type of pulp inflammation - absent = 9. Extension of pulp inflammation – absent = 9.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 3; disorganization of pulp tissue beneath the cavity = 2; disorganization of entire pulp tissue = 4.	Normal pulp organization was seen in 33.33% of the teeth in ProRoot MTA, 11.11% in TheraCal group (*P* = .06). Complete dentinal bridge formation rate was 11% and 56% in TheraCal and ProRoot MTA groups, respectively.
Intensity of pulp inflammation – absent = 9. Type of pulp - absent = 9. Extension of pulp inflammation – absent = 9.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 1; disorganization of pulp tissue beneath the cavity = 6; disorganization of entire pulp tissue = 2.	
Biodentine vs Theracal		
Intensity of pulp inflammation – absent =8, mild =1. Type of pulp inflammation – absent =8, mild = 1. Extension of pulp inflammation – absent = 8, mild = 1.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 6; disorganization of pulp tissue beneath the cavity = 3; disorganization of entire pulp tissue = 0.	Normal pulp organization was seen 11.11% in TheraCal®, and 66.67% in Biodentine group (P = .06). Biodentine group showed complete dentinal bridge formation in all teeth, whereas this rate was 11% in TheraCal group.
Intensity of pulp inflammation – absent = 9. Type of pulp - absent = 9. Extension of pulp inflammation – absent = 9.	Pulp tissue organization and morphology – normal or almost normal pulp tissue morphology = 1; disorganization of pulp tissue beneath the cavity = 6; disorganization of entire pulp tissue = 2	
ProRoot MTA vs I Root BP		
0 =0; 1 = 7; 2 = 4; 3 = 1; 4 = 0.	n/a	No significant difference between ProRoot® MTA and iRoot® BP in terms of pulp inflammation, formation of hard tissue bridge and its appearance was detected.
0 = 0; 1 = 8; 2 = 3; 3 = 1; 4 = 0.	n/a	

Study	Follow-up	Histology Results
		Bridge Formation
ProRoot MTA vs CEM		
Zarrabi et al., 2010	2W	Morphology of dentinal bridge - I = 3; II = 5; III = 0. Thickness – I = 5; II = 3; III = 0.
		Morphology of dentinal bridge – I = 5; II = 3; III = 0. Thickness – I = 4; II = 4; III = 0.
	8W	Morphology of dentinal bridge – I = 0; II = 6; III = 2. Thickness – I = 0; II = 5; III = 3.
		Morphology of dentinal bridge – I = 0; II = 4; III = 4. Thickness – I =0; II = 2; III = 6.
W MTA vs G MTA		
Agami et al., 2004		Non quantified
Eskandarizadeh et al., 2011	30D	Thickness of calcified bridge (TCB): 188±113. Presence of calcified bridge (PCB) (%): 10 (100).
		Thickness of calcified bridge: 134±21. Presence of calcified bridge: 9(90).
	60D	Thickness of calcified bridge: 191±105. Presence of calcified bridge: 10 (100).
		Thickness of calcified bridge: 275±67. Presence of calcified bridge: 10(100).
	90D	Thickness of calcified bridge: 330±196. Presence of calcified bridge: 10(100).
		Thickness of calcified bridge: 264±85. Presence of calcified bridge: 10(100).
G MTA vs G Angelus		
Accorinte et al., 2009	30D	Hard tissue bridge – complete (1) = 5; 2: partial bridge - little communication (2) = 1; lateral deposition of hard tissue on the walls of the cavity of pulp exposition (3) = 1; absence (4) = 1.
		Hard tissue bridge – I = 5; 3 = 2; 4 = 1.
	60D	Hard tissue bridge – I = 5; 3 = 1; 4 = 1.
		Hard tissue bridge – I = 6; 2 = 1; 3 = 3.
MTA Angelus vs Portland Cement		
Oliveira et al., 2013		Histological findings revealed the presence of dentine-like mineralised material deposition obliterating the root canal and some dentine barrier formation in the Potland Cement and MTA groups.

