Mestrado Integrado em Medicina Dentária

Faculdade de Medicina da Universidade de Coimbra

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Coimbra, Junho 2017

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Coimbra, Junho 2017

Summary

Abstract

Aim: The purpose of the present *in vitro* study was to assess the proper time to perform restorative procedures, immediately (12 minutes) and 7 days after bioceramic material (MTA or Biodentine^{TM}) placement, using a universal bonding system.

Materials and Methods: A total of 75 acrylic blocks were prepared and randomly divided into 5 groups ($n = 15$). For the 7-days groups, one week prior to the shear bond strength test, 2 groups were filled with either MTA (15 blocks – group 2) or Biodentine^{TM} (15 blocks – group 5). After storage in an incubator at 100% relative humidity and 37°C for 7 days, resin-based flowable composite (SDR^{TM}) was bonded to bioceramics using a universal bonding system (Prime&Bond active^{TM}), without acid etching. For the 12-minutes groups, 48 hours prior to the shear bond strength test, 3 groups were filled with either MTA (30 blocks – groups number 1 and 4) or Biodentine^{TM} (15 blocks – group number 3). Regarding groups 3 and 4, 12 minutes after bioceramic material placement, resin-based flowable composite was bonded to the capping materials using a universal bonding system, without acid etching. Concerning group 1, GC Fugi IX GP was used as restorative material 12 minutes after bioceramic material placement, without previous adhesive system application. Shear bond strength was measured using a universal testing machine (shear bond strength test) and data statistical analysis was performed using Dunn-Sidak post hoc test ($p<0,05$).

Results: Concerning the 12-minutes groups, Biodentine^{TM} group (3) showed the highest mean shear bond strength value, with statistically significant differences ($p<0,05$) when compared to both immediate MTA groups (1 and 4). Regarding the 12-minutes MTA samples, groups 1 (GIC as restorative material) and 4 (SDR^{TM} as restorative material) presented no differences according to the referred parameter. Within the 7-days groups (2 and 5), considering the mean shear bond strength value, there were no differences between both biomaterials. Among the two time intervals, 7-days MTA group (2) presented statistically significant differences ($p<0,05$) compared to the 12-minutes MTA group when bonded to SDR^{TM} (4), while no differences were reported regarding Biodentine^{TM} performance (3 and 5). Regarding both restorative material types and placement timing, SDR^{TM} restoration 7 days after bioceramic application (group 2) presented a statistically significant ($p<0,05$) higher mean bond strength value compared to GIC immediately placed (group 1).

Conclusions: Within the limitations of the present *in vitro* study, our findings suggest that restorative procedures might be preferably performed at a delayed (7-days) timeframe. Moreover, it may be concluded that the type of restorative material placed over MTA might not be a critical factor to achieve a successful outcome. Further studies in this line of research are needed with standardized experimental protocols to establish clear relations between the evaluated parameters.

Key-words: MTA; Biodentine^{TM}; GIC; bulk fill flowable composite; universal bonding system; shear bond strength

Resumo

Objetivo: Determinar o período temporal mais indicado para a execução de procedimentos restauradores, imediatamente (12 minutos) ou 7 dias após a aplicação do biocerâmico (MTA ou Biodentine^{TM}), utilizando um sistema adesivo universal.

Materiais e métodos: Um total de 75 blocos de acrílico foram preparados e distribuídos aleatoriamemte por 5 grupos (*n*=15). Sete (7) dias antes de serem submetidos aos testes mecânicos, 30 amostras foram preenchidas com MTA (grupo 2) ou Biodentine^{TM} (grupo 5). Após armazenamento em estufa a 37ºC durante uma semana na presença de 100% de humidade relativa, procedeu-se à aplicação de um sistema adesivo universal (Prime&Bond active^{TM}) sem condicionamento ácido prévio, seguido da colocação das cápsulas preenchidas com resina fluida (SDR^{TM}) sobre os cimentos inorgânicos. Relativamente aos restantes grupos, 48 horas previamente à execução dos testes de adesão, 45 amostras foram preenchidas com MTA (grupos 1 e 4) ou Biodentine^{TM} (grupo 3). Doze (12) minutos após colocação do biocerâmico, procedeu-se à aplicação de um sistema adesivo universal nos grupos 3 e 4, sem condicionamento ácido prévio, seguido da colocação das cápsulas preenchidas com resina fluida (grupos 3 e 4) ou cimento de ionómero de vidro (grupo 1) – GC Fugi IX GP - sobre os biocerâmicos. Os testes de adesão foram realizados numa máquina de testes universal e os valores obtidos submetidos a tratamento estatístico utilizando o teste Dunn-Sidak como teste post-hoc (diferenças estatisticamente significativas para p<0,05).

