

Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine

Domingos Pires, Mariana; Cordeiro, Joana; Vasconcelos, Isabel; Alves, Mariana; Quaresma, Sérgio André; Ginjeira, António; Camilleri, Josette

DOI:

[10.1016/j.dental.2021.03.021](https://doi.org/10.1016/j.dental.2021.03.021)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Domingos Pires, M, Cordeiro, J, Vasconcelos, I, Alves, M, Quaresma, SA, Ginjeira, A & Camilleri, J 2021, 'Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine', *Dental Materials*, vol. 37, no. 7, pp. e399-e406. <https://doi.org/10.1016/j.dental.2021.03.021>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Dental Materials

Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine --Manuscript Draft--

Manuscript Number:	DENTMA-D-20-00929R1
Article Type:	Full Length Article
Keywords:	Biodentine; ion release; Micro-hardness; Setting time; Tricalcium silicate; water-to-powder ratio
Corresponding Author:	Mariana Pires Universidade de Lisboa Faculdade de Medicina Dentaria PORTUGAL
First Author:	Mariana Pires
Order of Authors:	Mariana Pires Joana Cordeiro Isabel Vasconcelos Mariana Alves Sergio Andre Quaresma Antonio Ginjeira Josette Camilleri
Abstract:	<p>Objectives : The water to powder ratio and method of mixing is important for the properties of hydraulic cements. For this purpose a number of clinicians prefer premixed materials . Dental manufacturing companies provide predosed materials, however the manufacturer instructions are not always adhered to. The aim of this research is to investigate physical and chemical alterations of the tricalcium silicate-based cement Biodentine when manipulated according to the manufacturer’s instructions (control) or changing the doses and mixing of the material components.</p> <p>Methods: 6 groups were constituted according to different mixing and dosing of powder and liquid. The hydrated cements were characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). Calcium ion concentration of the leachate was also investigated. Assessment of the physical characteristics included setting time and microhardness.</p> <p>Results: Microstructural differences were visible only in the Biodentine mixed manually with water, in which early hydration rate was also affected, with lower calcium ion release. Increase of Biodentine liquid increased the calcium ion release, but also increased the setting time. Manual manipulation required more liquid (both water and Biodentine liquid) added to the mixture to guarantee a similar consistency to the control. A decrease in setting time was also noted. All groups showed higher values of microhardness at 24 hours compared to the freshly set materials. In the freshly set materials, there was an overall decrease in microhardness in all groups when compared to group control, particularly significant when increasing the dosage of Biodentine liquid.</p> <p>Significance: When mixing Biodentine, altering the mixing procedure in terms of type and amount of liquid added to the powder and mixing device chosen has an effect on the physical, chemical and mechanical characteristics and surface topography of the material, when compared to Biodentine mixed according to the manufacturer’s recommendations. Hence, the manufacturer’s instructions should be strictly followed.</p>
Response to Reviewers:	<p>Dear Editor-in-Chief from Dental Materials. Prof. Dr. David Watts,</p> <p>Herewith are, as required, the point-by-point responses to the reviewers regarding the submission of the manuscript entitled “Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine”.</p> <p>We deeply acknowledge the Reviewer’s comments, suggestions, and corrections, which we hope we have fully addressed.</p>

•The aim of this materials science study was to find out what happens if you deliberately violate the manufacturer's instructions when mixing the calcium silicate cement Biodentine.
Basically the work was well performed and the manuscript well written. However, from a scientific point of view the aim of this study is somewhat questionable. The manufacturer of Biodentine has already thought about why Biodentine is comparatively complicated to mix compared to other calcium silicate cements. The handling is clearly a competitive disadvantage and the manufacturer would certainly have done without it if it was not necessary. It is therefore not surprising that the last sentence in the abstract reads: "Hence, the manufacturer's instructions should be strictly followed." In principle, one should always follow the manufacturer's instructions when using dental materials and medications, if only for liability reasons.
On the other hand, I also know that some dentists violate the manufacturer's instructions out of misunderstood thriftiness and refrain from mixing in the capsule in order to mix small portions by hand. From this point of view, the present study is of interest as it could be shown that a violation of the manufacturer's guidelines has negative consequences for the quality of the product.
But it is disadvantageous that only one product of one manufacturer was examined in this study. In order to increase the quality of this study, it might be possible to additionally investigate what happens if MTA (ProRoot MTA or MTA Angelus) is mixed in a capsule like Biodentine instead of by hand. Does this then also have an influence on the material properties of the MTA?
Response: We appreciate the Reviewer's comments and suggestion. Logically, manufacturers test the optimum conditions for their materials, and it is based on those extensive tests that their usage and handling recommendations are emanated. However, there is also empiric knowledge from clinical practice that clinicians may sometimes overlook such recommendations, aiming at less material (and monetary) waste, and disregarding that what is perceived by them as small changes to the protocol might greatly influence the behavior of the material and, ultimately, its clinical performance. Unfortunately, we were unable to accommodate all the groups and variables that we had initially intended in our work. We have, however, included in the manuscript and references literature pertaining to changes in the mixing of MTA, which we deemed adequate and enriching to our manuscript and in line with the point the Reviewer has risen

•The first highlight is not a highlight but an explanation of the procedure. Please delete.
Response: We thank the reviewer for pointing out this mistake, which we have now corrected.

•It is mentioned in the introduction that Biodentine also contains dicalcium silicate. However, this has already been refuted. Please quote the corresponding literature here. Apparently, dicalcium silicate could not be detected in this study either.
Response: We apologize for this mistake, which we have now corrected and appropriately referenced.

•Unfortunately, the "Results" section is not well written. Some results remain nebulous. For example: "There was a tendency of increased final setting time in the groups where a full bottle of BD liquid was used...". What does the word "tendency" mean here? Was there a significant difference yes or no? Basically the statistical evaluation is missing. Not a single p-value is given here.
Response: We kindly thank the reviewer for the comments. We have now included p values and hope these changes clarify our Results section.

•Discussion, 2nd paragraph, "interestingly": There is a good reason why there is more liquid in the ampoule than necessary: 1. this ensures that there is enough liquid available for mixing and 2. with the rest of the liquid you have the option of mixing the already mixed Biodentine again with a drop of liquid if the cement is too solid. This is information from the manufacturer.
Response: We thank the reviewer for the comment and explanation, and we have corrected the text accordingly.

•Some typing errors should be corrected. For example, the references are not case-sensitive.

Response: We appreciate the Reviewer bringing this to our attention. We have carefully gone through the manuscript and corrected all typing errors encountered.