Histology Results		Conclusions
Inflammation Degree	Other Characteristics	
ProRoot MTA vs CEM		
Intensity of pulp inflamation – I = 3; II = 5; III = 0.	Odontoblast layer – I = 2; II = 6; III = 0.	Both MTA and CEM showed significantly better pulp response after 8 weeks compared with 2 weeks, with a thicker and more tubular pattern of the dentinal bridge, less pulp inflammation, and a palisade pattern of odontoblast cells. Although MTA and CEM groups had no significant difference in each measure in both time intervals, CEM induced a thicker dentinal bridge with less pulp inflammation at both 2 weeks and 8 weeks, compared with MTA.
Intensity of pulp inflamation – I = 0; II = 1; III = 7.	Odontoblast layer – I = 2; II = 6; III = 0.	
Intensity of pulp inflamation - I = 0; II = 6; III =2.	Odontoblast layer - I = 0; II = 4; III = 4.	
Intensity of pulp inflamation – I = 0; II = 1; III = 7.	Odontoblast layer - I = 0; II = 5; III = 3.	
W MTA vs G MTA		
Non quantified	Odontoblastic layer integrity, pulp calcification, and pulp vitality.	In the histologic study, both types of MTA successfully induced thick dentin bridge formation at the amputation sites. Teeth treated with gray MTA demonstrated pulp architecture nearest to normal pulp by preserving the odontoblastic layer and delicate fibrocellular matrix, yet few inflammatory cells or isolated calcified bodies were seen. Teeth treated with white MTA showed a denser fibrotic pattern, with more isolated calcifications in the pulp tissue along with secondary dentin formation.
Pulp inflammation - no inflammation (WI) = (50); minimal inflammation (MI) = (50).	n/a	Most WMTA specimens and all GMTA specimens showed either free of inflammation or minor pulpal inflammation at 60 and 90 day intervals. GMTA specimens have shown no significant difference to WMTA in terms of the presence and thickness of calcified bridge as well as the severity of pulp inflammatory response to the either of pulp capping materials at all time intervals of the present study (*P*>0.05).
WI = (40); MI = (40); moderate inflammation (MO) = (20).	n/a	
WI = (60); MI = (30); MO = 10.	n/a	
WI = (60); MI = (40).	n/a	
WI = (40); MI = (60).	n/a	
WI = (70); MI = (30).	n/a	
G MTA vs G Angelus		
Inflammatory response – no reaction (1) = 2; inflammatory reaction (2) = 6.	Giant cells – absent (1) = 8; great (4) = 1. Particles of the capping material – absent (1) = 6; mild number (2) = 1; moderate (3) = 1.	No significant difference was observed between the two materials (*P* > 0.05) in terms of overall histological features (hard tissue bridge, inflammatory response, giant cells and particles of capping materials). Overall, 94% and 88% of the specimens capped with MTA Angelus® and ProRoot®, respectively, showed either total or partial hard tissue bridge formation (P > 0.05).
Inflammatory response – 1 = 3; 2 = 4; abscess (3) = 1.	Giant cells – 1 = 7; moderate (3) = 1. Particles of the capping material - 1 = 7; 3 = 1.	
1 = 3; 2 = 5; Necrosis (4) =1.	Giant cells – 1 = 8; 4 = 1. Particles of the capping material – 1 = 8; Great (4) = 1.	
Inflammatory response – 1 = 7; 2 = 3.	Giant cells – 1 = 9; mild number (2) = 1. Particles of the capping material – 1 = 9; mild number (2) = 1.	
MTA Angelus vs Portland Cement		
Histological findings revealed the presence of dentine-like mineralised material deposition obliterating the root canal and some dentine barrier formation in the Potland Cement and MTA groups.		

Study	Follow-up	Histology Results
		Bridge Formation
MTA vs Retro MTA		
Bakhtiar et al., 2018	8W	Intensity of pulp inflammation – absent = 11. Type of pulp inflammation – no Inflammation = 11. Extension pulp inflamation – absent = 11.
		Intensity of pulp inflammation – absent = 8; mild = 3. Type of pulp inflammation – no Inflammation = 8; chronic = 3. Extension pulp inflamation – absent = 8; moderate =3.

Calcium silicates cements: ProRoot MTA (ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA); Biodentine (Biodentine™ - Septodont, Saint-Maur-des-Fossés Cedex, France); Theracal (TheraCal-LC® - Bisco Inc., Schamburg, IL, USA); iRoot® BP (iRoot BP - Innovative Bio Ceramix, Inc., Vancouver, BC, Canada); CEM (CEM - BioniquDent, Tehran, Iran); W MTA (White ProRoot® MTA - Dentsply Tulsa Dental, Johnson City, TN, USA); G MTA (Grey ProRoot® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA); MTA Angelus (MTA Angelus® - Londrina, Paraná, Brazil; Portland (Portland cement Votorantim-Cimentos, São Paulo, SP, Brazil); Retro MTA (RetroMTA® - BioMTA, Seoul, Korea).
Timing: I (Immediate); D (Day); W (Week).
n/a: not available.
Inclusion Criteria: Clinical trials that compares at least two calcium silicates cements; Limited to humans; Randomized Controlled Trials (RCT); Vital pulp treatments; Each tooth is evaluated as a whole; CASP-RCT (Critical Appraisal Skills Programme) e evaluation was equal or superior to 50%.
Exclusion criteria: Root resorptions, pulpectomies and apical barriers; Articles with same sample was considered the longest follow-up; Indirect pulp treatment.

Histology Results		Conclusions
Inflammation Degree	Other Characteristics	
	MTA vs Retro MTA	
Pulp tissue organization – normal pulp tissue = 6; disorganization beneath the cavity = 2. disorganization of the entire pulp tissue = 3. Dentinal bridge morphology – complete dentinal bridge = 7; discontinuous bridge = 4. Dentinal bridge thickness – more than 0.25 mm = 5; between 0.1–0.25 mm = 6.	n/a	In RetroMTA group, this study revealed the formation of a discontinuous bridge in most cases under the material within the pulp tissue with no significant inflammatory reaction in partially or completely disorganized dental pulp. This contrasts with ProRoot MTA, which resulted in complete dentin bridge formation in most of the teeth with no inflammation and normal pulp morphology.
Pulp tissue organization – normal pulp tissue = 1; disorganization beneath the cavity = 3; disorganization of the entire pulp tissue = 7. Dentinal bridge morphology – complete dentinal bridge = 3; discontinuous bridge = 7; no signs of mineralization = 1. Dentinal bridge thickness – between 0.1–0.25 mm = 5; less than 0.1 mm = 6.	n/a	