Resultados: O grupo 3 apresentou valores de força de adesão estatisticamente superiores (p<0,05) aos obtidos nos grupos 1 e 4, não se verificando diferenças aquando da comparação das forças de adesão dos grupo 1 e 4 entre si. Considerando a média dos valores de força de adesão obtidos nos grupos 2 e 5, não se verificaram diferenças entre os dois biomateriais. Considerando os dois intervalos temporais testados, o grupo 2 apresentou diferenças estatisticamente significativas (p<0,05) em relação ao grupo 4, sendo que não foram registadas diferenças relativamente aos grupos 3 e 5. Considerando o material restaurador utilizado e o timing de simulação da restauração, o grupo 2 apresentou diferenças estatisticamente significativas (p<0,05) em relação ao grupo 1.

Conclusão: Considerando as limitações associadas ao presente estudo *in vitro*, os resultados obtidos sugerem a recomendação da execução dos procedimentos restauradores 7 dias após a colocação do biocerâmico, podendo o tipo de material restaurador colocado sobre o MTA não ser determinante para o sucesso do tratamento. É imprescindível a realização de mais estudos com qualidade e validade científica, com aplicação de metodologias standard, para clarificar as relações entre as variáveis testadas.

Palavras-chave: MTA; Biodentine^{TM}; cimento de ionómero de vidro; compósito fluido; sistema adesivo universal; força de adesão

Introduction

Restorative procedures, as well as several traumatic injuries, may lead to pulp exposure and jeopardize both the vitality preservation and a successful prognosis.[1, 2] In some clinical situations, vital pulp therapy involves directly placing a biomaterial over the exposed pulp site - direct pulp capping.[2, 3] Besides stimulating dentin bridge formation, such treatment modality allows pulp sealing, preventing bacterial leakage. [4] Direct pulp capping is performed with the ultimate goal of maintaining pulp vitality by protecting the dental-pulp complex.[1, 3, 5] Furthermore, vital pulp therapy approaches include indirect pulp capping (biocompatible materials used as a protective barrier) and pulpotomy procedures (biomaterial placement following partial amputation of the dental pulp).[5] On the contrary, immature necrotic teeth clinical management might include regenerative endodontic procedures (REPs) performance, with bioceramic placement in the coronal portion of the root canal being required.[6]

Contemporary endodontics offers a variety of pulp capping agents with specific properties, advantages and drawbacks.   Although calcium hydroxide was previously used for pulp capping material, as the outcomes of its application remain somewhat unpredictable with literature providing evidence on undergoing dissolution over time (consequently presenting low sealing ability) and promoting dentin bridge formation showing multiple tunnel defects, it is still not broadly accepted.[1, 7, 8] Novel biomaterials, namely bioceramic materials, were recently introduced as alternatives to the historically used calcium hydroxide.[1]

Mineral trioxide aggregate (MTA), composed of tricalcium aluminate, dicalcium silicate, tricalcium silicate, tetracalcium aluminoferrite and bismuth oxide, was firstly introduced in 1993.[1] MTA exhibits multiple undoubtedly desirable properties that make it suitable for several clinical applications, namely: internal root resorption, furcation defects and root perforations repair, apexification, root-end filling, as well as root canal filling, vital pulp therapy and regenerative endodontic procedures.[1, 6, 9-14] Although this calcium silicate-based cement shows characteristics that identify it as the gold standard material for some clinical applications, such as low solubility, biocompatibility, hard-tissue inductive and conductive activity, antibacterial properties due to calcium hydroxide release following MTA setting, setting in a wet environment and the capacity to prevent bacterial leakage, MTA has some well-known drawbacks including long-term setting timeframe, potential of tooth discoloration, high cost and difficult handling.[1-3, 13-16] Considering the prolonged setting time and as MTA must undergo a hydration process to set (it is recommended to place a wet cotton pellet over the biomaterial in some procedures), its application requires more than a single appointment to complete treatment.[3, 5, 15] Aiming to overcome MTA disadvantages while preserving its intrinsic favorable characteristics, several formulations were produced by modifying the originally marketed composition.[11]

Considering the main clinical shortcomings regarding MTA, being the long setting its major drawback, a new calcium silicate-based material was introduced - Biodentine™.[1] Biodentine™ powder contains tricalcium silicate, calcium carbonate and zirconium oxide, whereas the water-based liquid formulation includes calcium chloride with a water-reducing agent.[1, 13, 17] This endodontic repair material presents itself as a multipurpose biocompatible product, having a wide range of clinical applications (same as MTA). Biological properties include antibacterial activity, biocompatibility, bioactivity, antibacterial and biomineralization properties, with a greater sealing ability being stated within its physical properties.[1, 13, 17] Additionally, shorter setting time due to calcium chloride addition (12 minutes approximately) and higher viscosity were reported when compared to classical MTA.[1, 17] Biodentine™ was developed as a recommended dentin substitute biomaterial and presents an alternative to MTA.[13, 17]

The long-term success of vital pulp therapy procedures relies on a proper sealing following biomaterial placement, essential to avoid microleakage.[8, 18] Final restoration should allow to reestablish both aesthetics and function.[5]

Regarding the bonding effectiveness of restorative materials to pulp capping agents, the preferable bonding strategy (self-etch or etch & rinse) to apply and the definition of a proper timing for definitive restoration remains a concern.[5, 14]

Concerning bonding interface, not many studies have reported conclusive data on the bond strength of composite resins to MTA and even fewer to Biodentine™, as well as from bioceramics to Fugi IX, a glass ionomer cement (GIC), used as a liner over the pulp capping agents.[1]

The purpose of the present *in vitro* study was to assess the proper time to perform restorative procedures, immediately (12 minutes) and 7 days after bioceramic material placement, using a universal bonding system.