- The Figure Legends are insufficient. Captions must always be self-explanatory. Abbreviations (BD, BD-A, BD-F and so on) must always be expanded. In Fig. 1, the individual photos must be labelled as a - e and then explained with text which mixing method was chosen in each case.

Response: We appreciate the Reviewer bringing this to our attention. We have expanded the figure legends and labelled Fig.1 as requested.

- It remains unclear whether there were significant differences in Figures 4, 5 and 6. If there are significant differences, the bars must be marked with an asterisk, for example.

Response: We thank the reviewer for this remark. We have completed images 4 and 6 to reflect and emphasize statistically significant differences between groups, in accordance also with the changes made in the results section. As for Fig 5, related to setting time results, the analysis done for these data was merely descriptive due to the limited number of samples tested. We have also included this observation in the manuscript to eliminate any misreading.

- Very important in this context: Ca ion release and microhardness are of secondary interest to the dentist in the dental practice when using Biodentine. What is of interest in daily practice is the shortest possible setting time of Biodentine. In Fig. 5, it now appears that BD-MW and BD-ML set much faster than BD. Is that correct? Is the difference significant? If so, this would be a great advantage in practice! However, this would contradict the manufacturer's approach and the statement made here: "Hence, the manufacturer's instructions should be strictly followed" must be critically reviewed from this point of view. This point must be discussed.

Response: We appreciate the Reviewer's comments. We agree that this would be a very interesting discussion, however, due to the limited samples in the setting time test, we limited our analysis to a descriptive one. Nonetheless, we have added a reference to the importance this would potentially have from a clinical perspective, and stressing the need for more studies on this aspect.

Dear Editor-in-Chief,

We come this way to submit for publication in “Dental Materials” the paper entitled “Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine”.

In consideration of the editors of the “Dental Materials” taking action in reviewing and editing this submission, the author(s) undersigned hereby transfer, assign or otherwise convey all copyright ownership to the “Dental Materials” in the event that such work is published in that Journal.

We affirm that we have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest is disclosed.

All authors had active part on the elaboration of this paper, and all authors read the final manuscript version.

Mariana Domingos Pires
(on behalf of all authors)

Dear Editor-in-Chief from Dental Materials. Prof. Dr. David Watts,

Herewith are, as required, the point-by-point responses to the reviewers regarding the submission of the manuscript entitled “Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine”.

We deeply acknowledge the Reviewer’s comments, suggestions, and corrections, which we hope we have fully addressed.

- The aim of this materials science study was to find out what happens if you deliberately violate the manufacturer's instructions when mixing the calcium silicate cement Biodentine. Basically the work was well performed and the manuscript well written. However, from a scientific point of view the aim of this study is somewhat questionable. The manufacturer of Biodentine has already thought about why Biodentine is comparatively complicated to mix compared to other calcium silicate cements. The handling is clearly a competitive disadvantage and the manufacturer would certainly have done without it if it was not necessary. It is therefore not surprising that the last sentence in the abstract reads: "Hence, the manufacturer's instructions should be strictly followed." In principle, one should always follow the manufacturer's instructions when using dental materials and medications, if only for liability reasons. On the other hand, I also know that some dentists violate the manufacturer's instructions out of misunderstood thriftiness and refrain from mixing in the capsule in order to mix small portions by hand. From this point of view, the present study is of interest as it could be shown that a violation of the manufacturer's guidelines has negative consequences for the quality of the product. But it is disadvantageous that only one product of one manufacturer was examined in this study. In order to increase the quality of this study, it might be possible to additionally investigate what happens if MTA (ProRoot MTA or MTA Angelus) is mixed in a capsule like Biodentine instead of by hand. Does this then also have an influence on the material properties of the MTA?

Response: We appreciate the Reviewer’s comments and suggestion. Logically, manufacturers test the optimum conditions for their materials, and it is based on those extensive tests that their usage and handling recommendations are emanated. However, there is also empiric knowledge from clinical practice that clinicians may sometimes overlook such recommendations, aiming at less material (and monetary) waste, and disregarding that what is perceived by them as small changes to the protocol might greatly influence the behavior of the material and, ultimately, its clinical performance. Unfortunately, we were unable to accommodate all the groups and variables that we had initially intended in our work. We have, however, included in the manuscript and references literature pertaining to changes in the mixing of MTA, which we deemed adequate and enriching to our manuscript and in line with the point the Reviewer has risen

- The first highlight is not a highlight but an explanation of the procedure. Please delete.

Response: We thank the reviewer for pointing out this mistake, which we have now corrected.

- It is mentioned in the introduction that Biodentine also contains dicalcium silicate. However, this has already been refuted. Please quote the corresponding literature here. Apparently, dicalcium silicate could not be detected in this study either.

Response: We apologize for this mistake, which we have now corrected and appropriately referenced.

- Unfortunately, the "Results" section is not well written. Some results remain nebulous. For example: "There was a tendency of increased final setting time in the groups where a full bottle of BD liquid was used...". What does the word "tendency" mean here? Was there a significant difference yes or no? Basically the statistical evaluation is missing. Not a single p-value is given here.

Response: We kindly thank the reviewer for the comments. We have now included p values and hope these changes clarify our Results section.

- Discussion, 2nd paragraph, "interestingly": There is a good reason why there is more liquid in the ampoule than necessary: 1. this ensures that there is enough liquid available for mixing and 2. with the rest of the liquid you have the option of mixing the already mixed Biodentine again with a drop of liquid if the cement is too solid. This is information from the manufacturer.

Response: We thank the reviewer for the comment and explanation, and we have corrected the text accordingly.

- Some typing errors should be corrected. For example, the references are not case-sensitive.

Response: We appreciate the Reviewer bringing this to our attention. We have carefully gone through the manuscript and corrected all typing errors encountered.

- The Figure Legends are insufficient. Captions must always be self-explanatory. Abbreviations (BD, BD-A, BD-F and so on) must always be expanded. In Fig. 1, the individual photos must be labelled as a - e and then explained with text which mixing method was chosen in each case.

Response: We appreciate the Reviewer bringing this to our attention. We have expanded the figure legends and labelled Fig.1 as requested.

- It remains unclear whether there were significant differences in Figures 4, 5 and 6. If there are significant differences, the bars must be marked with an asterisk, for example.

Response: We thank the reviewer for this remark. We have completed images 4 and 6 to reflect and emphasize statistically significant differences between groups, in accordance also with the changes made in the results section. As for Fig 5, related to setting time results, the analysis done for these data was merely descriptive due to the limited number of samples tested. We have also included this observation in the manuscript to eliminate any misreading.