The tested null hypothesis states there are no statistically significant differences between the five tested groups regarding the adhesive joints shear bond strength value.

Materials and Methods

Review

A research was made using PubMed Database with the following keywords and Boolean connectors and a timeframe of 10 years: "tricalcium silicate"[All Fields] OR "biodentine"[All Fields] OR MTA[All Fields] AND ("Dental Cements"[Mesh] OR "adhesive"[All Fields]) AND "Composite Resins"[Mesh] ("2007/05/20"[PDat] : "2017/05/16"[PDat]).

Methods

1- Sample preparation and restorative material placement

Table I. – Materials' manufacturer, composition, application procedure, lot number and expiration date information

Material	Manufacturer	Composition	Preparation/application procedure	Lot number	Expiration date
Biodentine™ Bioactive dentine substitute	Septodont, Saint-Maur-des-Fossés Cedex, France	Powder: tricalcium silicate, zirconium oxide, calcium oxide, calcium carbonate and colourings Aqueous solution: calcium chloride and polycarboxylate	1.Pour 5 drops of liquid into the capsule 2.Place the capsule on a mixing device 3.Mix for 30 seconds	B19588	01-2019
ProRoot MTA® (White) Mineral Trioxide Aggregate	Dentsply Tulsa Dental, Johnson City, TN	Tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate and calcium sulfate dehydrate or gypsum	Mixed powder/liquid ratio 1:3	121780	02-2018
Prime&Bond Active™ Universal adhesive system	Dentsply DeTrey GmbH, Konstanz, Germany	Phosphoric acid modified acrylate resin, multifunctional acrylate, bifunctional acrylate, acidic acrylate, isopropanol, water, initiator, stabilizer	1.Apply the bonding system to all surface 2.Rest for 20 seconds 3.Air drying for at least 5 seconds to 4.promote solvent evaporation 5.Light-curing for 10 seconds	1703000402	01-2019
SDR™ Flowable composite	Dentsply DeTrey GmbH, Konstanz, Germany	Barium-alumino-fluoro-borosilicate 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 seconds	02023	02-2020
GC Fuji IX GP (A3) Glass ionomer restorative cement	GC Corporation, Tokyo, Japan	Powder: aluminofluorosilicate glass, polyacrylic acid powder Liquid: polyacrylic acid, distilled water, polybasic carboxylic acid	Mixed poder/liquid ratio 1:1	1610031	10-2019

A total of 75 acrylic blocks (3 cm height × 1,5 cm diameter) presenting a 5 mm diameter and 2 mm deep central hole (standard position and dimensions obtained using a *putty* - Virtual Refill Putty Fast Set, Ivoclar Vivadent - mold) were prepared and polished. The blocks were then randomly divided

into 5 groups ($n = 15$) for the analysis and samples within each group were numerically identified from 1 to 15.

Table II. – Study groups

Group	Timing for restorative material placement (after bioceramic application)	Calcium silicate-based material	Bonding system	Restorative material
1	12 minutes	ProRoot MTA®	-	GC Fugi IX GP
2	7 days	ProRoot MTA®	Prime&Bond Active™	SDR™
3	12 minutes	Biodentine™	Prime&Bond Active™	SDR™
4	12 minutes	ProRoot MTA®	Prime&Bond Active™	SDR™
5	7 days	Biodentine™	Prime&Bond Active™	SDR™

For the 7 - days groups, one week prior to the shear bond strength test, 2 groups were filled with either MTA (15 blocks – group number 2) or Biodentine™ (15 blocks – group number 5). Powder and liquid of each calcium silicate-based material was prepared and mixed according to the manufacturer's instructions, and applied in the acrylic blocks using a spatula, with the blocks supported on a vibratory base. All 30 samples were stored in an incubator at 100% relative humidity and 37°C for 7 days to allow complete setting. After the 7-days scheduled storage, application of a universal bonding system over the capping material was performed according to the manufacturer's instructions, without acid etching. Air drying was performed previously to bonding procedure in MTA specimens, whereas Biodentine™ samples surface was polished using 100 granulometry strips (Hawe Finishing and Polishing Strips, Kerr). Polishing procedure was followed by rinsing and air drying. Following the bonding procedure, a cylindrically shaped capsule with an internal diameter of 2,54mm and 4,39mm height was filled with Surefil SDR™ bulk fill flowable composite and centrally placed over the bonding site. The restorative composite material was then light cured for 80 seconds with a polywave LED curing light source (Bluephase® Style, Ivoclar Vivadent) – 20 seconds/quadrant.

For the 12-minutes groups, 48 hours prior to the shear bond strength test, 3 groups were filled with either MTA (30 blocks – groups number 1 and 4) or Biodentine™ (15 blocks – group number 3). Powder and liquid of each calcium silicate-based material was prepared and placed in the acrylic blocks similarly to the 7-days groups. Regarding groups 3 and 4, 12 minutes after bioceramic material placement, application of a universal bonding system over the capping material was performed according to the manufacturer's instructions, without acid etching. In group 1 no adhesive was used. Following the previously described procedures, a cylindrically shaped capsule with an internal diameter of 2,54mm and 4,39mm height was filled with either SDR™ flowable composite (groups 3 and 4) or Fugi IX (group 1) and centrally placed over the restoration site. Fugi IX powder and liquid were mixed according to the manufacturer's instructions. Concerning groups 3 and 4, the restorative composite material was placed and light cured for 80 seconds with a polywave LED curing light source (Bluephase® Style, Ivoclar Vivadent) – 20 seconds/quadrant. All 45 samples were stored in an incubator at 100% relative humidity and 37°C for 48 hours to allow complete setting before SBS testing.

It is noteworthy that, likewise both MTA and Biodentine™ application, restorative material placement was performed by one single operator. To summarize, 2 different operators were involved in samples' preparation.

Previously to the shear bond strength test, groups testing sequence was randomly defined.

2- Shear bond strength test

The samples were tested in shear mode using a universal testing machine (Model AG-I, Shimadzu Corporation, Kyoto, Japan). The compression load resulting in the shear bond strength was performed parallel and near of the adhesive interface. The shear force was applied by a chisel-shaped rod at a crosshead speed of 0,5mm/minute, up to bond disruption. Shear bond-strength values, expressed in MegaPascals (MPa), were calculated by dividing peak break force (N) by the cross-sectional area of the bonded interface of each group.

3- Fracture analysis

Following the SBS test, the fractured surfaces were evaluated by a single blind operator using a stereomicroscope (Nikon SMZ1500 – objective HR Plan Apo 1X WD 54; Nikon, Japan) and the fracture pattern was registered. Fracture mode was classified as (0) adhesive fracture, (1) cohesive fracture in calcium silicate-based material, (2) cohesive fracture in the restorative material and (3) mixed failure (comprising both adhesive and cohesive fracture).

4- Statistical analysis

The description of the results within each group was performed using the mean, standard deviation, maximum and minimum values. Besides the aforementioned analysis box-plots were also constructed aiming to show the obtained shear bond strength values. The normality of data distribution testing was carried out using the Shapiro-Wilk test. Kruskal-Willis tests were used to detect significant differences between the median across the groups as data did not follow the normal distribution. Post-hoc comparisons were accomplished using the Dunn Sidak test. Statistical analysis was performed using the commercially available IBM SPSS v.24 software. The pre-test failures occurred in only three specimens from group 1. Therefore, they were not included in the analysis. The outcomes regarding shear bond strength were expressed in MPa.

Results

Review

The titles and abstracts of the 51 initially found publications were analysed and 42 were excluded for being unrelated to the subject. Two (2) publications were not full text available and the authors did not answer the request sent by email, thus being excluded. Fifteen (19) cross references were added. Therefore, 26 papers were included in this review.

Methods

Table III. - Mean, standard deviation, maximum and minimum values of shear bond strength (MPa) of the tested groups.

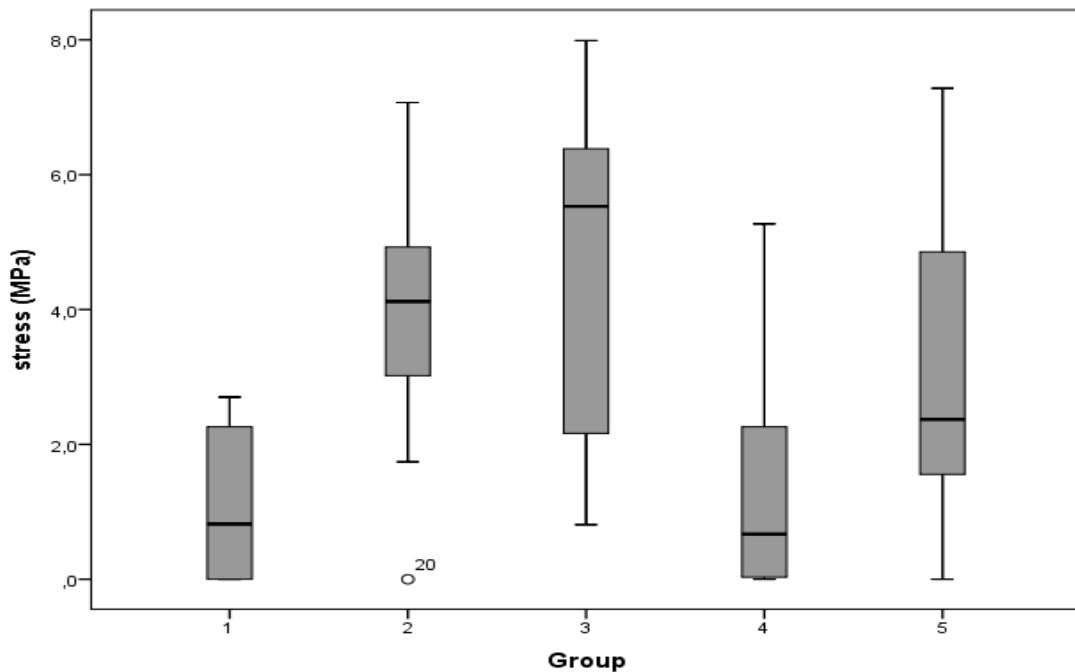|		N	Mean	Standard deviation	Minimum	Maximum
Group	1	12	1.14	1.12	.00	2.70
	2	15	3.86	1.72	.00	7.07
	3	15	4.44	2.49	.81	7.99
	4	15	1.33	1.56	.00	5.27
	5	15	3.09	2.23	.00	7.28