- Very important in this context: Ca ion release and microhardness are of secondary interest to the dentist in the dental practice when using Biodentine. What is of interest in daily practice is the shortest possible setting time of Biodentine. In Fig. 5, it now appears that BD-MW and BD-ML set much faster than BD. Is that correct? Is the difference significant? If so, this would be a great advantage in practice! However, this would contradict the manufacturer's approach and the statement made here: "Hence, the manufacturer's instructions should be strictly followed" must be critically reviewed from this point of view. This point must be discussed.

Response: We appreciate the Reviewer's comments. We agree that this would be a very interesting discussion, however, due to the limited samples in the setting time test, we limited our analysis to a descriptive one. Nonetheless, we have added a reference to the importance this would potentially have from a clinical perspective, and stressing the need for more studies on this aspect.

Highlights

- Manual mixing with water results in microstructural changes, with lower setting time and lower calcium ion release.
- Manual mixing required more liquid added to the mixture to obtain a similar consistency and flow to the control
- Increasing the amount of Biodentine liquid increases the calcium ion release but also increases the setting time
- Following the manufacturer's instruction for the mixing of Biodentine is mandatory to guarantee the materials characteristics and clinical performance

Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine

Abstract

Objectives: The water to powder ratio and method of mixing is important for the properties of hydraulic cements. For this purpose a number of clinicians prefer premixed materials. Dental manufacturing companies provide predosed materials, however the manufacturer instructions are not always adhered to. The aim of this research is to investigate physical and chemical alterations of the tricalcium silicate- based cement Biodentine when manipulated according to the manufacturer's instructions (control) or changing the doses and mixing of the material components.

Methods: 6 groups were constituted according to different mixing and dosing of powder and liquid. The hydrated cements were characterized using scanning electron microscopy (SEM), X- ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT- IR). Calcium ion concentration of the leachate was also investigated. Assessment of the physical characteristics included setting time and microhardness.

Results: Microstructural differences were visible only in the Biodentine mixed manually with water, in which early hydration rate was also affected, with lower calcium ion release. Increase of Biodentine liquid increased the calcium ion release, but also increased the setting time. Manual manipulation required more liquid (both water and Biodentine liquid) added to the mixture to guarantee a similar consistency to the control. A decrease in setting time was also noted. All groups showed higher values of microhardness at 24 hours compared to the freshly set materials. In the freshly set materials, there was an overall decrease in microhardness in all groups when compared to group control, particularly significant when increasing the dosage of Biodentine liquid.

Significance: When mixing Biodentine, altering the mixing procedure in terms of type and amount of liquid added to the powder and mixing device chosen has an effect on the physical, chemical and mechanical characteristics and surface topography of the material, when compared to Biodentine mixed according to the manufacturer's recommendations. Hence, the manufacturer's instructions should be strictly followed.

Keywords

Biodentine, ion release, micro-hardness, setting time, tricalcium silicate, water-to-powder ratio

**Effect of different manipulations on the physical, chemical and microstructural characteristics of
Biodentine**

Mariana Domingos Pires^a, Joana Cordeiro^a, Isabel Vasconcelos^a, Mariana Alves^a, Sérgio André Quaresma^a,
António Ginjeira^{a,b}, Josette Camilleri^c

- a) Department of Endodontics, Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal
- b) Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Faculdade de Medicina Dentária,
Universidade de Lisboa, Portugal
- c) School of Dentistry, University of Birmingham, Birmingham, UK

Corresponding author

Dr. Mariana Domingos Pires

Faculdade de Medicina Dentária da Universidade de Lisboa

Cidade Universitária

1649-003 Lisboa, Portugal

Tel: (+351) 91 89 68 9 21

e-mail: mariana.dpires@gmail.com

1. Introduction

There are numerous hydraulic calcium silicate cements (HCSCs) currently in use in endodontics, with mineral trioxide aggregate (MTA) having been the first to be introduced and the most extensively studied. MTA is composed of Portland cement and bismuth oxide in an 4/1 ratio [1,2]. In 2009, Septodont (Saint-Maur-des-Fossés, France) introduced Biodentine (BD), an optimized tricalcium silicate-based dental material to be used as a bioactive dentin substitute [3]. Biodentine is a two component material; powder and liquid. The powder, presented in a capsule, is mainly composed of tricalcium silicate, **with a smaller percentage of calcium carbonate, and zirconium oxide as a radiopacifier** [4]. The liquid is mostly composed of water but also contains calcium chloride as a setting accelerator and a water reducing agent [4,5].

The material is claimed to possess better physical and biological properties compared to other tricalcium silicate-based cements [6]. The advantages over MTA include the elimination of trace elements and of aluminum-based phases by using pure tricalcium silicate in its composition [4,7], absence of tooth discoloration by including zirconium oxide instead of bismuth oxide [8,9,10] and a shorter setting time (approximately 10-12 minutes), as a result of the calcium chloride in the aqueous solution [4, 11]. It also shows higher strength when compared to other similar materials thanks to the lower water to cement ratio possible due to the incorporation of the polycarboxylate in the aqueous solution [4, 12, 13, 14,15] and a higher initial rate of calcium ion release [16,17,18].

The mixing and handling characteristics of the powder/liquid system is very technique sensitive and leads to considerable waste [19]. The manufacturer's recommended mixing instructions for BD include placing five drops of the liquid in the ampoule (which does not correspond to the total amount of liquid) in the open capsule, and placing the closed capsule in a mixing device available from Septodont (Saint-Maur-des-Fossés, France) at 4000-4200 rotations per minute for thirty seconds [20].

In an effort to reduce waste, clinicians and dental assistants may be tempted to manipulate BD with some alterations to the recommendations by the manufacturer [21]. Such alterations may have an impact on the characteristics of the final hydrated material and, thus, its clinical performance [22].

The aim of this research was to investigate whether altering the manufacturer's recommendations for the mixing of Biodentine (Septodont, Saint-Maur-des-fosses, France) would affect the material's physical and chemical properties.