Figure 1. - Box-plot showing shear bond strength values distribution in the tested groups.

Table IV. - Group comparison p values

Group comparison	p
1-2	0.011
1-3	0.002
1-4	1.000
1-5	0.193
2-3	1.000
2-4	0.023
2-5	1.000
3-4	0.004
3-5	1.000
4-5	0.382

Table V. - Group comparison according to p values

Bioceramic Restorative material	Restorative material placement timing	
	Immediate (12-minutes)	**Delayed** (7-days)
Biodentine[TM] SDR[TM]	Group 3 *4,44±2,49[a]	Group 5 *3,09±2,23[a,b]
ProRoot MTA® SDR[TM]	Group 4 *1,33±1,56[b]	Group 2 *3,86±1,72[a]
ProRoot MTA® GC Fugi IX GP	Group 1 *1,14±1,12[b]	

*Mean bond strength value ± standard deviation (MPa)
Note: Mean bond strength values sharing the same superscript letter were not statistically significant different ($p>0,05$)

There are statistical meaningful differences ($\chi^2(4) = 23.72; p < 0.001$) regarding the shear bond strength in the tested groups.

Concerning the 12-minutes groups, Biodentine[TM] group (3) showed the highest mean shear bond strength value, with statistically significant differences ($p<0,05$) when compared to both immediate MTA groups (1 and 4). Regarding the 12-minutes MTA samples, groups 1 (GIC as restorative material) and 4 (SDR[TM] as restorative material) no meaningful differences were detected in the referred parameter. Therefore, no statistically significant differences were verified between both restorative materials.

Within the 7-days groups (2 and 5), considering the mean shear bond strength value, there were no statistically differences between both biomaterials.

Among the two time intervals, 7-days MTA group (2) presented statistically significant differences ($p<0,05$) compared to the 12-minutes MTA group when bonded to SDR[TM] (4), while no differences were reported regarding Biodentine[TM] performance (3 and 5). Regarding both restorative

7

material types and placement timing, SDR^{TM} restoration 7 days after bioceramic application (group 2) presented a statistically significant (p<0,05) higher mean bond strength value compared to GIC immediately placed (group 1).
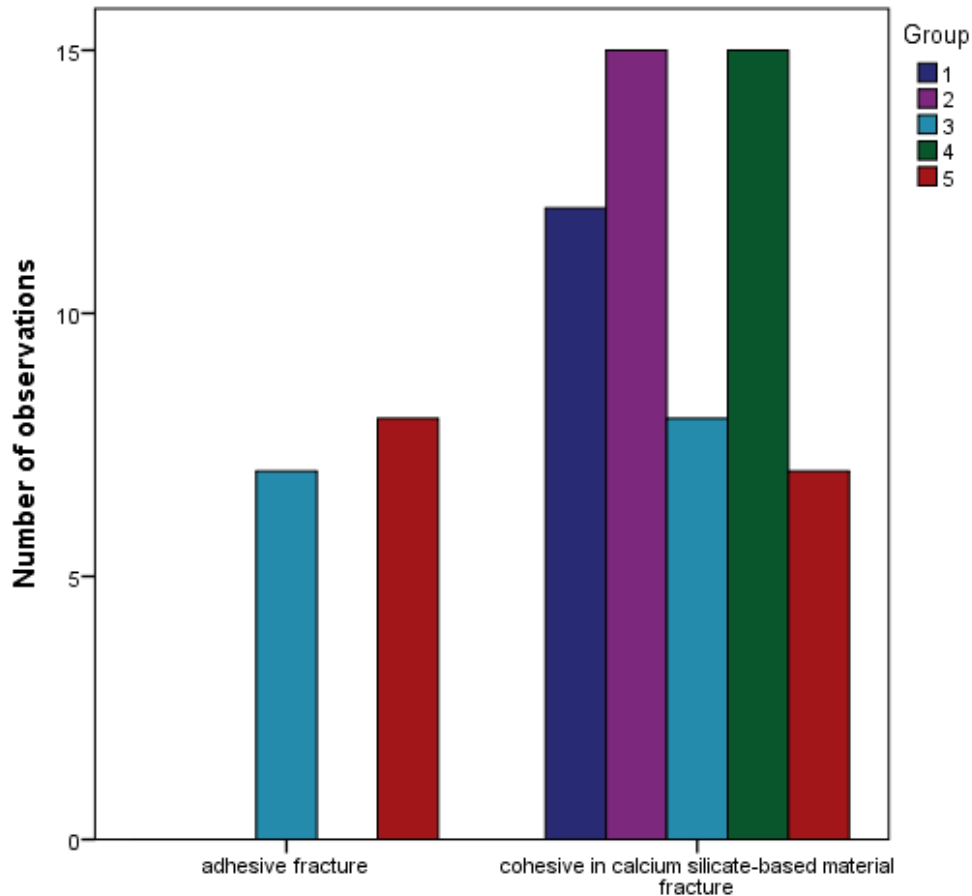

Figure 2. - Fracture mode of the tested specimens following shear bond strength test

No specimens failed cohesively within the restorative material (GIC or SDR^{TM}) and no mixed failures were verified.

All MTA samples exhibited cohesive failures regardless the restorative material placement timing.

Biodentine^{TM} fracture pattern analysis revealed a primarily cohesive fracture within the subtract in the 12-minutes group, whereas the adhesive failure mode presented a higher rate in the 7-days group.

Discussion

Nowadays several endodontic procedures include calcium silicate-based materials placement, with a following effective coronal seal being required.[8, 11] Concerning final rehabilitation, many clinical situations require composite resin direct restorations following vital pulp therapy and regenerative endodontic procedures.[6, 11] Consequently, proper bonding of the restorative material to pulp capping agents is essential to produce gap-free successful restorations, allowing to achieve an adhesive interface capable of spreading stress over the entirely bonding site.[1, 19] Currently there is no recommended restorative-pulp capping materials' bonding protocol described in the literature. Therefore different authors present many study-design variations regarding the type of restorative materials and differences in the formulation of different used resin-based composites and adhesive systems, restoration timing, chosen biomaterial, polishing of biomaterial surface, bonding surface area, as well as several other method/protocol details, which may affect the achieved results. This fact presents a barrier for result analysis and comparison among different studies.

Bond strength regarding the adhesive joint between composite resins/glass ionomer cements and bioceramics is one critical factor for a favorable treatment outcome, with values ranging between 17 and 20 MPa being stated as needed to achieve an interface capable of withstanding contraction forces, producing gap-free restoration margins.[10, 12, 13] However, there is still no definite recommendation within literature regarding the preferable bonding protocol (self-etch or total-etch) to apply. Moreover, there are no studies evaluating bond strength of restorative materials when bonded to MTA or Biodentine™ with universal adhesive systems applied, presenting a limitation for result comparison.

Neelakantan *et al.* assessed the bond strength between resin-based composites and MTA applying 3 different bonding systems and presented the highest bond strength values with the one-step self-etching adhesive at all three tested intervals.[10] Similarly, Odabas *et al.* demonstrated highest bond strength when self-etch adhesive systems were applied.[17]

On the other hand, Yelamali *et al.* reported greater bond strength values when a two-step etch & rinse adhesive was applied over MTA, although the values were not statistically significant when compared to a two-step self-etching system.[12] Bayrak *et al.* also reported higher shear bond strength obtained with etch-and-rinse adhesive systems.[20] In agreement with the former authors, Tunç *et al.* evaluated the bond strength of a composite and a compomer to white MTA with two bonding systems and concluded the total-etch one-bottle adhesive system bonded significantly stronger than the self-etch one-step approach.[21]

Different from the previous findings and according to Hashem *et al.*, a total-etch or self-etch bonding system may be used, since no significant differences were pointed regarding the bonding

technique.[22] These findings are in agreement with the results of Oskoee *et al.*, as well as Alzraikat *et al.*[5, 8, 19]

As referred, morphological changes following different surface treatment remain unclarified.[11] Concerning the studies presenting superior results obtained with the self-etch approach, researchers hypothesized the rinsing after phosphoric acid etching included in etch & rinse technique might lead to MTA microstructure modifications and consequently lower bond strength records.[10] In fact, modified microhardness, as well as affected compressive strength and displacement/dissolution possibility of MTA following acid etching procedures performed on freshly mixed MTA were reported.[5, 8, 23] Oskoee *et al.* described MTA surface degradation and cohesive strength reduction following acid etching.[8] Tsujimoto *et al.* reported no MTA microhardness disturbance in a 10-minute group performing a self-etching procedure.[14] Additionally, a scanning electron microscope evaluation showed that the setting of a low pH environment when acid etching is performed originates amorphous surface-structures and needle-shaped crystals removal.[7, 22] It has been suggested that a selective removal of the matrix around the crystals produces a MTA surface with characteristics that might present an ideal substrate for bonding, by enhancing adhesion through micro-mechanical retention.[19, 22]

On the contrary, previous studies mentioned that a more destructive potential of stronger acidic treatment results in deeper and more retentive microporosity, ultimately associated with enhanced bond strength.[11, 13] Likewise, Kayahan *et al.* reported a potential to enhance bonding of resinous materials associated with etching created surface changes.[23]

Further studies are needed to provide information on the structural MTA / Biodentine[TM] changes that arise from several bonding strategies performances (with different chemical and mechanical surface treatment methods) and their possible effects on bond strength to the restorative material, as well as clarify the adhesive mechanisms involved.[11, 22]

In the present study, SDR[TM] was bonded to both MTA and Biodentine[TM] applying a universal adhesive system.[24] Previous acid etching was not performed considering surface treatment effects remain unclear within literature.