2. Materials and Methods

This study evaluated Biodentine (Septodont, Saint-Maur-des-fossés, France) (BD) manipulated in different ways. The six study groups included the following:

- Group 1 (BD): 1 capsule of BD and 5 drops of liquid, vibrated for 30 seconds (s) in the BD vibrator (BDV), according to manufacturer's instructions;

- Group 2 (BD-A): 1 capsule of BD and 5 drops of BD liquid vibrated for 30 s in the amalgamator (Amg) (Vibracap RS, Paris, France);
- Group 3 (BD-F): 1 capsule of BD and full amount of BD liquid provided, vibrated for 30 s in the BDV
- Group 4 (BD-AF): 1 capsule of BD and full amount of BD liquid, vibrated for 30 s in the Amg
- Group 5 (BD-MW): 1 capsule of BD and 6 drops of water manually mixed on a glass plate until a homogeneous consistency (similar to group 1) was obtained
- Group 6 (BD-ML): 1 capsule of BD and 6 drops of BD liquid manually mixed on a glass plate until a homogeneous consistency (similar to group control) was obtained

Cylindrical specimens 6 mm in diameter and 2 mm high were prepared in stainless-steel molds. The BD was mixed in the different ways defined in Groups 1-6 and tested immediately after setting and again after 24 hours (h) kept at 37°C in Hank's balanced salt solution (HBSS; Sigma Aldrich, Gillingham, UK). All mixing was done by a single operator (JC). The specimens were characterized to assess whether the changes in dosing and mixing affected the material chemistry and microstructure. This was done by scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis, and Fourier transform infrared (FT-IR) spectroscopy. Leachate analysis was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES). The effect of modified mixing and dosing on the physical properties was also assessed, including setting time and microhardness.

2.1 Material characterization

The materials were tested immediately after setting and also after 1 day immersion in Hank's balanced salt solution (HBSS). The characterization by SEM, ATR and one batch for microhardness were tested immediately after the materials had achieved their final setting time testing using an indenter. The XRD, calcium ion release and remaining batch for microhardness were assessed after 24h immersed in 5 mL HBSS and placed into a cabinet at 37 °C.

2.1.1 Scanning electron microscopy:

The ultrastructure was assessed by SEM. Immediately after setting, the specimens were vacuum desiccated and progressively polished. The polishing was achieved using silicon carbide grinding paper of grit sizes 1000 (P2500, CarbiMet, Buehler, Esslingen, Germany) and 280 (P320, CarbiMet, Buehler, Esslingen, Germany) under tap water. The specimens were attached to aluminum stubs, gold coated (K550X Sputter coater, Emitech, Sussex, UK) and viewed under the SEM (EVO MA10, Zeiss, Oberkochen, Germany). Scanning electron micrographs were captured of the different material microstructural components at different magnifications - 500, 1000 and 2000 - in backscatter electron mode.

2.1.2 X-ray diffraction (XRD) analysis

Phase analysis was carried out on the set materials using X-ray diffraction analysis. The diffractometer (D8 ADVANCE, Bruker, Massachusetts, USA) used Cu K α radiation at 40 mA and 40 kV. After 24h immersion in HBSS the materials were desiccated for 30 min, crushed using a mortar and pestle prior to testing. Samples were presented in powder form and the detector was set to rotate between 5 and 50°, a sampling width of 0.058° and scan speed of 1°/min. Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

2.1.3 ATR-Fourier transform infrared (FT-IR) spectroscopy:

After final setting the specimens were desiccated for 30 min and afterwards crushed to powder with a mortar and pestle. The resulting powder was analyzed using a Fourier transform infrared spectrophotometer (Nicolet 6700, Thermo Scientific, Tewksbury, USA) using transmitted infrared spectroscopy with frequencies between 4000 and 400 cm⁻¹ (wavenumbers).

2.1.4 Calcium Ion Release

After setting the specimens were immersed in 5 mL Hank's balanced salt solution (HBSS, Sigma Aldrich, Gillingham, UK) for 24 h. Calcium ion release was assessed by ICP-OES (Optima 8000, PerkinElmer, Waltham, USA) and determined as the average of three measurements per sample in parts per million (ppm).

2.2 Assessment of physical characteristics

2.2.1 Setting Time:

The cements were mixed as previously described and placed in a stainless-steel mold of 10 mm inner diameter and 5 mm height and pressed between two glass plates. The assembly was placed into a cabinet (Shake 'n' Stack, Hybaid, ThermoFisher Scientific, Massachusetts, USA) at 37 °C and relative humidity of 95 ± 5 % for 5 min. An indenter with 100 ± 5 g in weight using a round needle with a flat end, 1 ± 0.1 mm in diameter was used to assess the initial setting time. This indenter was lowered vertically onto the surface of the test cement. This process was repeated every 60 seconds until the indenter failed to make a visible imprint on the sample surface. At this point, the 100 g indenter was replaced by the 400 g indenter to test for final setting time. The previous procedure was repeated until the indenter failed to make a visible imprint on the sample surface. The initial setting time was defined as the time elapsed between the end of mixing and the time when the 100 g needle failed to make a visible indentation on the test cement; and final setting time was defined as the time elapsed between the end of mixing and the time when the 400 g needle failed to make a visible indentation on the test cement.

2.2.2 Microhardness:

Six specimens of each group were mixed according to the previous description. Three were tested after setting and the remaining three were placed in plastic containers with 5 mL HBSS and placed into a cabinet at 37 °C and relative humidity of 95 ± 5 % for 24 h. Microhardness testing (Mitutoyo, Mitutoyo Asia Pacific td. Singapore) was performed using a diamond shaped indenter on polished surfaces. A load of 4,903 N was applied for ten seconds. Vickers Hardness Number (VHN) was recorded from an average of three indentions per specimen.

2.3 Statistical analysis:

The data **obtained for calcium ion release and microhardness** were subjected to statistical analysis using SPSS version 25.0.0 (SPSS Inc, Chicago, IL, USA). The Kolmogorov–Smirnov showed that the data were normally distributed and, therefore, parametric statistical tests were performed (one-way analysis of variance – ANOVA- followed by Tukey’s *post-hoc* test for multiple comparisons). The level of statistical significance was set at $p < 0.05$.

Only one specimen for group was tested for setting time, which conveyed insufficient data to perform a statistical analysis other than descriptive.

3. Results

3.1 Material characterization

The scanning electron micrographs (Fig. 1) of all the groups were similar. Microstructural differences were visible only in the Biodentine mixed manually with water (BD-MW). While all the groups exhibited cement particles with the hydration halo, in the BD-MW group no hydration product around the cement particles was visible, indicating a slower hydration process compared to the other groups.