The purpose of the present *in vitro* study was to assess the proper time, immediately (12 minutes) and 7 days (delayed) after bioceramic material placement, to perform restorative procedures. An insight on this parameter would present undoubted clinical relevance. Indeed, currently multiple visit procedures could be suitable to be performed in a single-visit, decreasing both time and cost, if early time intervals were proven to be the preferable time frame to place final direct restorations.

Presently, there are no available guidelines regarding a recommended time frame for composite or glass ionomer cement placement.[10] However, considering the average setting time of both tested biomaterials, it is admittedly important to understand its physical/chemical property modifications during the setting process to determine either if it influences or not the definition of a proper timing to perform restorative procedures.

While Biodentine[TM] exhibits a 12 minutes setting time, MTA presents a prolonged setting time of 2 hours and 45 minutes.[10, 14] Few researchers evaluated shear bond strength of MTA and/or

Biodentine™ to resin-based composite at different time intervals.[10, 14, 15, 17, 22] Neelakantan *et al.* evaluated shear bond strength of a resin-based composite/MTA bonding at 3 different restorative procedure timings (immediately, 45 minutes and 24 hours) and showed that immediate composite placement, regardless the bonding system used, leads to higher bond strength levels.[10] The researchers suggested that a more porous structure verified in an early setting stage promotes better adhesive system penetration, which might present a valid explanation for the accomplished results.[10] The findings of Neelakantan *et al.* are in agreement with the reports shared by Tsujimoto *et al.*, whose study aimed to investigate a proper timing to restore over MTA through SEM and Vickers microhardness evaluation, considering the following time intervals: 10 minutes, 1 day and 7 days.[14] This author states that the recommended clinical procedure might include almost immediate placement of the final restoration after MTA mixing during a single appointment.[14] Concerning Biodentine™, Hashem *et al.* allowed aging of the substract for early time intervals (immediate, 5 minutes, 20 minutes and 24 hours) and delayed time intervals (2 weeks, 1 month, 3 months and 6 months) after biomaterial placement, and reported that it is preferable to delay more than 2 weeks the restorative material placement, in order to allow an intrinsic maturation capable of resisting resin composite contraction forces.[22] Additionally, Odabas *et al.* showed higher bond strength values at a 24 hour-period within the 2 time tested intervals (12 minutes and 24 hours).[17]

In the present study, concerning the 12-minutes groups, Biodentine™ group (group 3) showed the highest mean shear bond strength value, with statistically significant differences ($p<0,05$) when compared to both immediate MTA groups (1 and 4). Regarding the 12-minutes MTA groups, although groups 1 (GIC as restorative material) and 4 (SDR™ as restorative material) presented no differences according to the referred parameter, it is noteworthy that four GIC specimens presented fracture during handling and three exhibited a superior bonded area due to GIC excess presence that resulted in abnormally high bond strength values compared with the samples without material excess. Therefore, these values may be misleading and not reflect reality. Within the 7-days groups (2 and 5), considering the mean shear bond strength value, there were no differences recorded between both biomaterials.

Among the two tested time intervals, 7-days MTA group (group 2) presented statistically significant differences ($p<0,05$) compared to the 12-minutes MTA group when bonded to SDR™ (group 4), while no differences were reported regarding Biodentine™ performance (groups 3 and 5). Our findings concerning MTA groups oppose to those presented by Neelakantan *et al.*, while the results obtained regarding Biodentine™ are in agreement with literature.[10] Regarding both restorative material type and placement timing, SDR™ restoration 7 days after bioceramic application (group 2) presented a statistically significant ($p<0,05$) higher mean bond strength value compared to GIC immediately placed (group 1), which might be related with the underlying adhesive mechanisms. These findings suggest that restorative procedures might be preferably performed at a delayed timeframe.

Regarding retention, both chemical and micromechanical adhesion were suggested as mechanisms for GIC bonding to a calcium silicate-based cement.[10] Diving deeper into the mechanism, porous MTA surfaces might allow for a micromechanical interlocking and its formulation comprises high

levels of mineral oxides to which GIC may chemically bond. Apart from these findings and bearing in mind the chemical adhesion mechanism of universal adhesives, although there are no literature references whatsoever to this topic, it is important to clarify in further studies if the 10-MDP would also chemically interact via ionic bonding to calcium present in calcium silicate-based cements, as it does to the one present in hydroxyapatites.[25]

Previous studies reported bond strength to be acceptable when cohesive fracture occurs rather than adhesive, which is required to accomplish successful restorative procedures.[5, 19] Currently, a consensus has not been reached among researchers regarding a preferable restorative material to place over the biomaterial.[10]

In our study, no specimens failed cohesively within composite resin, similarly to Odabas *et al.* findings.[17]

Failure mode analysis highlighting a cohesive fracture pattern within pulp capping material might reflect its low cohesive resistance compared to a high bond strength value. This explanation was proposed in previous studies.[8, 19] In fact, in the present study all MTA samples exhibited cohesive failures regardless the restorative material placement timing, therefore suggesting a favorable bond between MTA and both tested restorative materials.