X-ray diffraction analysis is presented in Fig. 2, where the different phases, namely tricalcium silicate (ICDD: 31-0301), zirconium oxide (ICDD: 37-1484) and calcium carbonate (ICDD: 005-0686) are shown. The materials were identical except for the BD-MW group, which showed a calcium hydroxide peak, visible at $18^\circ 2\theta$. No other group showed this peak.

The FT-IR plots of the six groups are shown in Fig. 3. FT-IR spectroscopy spectrum of BD was analyzed between $4000\text{--}400\text{ cm}^{-1}$. Broad peaks at the region 3400 (water or hydroxide), 2500 and sharper peaks at 1420 and close to 700 cm^{-1} (carbonate) could be identified. The band in the 3400 cm^{-1} region is typical of free OH, which can be present due to water or calcium hydroxide present in the material. This band was modified for all the groups except BD-AF when compared with the control group (BD). At 700 cm^{-1} , typical of carbonate, there was also changes for the BD-AF group, with less absorbance measured.

The results for calcium release (mg/L) are presented in Fig. 4. The use of different mixing methods, (vibrator, the amalgamator or manual mixing) did not affect the calcium ion release as long as the liquid was not changed. The addition of more BD liquid (BD-F, BD-AF) **significantly** increased the calcium ion release ($p=0,001$ and $p=0,003$, respectively), and manual mixing with water deteriorated the calcium ion release.

3.2 Assessment of physical characteristics

The results for initial and final setting times, in minutes, are shown in Fig. 5. There was a tendency of increased final setting time in the groups where a full bottle of BD liquid was used (groups BDF, BDAF), and a decrease in both initial and final setting time in the groups which were manually mixed (BDMW, BDML).

The results for the measurements of microhardness are presented in Fig. 6. All groups showed higher values of microhardness at 24 h compared to the freshly set materials. In the freshly set materials, there was an overall decrease in microhardness in all groups when compared to group control. This decrease was statistically significant with manual mixing with water (BDMW, $p=0,012$) and when the amount of liquid was increased (BD-F, $p=0,010$). The addition of excess liquid also affected the 24 h microhardness values (BD-AF, $p=0,022$). The manual mixing with water recovered after 24 h and exhibited similar microhardness values to the control.

4. Discussion

Biodentine is considered a valid substitute for MTA and MTA-based materials in similar clinical procedures, showing enhanced physical and handling characteristics [23].

Currently, Biodentine is available in pre-set capsules with powder (700 mg) in which, after opening, five drops of the content of the ampule that carries the liquid are to be placed. The capsule should then be re-closed and vibrated at 4000-4200 rotations per minute for 30 s. A specific vibrator is also available from Septodont [20].

Clinically, the quick setting time of Biodentine, and the amount of product obtained from a single capsule, make waste inevitable [24]. A dose of approximately 300 mg is available for most other products. Also, contrary to other pre-mixed hydraulic calcium silicates, Biodentine's currently available presentation and mixing instructions make it susceptible to alterations introduced by the handler [25]. Modifications in the proportions of the product used for mixing and the mixing procedure itself might produce inconsistent setting and diminished physical properties, which translate into poor clinical outcome [26, 27]. Changes to the powder-to-water ratios have been shown to influence MTA's physical properties [21, 28, 29]. **Alterations in the mixing of MTA have also been previously assessed, showing that mechanical instead of manual mixing**

produces a material with better handling characteristics, but not necessarily better characteristics) [30, 31]. To date, no study has identified and analyzed possible changes to Biodentine's properties if the proportions of the two components are altered.

Setting time is one of the most clinically relevant features of a material. It is defined as the length of time for a material to transition from a fluid state into a hardened state [32]. The set material will counteract dislocation, maintain shape and withstand stress, ultimately resulting in maintenance of the seal. An adequate setting time is also important to ensure proper handling characteristics [32,33]. There are several disadvantages that result from the increased setting time. On one hand, a long setting time could cause clinical problems because of the cement's inability to maintain shape and tolerate stresses during this period [33]. On the other hand, a slower setting time may compromise the possibility of placing the definitive restoration in the same appointment after direct pulp capping and negatively affect the sealing ability. Also, in supracrestal areas and when used in sealing root perforations, the material is highly susceptible to washout [32]. Setting time is dependent not only on the material's components, including particle size, radiopacifiers, and additives [21,34], but also on environmental characteristics such as relative humidity and the presence of blood and tissue fluids [34, 35, 36]. Regardless of methodological differences, the literature is consensual in stating that Biodentine has reduced setting time when compared to MTA [14, 15, 37, 38, 39], as advertised by the manufacturer. Clinically, such reduction translates into reduced chair time in procedures where Biodentine is included, while maintaining the advantages of the gold standard MTA.

Calcium chloride has been shown to accelerate the hydration and setting reactions both in MTA and tricalcium silicate materials [40, 41, 42]. On the other hand, the polycarboxylate in BDL, a hydrosoluble polymer, separates the reacting particles and counteracts the accelerating effect of the calcium chloride, thus creating a balance that results in adequate handling characteristics [16]. The hydrosoluble polymer also allows for a lower water/cement ratio and this enhances the compressive strength of the material [43, 44]. Altering the proportions of these components will, therefore, have an impact on setting time of BD since this balance will be disrupted.

Initial setting time of BD was 12 min, which is in accordance with previous reports [37, 45, 46]. Reported results from other studies vary from 6.5 [46] to 30 min [35], with such variations being attributed to different methodologies applied. BD final setting time was 19 min. Only one other study reports on the final setting time of Biodentine [48], with values of 85.66 ± 6.03 min, a difference which is, again, related with different methodologies. Alhodiry *et al.* [35] evaluated only the initial setting time by using a Gilmore needle with 2 mm flat end and 100 g mass. After mixing, the specimens were left at room temperature and evaluated every 5 minutes. Kaup *et al.* [48] used a single indenter with 100 g to determine the final setting time. In this study, final setting time was even lower when the mixture was done with tap water (BD-MW). However, more

water – six drops instead of five - was necessary to obtain a similar mixture, in terms of consistency and flow, to the control, a consequence of the elimination of the hydrosoluble polymer in BDL, which allows the reduction of water content needed to achieve adequate material flow and workability [16]. Replacement of BDL with tap water resulted in a considerable decrease in both initial and final setting times when compared to the control group (BD), also presumably due to the elimination of the polymer, which can be seen clinically as a limitation since early setting might compromise adaptation of the BD and deficient compaction of the material. On the other hand, if the characteristics of the set material were maintained and a considerable reduction of setting time and, hence, chair time, could be obtained, this alteration could represent a major benefit for the clinicians. Further studies, with larger sample sizes, would be needed to assess the impact of this change in Biodentine.

Surface microhardness can be used as an indirect indicator of the extent of the setting process and the overall resistance to deformation [34], being a principal parameter for the mechanical properties of the material. Clinically, it influences the behavior of the material when used for pulp capping under a restoration [46]. Biodentine vickers microhardness has been shown to be identical to sound human dentine but 2-fold superior when compared to other tricalcium silicate materials [48], a feature achievable thanks to the water reducing effect of the polycarboxylate polymer in BDL, which results in decreased porosity and in a material structurally more resistant to compressive forces [6, 16, 45, 49, 50]. The decrease in microhardness for the freshly set materials was statistically significant when the mixing was manual with water added (BD-MW) and when the amount of liquid was increased (BD-F). The addition of excess liquid also affected the 24h microhardness values. BD-MW recovered after 24h, exhibiting similar microhardness values to the control. These results indicate that altering the amount of the liquid and, hence, the additives, might be more prejudicial to the material's compressive strength than completely eliminating these components. Previous studies about factors that affect the microhardness of MTA have shown that the presence of humidity does not affect the MH, despite the presence of serum and blood which adversely affect the microhardness of MTA [27, 51]. This fact may explain why the MH after 24h of BD-MW is similar to the control group.

SEM allows material microstructure observation and surface visualization [4] and has been used to assess hydration mechanisms in both MTA and tricalcium silicate materials [16]. The hydration process is a complex phenomenon that can influence the biological, chemical and physical properties of the cements [26]. described for MTA that most of the residual unhydrated cement grains had a dense rim of hydration product made up of pure calcium silicate hydrate. Also for BD, microstructural differences were detected in the sample mixed manually with water (BD-MW), where probably the hydration process was slower. Indeed, in the group where water was added and manually mixed with powder, the mixture and an homogeneous consistency were difficult to obtain. This explains the necessity to add 6 drops instead of 5 drops, unlike other groups. This variation in the volume of water might produce uncontrolled and undesirable characteristics, such as incomplete setting and poorer handling properties [27]. Encapsulation of pre-set proportions of powder and water appears advantageous as provides the right powder to liquid ratio and avoids the mixing technique,

which is a more operator dependent technique, reducing the variability that might occur when the material is manually mixed.

Material hydration can be further monitored using XRD analysis, since most of the hydration by-products are crystalline, which gives qualitative information of the elemental constitution of the test materials [50]. The FT-IR technique serves an adjunct to XRD in identifying phase constituents in the samples.

Despite Biodentine's high values for calcium ions leached, the low porosity of the material – achieved through the water reducing effect of the polycarboxylate in BDL – explains the lack of superficial calcium hydroxide deposition [34, 53, 54]. The calcium hydroxide peak detected in BD-MW shows that there was deposition of this crystal in the material's surface; replacing BDL with tap-water (BD-MW), with elimination of the hydrosoluble polymer, results in such difference.

The calcium hydroxide, a hydration reaction by-product, accounts for most of the biological properties of hydraulic calcium silicate cements [43, 44, 46]. It is responsible for stimulating the differentiation of the dental pulp and facilitates the mineralisation by means of deposition of dentin-like barrier upon the surface of the pulp [55]. Formation of calcium hydroxide in the early stage is essential for the progression of the hydration process and, consequently, the material's setting and strength [56]. The literature consistently shows higher values of calcium ion release of BD when compared to MTA [16], due, in part, to the purity of the material's composition and the addition of calcium chloride to the mixture [15]. It has been shown that calcium ion release affects positively the antibacterial effectiveness [46]. In our study, altering the amount of BDL (BD-F and BD-AF) resulted in an increase of calcium ions release. Again, these results are attributed to the alteration of the balancing effect exerted by the calcium chloride and polycarboxylate in BDL. These results are in accordance with previous studies in which it was altered the water-powder ratio. When the amount of water was increased in the mixing of the MTA, more calcium ion was released [29]. In fact, when the liquid was not changed, changing the mixing technique did not affect calcium release. On the other hand, when BDL was replaced with tap water (BD-MW) calcium ions released decreased. In a recent study Koutroulis *et al.* [46] characterizes several TCS-based materials. When testing Biodentine and compared with a prototype TCS/ZO, it revealed an enhanced hydration and calcium leaching profile specially due to the presence of additives, such as calcium chloride and calcium carbonate.

5. Conclusions

When mixing Biodentine, altering the mixing procedure in terms of type and amount of liquid added to the powder and mixing device chosen has an effect on the physical, chemical and mechanical characteristics and surface topography of the material, when compared to Biodentine mixed according to the manufacturer's recommendations. Hence, our results are an indication that the manufacturer's instructions should be strictly followed in order to ensure adequate properties of Biodentine and, ultimately, its effects.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors thank Septodont for supplying the materials, and Jianguo Liu for the technical support in scanningelectron microscopy.

References

- [1] Torabinejad M, White DJ (1993). Tooth filling material and method of use. US Patent Patent Number: 5.415.547A
- [2] Torabinejad M, White DJ (1995). Tooth filling material and use. US Patent Patent Number: 5,769,638.
- [3] Septodont Biodentine Active Biosilicate Technology Scientific File. In Vitro 2010. Available online: <http://www.oraverse.com/bio/img/Biodentine-ScientificFile.pdf> (last accessed on 25 September 2020)
- [4] Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. *Dent Mater* 2013; 29(5): 580/-93.
- [5] About I. Biodentine: from biochemical and bioactive properties to clinical applications. *G Ital Endod* 2016; 30: 81-8
- [6] Rajasekharan S, Martens LC, Cauwels RGEC, Verbeeck RMH. Biodentine™ material characteristics and clinical applications: A review of the literature. *Eur Arch Paediatr Dent* 2014; 15(3): 147–58.
- [7] Camilleri J. Characterization and hydration kinetics of tricalcium silicate cement for use as a dental biomaterial. *Dent Mater* 2012; 27 :836–44.
- [8] Vallés M, Mercadé M, Duran-Sindreu F, Bourdelande JL, Roig M. **Influence of light and oxygen on the color stability of five calcium silicate–based materials.** *J Endod* 2013; 39(4): 525–28.
- [9] Marciano MA, Costa RM, CamilleriJ, Mondelli RFL, Guimarães BM, Duarte MAH. Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. *J Endod* 2014; 40(8): 1235–40.
- [10] Vallés M, Roig M, Duran-Sindreu F, Martínez S, Mercadé M. Color stability of teeth restored with biodentine: A 6-month in Vitro Study. *J Endod* 2015; 41(7): 1157–60.
- [11] Nagataki S. Hardening accelerators. Paillere AM, editor. Applications of admixtures in concrete. 1995 Rilem report 10. E & FN Spon, London, UK
- [12] Darvell BW, Wu RCT. MTA - An Hydraulic Silicate Cement: Review update and setting reaction. *Dent Mater* 2011; 27(5): 407–22
- [13] Formosa LM, Mallia B, Camilleri J. The effect of curing conditions on the physical properties of tricalcium silicate cement for use as a dental biomaterial. *Int Endod J* 2012; 45(4): 326–36.
- [14] Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent Mater* 2013; 29(2): e20-8.
- [15] Rajasekharan S, Martens LC, Cauwels RGEC, Anthonappa RP. Biodentine™ material characteristics and clinical applications: a 3 year literature review and update. *Eur Arch Paediatr Dent* 2018 19(1): 1-22
- [16] Camilleri J. Hydration characteristics of Biodentine and Theracal used as pulp capping materials. *Dent Mater* 2014; 30(7): 709–15.
- [17] Camilleri J, Laurent P, About I. Hydration of biodentine, theracal LC, and a prototype tricalcium silicate-based dentin replacement material after pulp capping in entire tooth cultures. *J Endod* 2014; 40(11): 1846–54.