As previously referred, there was a significant difference between the mean bond strengths at 12-minutes and 7-days for the MTA groups bonded to SDR[TM], with the highest value obtained in the delayed timing group. Therefore, considering all failures were cohesive in the calcium silicate-based cement and a statistically higher bond strength value was obtained at 7-days, MTA might present a superior cohesive resistance 7 days after placement.

Concerning the GIC group, pre-test failures, as well as several fractures during handling, were verified, suggesting low bond strength values. In agreement with the results reported in a pilot study published by our research group in MSci thesis format, when pre-test failures were verified, surface evaluation and analysis revealed a cohesive fracture pattern within MTA.[26] Therefore, even though the bond strength levels between MTA and GIC are suggested to be low, they might be superior to the MTA cohesive strength.

Biodentine[TM] failure pattern analysis revealed a primarily cohesive fracture within the substract in the 12-minutes group, whereas the adhesive failure mode presented a higher rate in the 7-days group. Considering Biodentine[TM] samples showed no bond strength differences when both time intervals were compared, a slightly increased number of adhesive failures within the pulp capping material in the delayed group might suggest lower bond strength level and/or a higher biomaterial cohesive strength. It is worth mentioning that all the 7-days Biodentine[TM] sample surfaces were polished as they presented an irregular and heterogeneous aspect after one week of storage that we suspected could lead to biased results. Although using polishing strips allowed to flatten the Biodentine[TM] surface and thus enable a standardization of the contact area between the bioceramic and the restorative material, polishing might explain why delayed Biodentine[TM] group exhibited a higher number of samples with adhesive fracture patterns, since a possible existing micromechanical retention might have been reduced, occluded or even eliminated with the referred procedure.

When comparing both calcium silicate-based cement groups (MTA vs Biodentine™), regardless the restorative material or timing, the differences regarding the fracture pattern may be due to a different intrinsic cohesive strength between MTA and Biodentine™, the last presenting probably better physical and mechanical properties.

Conclusions

Within the limitations of the present *in vitro* study, our findings suggest that restorative procedures might be preferably performed at a delayed (7 - days) timeframe. Moreover, it may be concluded that the type of restorative material placed over MTA might not be a critical factor to achieve a successful outcome.

Further studies in this line of research are needed with standardized experimental protocols to establish clear relations between the evaluated parameters.

Acknowledgements

Ao meu orientador, Prof. Doutor Paulo Palma, agradeço a disponibilidade, entusiasmo pela investigação, ajuda e apoio permanentes. Obrigada pelos conhecimentos transmitidos e pelas palavras de incentivo constantes, não só ao longo da realização deste trabalho mas também no decurso da minha vida académica. Um muito obrigada ainda pelas orientações e conselhos tecidos, pela amizade e preocupação.

Ao Mestre Rui Falacho, uma das minhas referências na Medicina Dentária e co-orientador deste trabalho final, com quem aprendi muito do que sei hoje, agradeço o incentivo permanente em procurar a excelência. Uma inspiração enquanto médico dentista, docente e pessoa. Um obrigada ainda pela disponibilidade, motivação e dedicação na construção do presente trabalho, assim como pelos conselhos valiosos e pelo rigor exigido.

Um agradecimento ao Prof. Doutor João Carlos Ramos, pelos conselhos, críticas e sugestões, assim como pelos conhecimentos partilhados e ânimo transmitido, que contribuíram para o enriquecimento e elevação da qualidade científica do presente trabalho. De referir e agradecer ainda a ponte estabelecida com a Universidade de Aveiro, possibilitando e facilitando a execução dos testes mecânicos necessários à elaboração deste trabalho.

À técnica de laboratório Cláudia Brites, sem a qual a realização deste trabalho não seria possível, um agradecimento pela participação na execução laboratorial. Um agradecimento especial pela disponibilidade, ajuda, preocupação, companhia, palavras amigas e alegria de sempre.

Ao Prof. Doutor Francisco Caramelo, agradeço o valioso e imprescindível contributo no tratamento estatístico dos dados, bem como a disponibilidade que sempre demonstra para com todos os pedidos que lhe são endereçados.

Ao Prof. Doutor José Maria Ferreira**,** Departamento de Engenharia de Materiais e Cerâmica, CICECO, Universidade de Aveiro e seus colaboradores pela ajuda e disponibilização dos meios laboratoriais usados na realização deste trabalho.

À minha família, em especial pais e irmãos, sempre presentes, obrigada pela proteção, paciência, ajuda e apoio nos momentos mais desafiantes, assim como pelo reconhecimento do meu trabalho e esforço desenvolvidos no decurso da minha vida académica e pelo incentivo à formação que sempre demonstraram.

Aos amigos de sempre.

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