- [18] Gandolfi MG, Siboni F, Botero T, Bossù M, Riccitiello F, Prati C. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. *J Appl Biomater Funct Mater* 2015; 13(1): 43-60
- [19] Debelian G, Trope M. The use of premixed bioceramic materials in endodontics. *G Ital Endodo* 2016; 30(2): 70–80.
- [20] No Authors Listed (2009). Biodentine Safety Data Sheet. Saint Maur des Fosses, France: Septodont. URL accessed on 3/2/2020 at: http://www.sirowahelsinki.fi/uudet_septodont_pdf/Biodentine_safetydata.pdf.
- [21] Koutroulis A, Batchelor H, Kuehne SA, Cooper PR, Camilleri J. (2019a). Investigation of the effect of the water to powder ratio on hydraulic cement properties. *Dent Mater* 2019 (a); 35(8): 1146–54.
- [22] Nekoofar MH, Haddad DC, Nolde J, Aseeley Z. Water content of ampoule packaged with ProRoot MTA: Letter to the Editor. *Int Endod J* 2009; 42(6): 549–51.
- [23] Athanasiadou E, Paschalidou M, Theocharidou A, Kontoudakis N, Arapostathis K, Bakopoulou A. Biological interactions of a calcium silicate based cement (Biodentine™) with Stem Cells from Human Exfoliated Deciduous teeth. *Dent Mater* 2018; 34(12): 1797–1813.
- [24] Taha NA, Abdelkader SZ. Outcome of full pulpotomy using Biodentine in adult patients with symptoms indicative of irreversible pulpitis. *Int Endod J* 2018; 51(8): 819–28.
- [25] Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, Pashley D H, Tay FR. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. *J Endod* 2011; 37(5): 673–7.
- [26] Camilleri J. Hydration mechanisms of mineral trioxide aggregate. *Int Endod J* 2007; 40: 462–70.
- [27] Nekoofar MH, Aseeley Z, Dummer PMH. The effect of various mixing techniques on the surface microhardness of mineral trioxide aggregate. *Int Endod J* 2010; 43(4): 312–20.
- [28] Fridland M, Rosado R. Mineral trioxide aggregate (MTA) solubility and porosity with different water-to powder ratios. *J Endod* 2003; 29: 814–7.
- [29] Cavenago BC, Pereira TC, Duarte MAH, Ordinola-Zapata R, Marciano MA, Bramante CM, Bernardineli N. Influence of powder-to-water ratio on radiopacity, setting time, pH, calcium ion release and a micro-CT volumetric solubility of white mineral trioxide aggregate. *Int Endod J* 2014; 47(2): 120–26.
- [30] Basturk FB, Nekoofar MH, Günday M, Dummer PM. The effect of various mixing and placement techniques on the compressive strength of mineral trioxide aggregate. *J Endod.* 2013 Jan;39(1):111-4.
- [31] Sisli SN, Ozbas H. Comparative micro-computed tomographic evaluation of the sealing quality of proroot mta and mta angelus apical plugs placed with various techniques. *J Endod.* 2017 Jan;43(1):147-151.
- [32] Shen YA, Peng B, Yang Y, Ma J, Haapasalo M. What do different tests tell about the mechanical and biological properties of bioceramic materials? *Endod Topics* 2015; 32 (1):47-85.

- [33] Sluyk SR, Moon PC, Hartwell GR. Evaluation of setting properties and retention characteristics of mineral trioxide aggregate when used as a furcation perforation repair material. *J Endod* 1998; 24 (11): 768-71.
- [34] Yang, D. K., Kim, S., Park, J. W., Kim, E., & Shin, S. J. (2018). Different setting conditions affect surface characteristics and microhardness of calcium silicate-based sealers. *Scanning* 2018; 7136345
- [35] Alhodiry W, Lyons MF, Chadwick RG. Effect of saliva and blood contamination on the bi-axial flexural strength and setting time of two calcium-silicate based cements: Portland cement and biodentine. *Eur J Prosthodont Restor Dent* 2014; 22(1): 20-23.
- [36] Duarte MAH, Marciano MA, Vivian RR, Tanomaru Filho M, Tanomaru JMG, Camilleri, J. Tricalcium silicate-based cements: properties and modifications. *Braz Or Res*, 32(suppl 1): e70.
- [37] Dawood AE, Manton DJ, Parashos P, Wong RHK, Palamara JEA, Stanton DP, Reynolds EC. The physical properties and ion release of CPP-ACP-modified calcium silicate-based cements. *Aust Dent J* 2012; 60(4): 434-44.
- [38] Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus biodentine: Review of literature with a comparative analysis. *J Clin Diagn Res* 2017; 11(8): ZG01-ZG05.
- [39] Quintana RM, Jardine AP, Grechi TR, Graziotin-Soares R, Ardenghi DM, Scarparo R K, Kopper PMP. Bone tissue reaction, setting time, solubility, and pH of root repair materials. *Clin Oral Investig* 2018; 23(3): 1359-66.
- [40] Bortoluzzi EA, Broon NJ, Bramante CM, Felipe WT, Tanomaru Filho M, Esberard RM. The influence of calcium chloride on the setting time, solubility, disintegration, and pH of mineral trioxide aggregate and white Portland cement with a radiopacifier. *J Endod* 2009; 35(4): 550-4.
- [41] Malkondu Ö, Kazandağ MK, Kazazoğlu E. A review on biodentine, a contemporary dentine replacement and repair material. *Biomed Res Int* 2014; 10: 160951
- [42] Saghiri MA, Orangi J, Asatourian A, Gutmann JL, Garcia-Godoy F, Lotfi M, Sheibani N. Calcium silicate-based cements and functional impacts of various constituents. *Dent Mater J* 2017; 36(1): 8-18.
- [43] Camilleri J. The physical properties of accelerated Portland cement for endodontic use. *Int Endod J* 2008 (a); 41:151-7.
- [44] Camilleri J..Modification of MTA. Physical and mechanical properties. *Int Endod J* 2008 (b); 41:843-9.
- [45] Jang YE, Lee BN, Koh JT, Park YJ, Joo NE, Chang HS, Hwang IN, Oh WM, Hwang YC. Cytotoxicity and physical properties of tricalcium silicate-based endodontic materials. *Restor Dent Endod* 2014; 39(2): 89-94.
- [46] Koutroulis A, Kuehne SA, Cooper PR, Camilleri J. (2019b). The role of calcium ion release on biocompatibility and antimicrobial properties of hydraulic cements. *Sci Rep* 2019 (b); 9(1): 19019.
- [47] Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A. Comparison of physical and mechanical properties of mineral trioxide aggregate and Biodentine. *Indian J Dent Res* 2014; 25(6): 692-7.

- [48] Kaup M, Schäfer E, Dammaschke T. An in vitro study of different material properties of Biodentine compared to ProRoot MTA. *Head Face Medi* 2015; 11(1): 16
- [49] Kayahan MB, Nekoofar MH, McCann A, Sunay H, Kaptan RF, Meraji N, Dummer PMH. Effect of acid etching procedures on the compressive strength of 4 calcium silicate-based endodontic cements. *J Endod* 2013; 39(12): 1646–1648.
- [50] Lucas CP, Viapiana R, Bosso-Martelo R, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M. Physicochemical Properties and Dentin Bond Strength of a Tricalcium Silicate-Based Retrograde Material. *Braz Dent J*. 2017 Jan-Feb;28(1):51-56.
- [51] Kang JS, Rhim EM, Huh SY, Ahn SJ, Kim DS, Kim SY, Park SH. The effects of humidity and serum on the surface microhardness and morphology of five retrograde filling materials. *Scanning*. 2012 Jul-Aug;34(4):207-14.
- [52] Camilleri J. Staining potential of Neo MTA Plus, MTA Plus, and Biodentine used for pulpotomy procedures. *J Endod*. 2015 Jul;41(7):1139-45.
- [53] Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. *J Endod*. 2012 Jun;38(6):842-5.
- [54] Zhao W, Wang J, Zhai W, Wang Z, Chang J. The self-setting properties and in vitro bioactivity of tricalcium silicate. *Biomaterials*. 2005 Nov;26(31):6113-21.
- [55] An S, Gao Y, Ling J, Wei X, Xiao Y. Calcium ions promote osteogenic differentiation and mineralization of human dental pulp cells: implications for pulp capping materials. *J Mater Sci Mater Med*. 2012 Mar;23(3):789-95.
- [56] Nekoofar MH, Davies TE, Stone D, Basturk FB, Dummer PM. Microstructure and chemical analysis of blood-contaminated mineral trioxide aggregate. *Int Endod J*. 2011 Nov;44(11):1011-8.

Figure Legends

Figure 1 Representative back-scatter scanning electron micrographs (2000X magnification) of Biodentine mixed according to manufacturer's instructions (BD, figure 1A) or vibrated in an amalgamator (BD-A, figure 1B); with full amount of Biodentine liquid, mixed in the dedicated vibrator (BD-F, Figure 1C) or in an amalgamator (BD-AF, Figure 1D); manually mixed with 6 drops of water (BD-MW, Figure 1E) or 6 drops of Biodentine liquid (BD-ML, Figure 1F).

Figure 2 X-ray diffraction plots of Biodentine with modified mixing and dosing showing the crystalline phases present (CH; calcium hydroxide; CC: calcium carbonate; TCS: tricalcium silicate; ZO: zirconium oxide; BD: control; BD-A: Biodentine vibrated in an amalgamator; BD-F: full amount of Biodentine liquid incorporated; BD-AF: full amount of Biodentine incorporated and vibrated in an amalgamator; BD-MW: manually mixed with 6 drops of water; BD-ML: manually mixed with 6 drop of Biodentine liquid)

Figure 3 FT-IR spectra of Biodentine with modified mixing and manipulation showing changes indicated by arrows (BD: control; BD-A: Biodentine vibrated in an amalgamator; BD-F: full amount of Biodentine liquid incorporated; BD-AF: full amount of Biodentine incorporated and vibrated in an amalgamator; BD-MW: manually mixed with 6 drops of water; BD-ML: manually mixed with 6 drop of Biodentine liquid)

Figure 4 Plots of calcium ion release in solution by the Biodentine with modified dosing and mixing (BD: control; BD-A: Biodentine vibrated in an amalgamator; BD-F: full amount of Biodentine liquid incorporated; BD-AF: full amount of Biodentine incorporated and vibrated in an amalgamator; BD-MW: manually mixed with 6 drops of water; BD-ML: manually mixed with 6 drop of Biodentine liquid). Distinct alphabetic letters indicates statistically significant difference between the materials.

Figure 5 Initial and final setting times of the Biodentine with different dosing and manipulation (BD: control; BD-A: Biodentine vibrated in an amalgamator; BD-F: full amount of Biodentine liquid incorporated; BD-AF: full amount of Biodentine incorporated and vibrated in an amalgamator; BD-MW: manually mixed with 6 drops of water; BD-ML: manually mixed with 6 drop of Biodentine liquid)

Figure 6 Microhardness values (VHN: Vickers Hardness Number) of Biodentine with modified dosing and mixing tested immediately after set and after 24 hours (BD: control; BD-A: Biodentine vibrated in an amalgamator; BD-F: full amount of Biodentine liquid incorporated; BD-AF: full amount of Biodentine incorporated and vibrated in an amalgamator; BD-MW: manually mixed with 6 drops of water; BD-ML: manually mixed with 6 drop of Biodentine liquid). Distinct alphabetic letters indicates statistically significant difference between the materials.











