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Original Article

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A new proposal for evaluating of the solubility of bioceramic materials in dentin tubes after immersion in PBS: a laboratory investigation

Nova proposta para avaliação da solubilidade de materiais biocerâmicos em tubos de dentina após imersão em PBS: um estudo laboratorial

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Resumo

Introdução: Materiais reparadores devem apresentar baixa solubilidade. Solução salina tamponada com fosfato (PBS) permite simular condição clínica e interação com dentina pode ser importante para correta avaliação da perda de massa de cimentos biocerâmicos. **Objetivo:** Avaliou o efeito da imersão em água destilada (AD) ou PBS na solubilidade de Bio-C Repair (BCR, Angelus) ou MTA Repair HP (MTAHP, Angelus) usando modelo de tubo de dentina. **Material e método:** Tubos de dentina bovina foram confeccionados com 4 mm de comprimento, 1,5 mm de diâmetro interno e 1 mm aproximadamente de espessura de parede. Os espécimes foram imersos em AD por 24h, posteriormente preenchidos com BCR ou MTAHP (n = 14) e armazenados em estufa a 37°C e umidade 95% por 24h. Após serem pesados em balança de precisão para determinação da massa inicial, os corpos de prova foram imersos em AD (pH 6,5) ou PBS (pH 7,0) (n = 7) por 28 dias. Tubos vazios também foram utilizados para o cálculo de perda de massa da dentina (n=4). Após esse período, os espécimes foram pesados até a estabilização da massa final (0,001g). A solubilidade de cada material foi avaliada. Testes estatísticos ANOVA e Tukey foram realizados (α=0,05). **Resultado:** BCR e MTAHP apresentaram ganho de massa em AD e perda de massa em PBS (p<0,05). **Conclusão:** A solução de imersão influencia a solubilidade de BCR e MTAHP usando modelo de tubo de dentina. Nova proposta metodológica poderá ser uma alternativa às normas ISO para testar a solubilidade de cimentos biocerâmicos.

Descritores: Calcarea silicata; endodontia; materiais dentários; propriedades físicas.

Abstract

Introduction: Repair materials must have low solubility. Phosphate buffered saline (PBS) allows simulating clinical condition and interaction with dentin may be important for the correct evaluate of mass loss of bioceramic cements. **Objective:** To evaluate the effect of distilled water (DW), or PBS immersion on the solubility of Bio-C Repair (BCR, Angelus) or MTA Repair HP (MTAHP, Angelus) using a dentin tube model. **Material and method:** Bovine dentin tubes with a length of 4 mm, an internal diameter of 1.5 mm and walls thickness of approximately 1 mm were made. The specimens were immersed in DW for 24h, then filled with BCR or MTAHP (n = 14) and stored in an oven at 37°C and 95% humidity for 24h. After being weighed on a precision balance to determine the initial mass, the specimens were immersed in DW (pH 6.5) or PBS (pH 7.0) (n = 7) for 28 days. Empty tubes also were used for calculating the mass loss of the dentin (n=4). After this period, the specimens were weighed until stabilization of the final mass occurred (0.001g). The solubility of each material was evaluated. ANOVA and Tukey statistical tests were performed (α=0.05). **Result:** BCR and MTAHP showing gain of mass in DW and mass loss in PBS ($p<0.05$). **Conclusion:** The immersion solution influenced the solubility of BCR and MTAHP using dentin tube model. The new methodological proposal could be an alternative to ISO standards for testing the solubility of bioceramic cements.

Descriptors: Calcarea silicata; endodontics; dental materials; physical properties.

INTRODUCTION

Repair materials are indicated for retrofilling, pulp capping and root perforation sealing[1]. Low solubility and dimensional stability are important properties, since dissolution or contraction favor infiltration/leakage, thereby compromising successful treatment[2]. Although bioceramic materials have adequate biological, physicochemical, and antibacterial properties[3,4], solubility above values recommended by the *International Organization for Standardization* - ISO 6876:2012 standards have been reported[5,6].

Solubility is determined by the percentage of mass loss after immersion in distilled water, with values lower than 3.0% recommended according to ISO 6876 and *American National Standards Institute e American Dental Association* (ANSI-ADA) standards[7,8]. However, some factors may influence the analysis of bioceramic material solubility[9]. Loss of mass after immersion in distilled water may occur due to dehydration during the drying process[10]. Furthermore, bioceramic materials capture fluids from the environment, and absorb water during the setting process[9], which can influence the results. Immersion of bioceramics in simulated body fluids, such as phosphate-buffered saline solution (PBS) makes it possible to obtain lower solubility[11]. The lower mass loss may be related to mineral deposition on the surface of these materials when immersed in PBS, leading to the formation of a superficial layer of hydroxyapatite[11]. Moreover, interaction of the material with PBS in the presence of dentin makes it possible for the biomineralization process to occur, thereby increasing the sealing and bond strength of the material[12]. However, there are still no studies using dentin tubes model to assess the solubility of bioceramic repair materials after immersion in distilled water or PBS.

Bio-C Repair (BCR; Angelus, PR, Brazil) is a ready-to-use bioceramic repair cement. This cement induces biomineralization[13] and shows cytocompatibility[13,14]. BCR has greater filling capacity and less volumetric change than MTA Repair HP[15], in addition it has adequate radiopacity[14], less porosity and gaps in the material/dentin interface[16]. However, BCR has shown a higher percentage of voids when compared with White MTA (Angelus, PR, Brazil)[17]. However, there are no reports in the literature regarding the interaction of this material with dentin after immersion in different solutions.

MTA Repair HP (MTAHP; Angelus, PR, Brazil) is a cement based on calcium silicate with presentation powder/liquid form. The plasticizer associated to water improved the material consistency[18] and diminished the setting time[19]. MTAHP has biocompatibility and ability to induce biomineralization[18,20], in addition the resistance to compression[21], adequate flow[18,22], antimicrobial activity[23,24], and radiopacity[5,18,24], without promoting tooth discoloration[23]. Its solubility has been described as being low[18,24], however, values above those recommended by ISO have been reported[5]. Nevertheless, the assessment of MTAHP solubility using dentin models in different immersion solution is still unknown in the literature.

Assessment of the solubility of bioceramic cements using dentin tube models can provide approaching values to the clinical scenario. Therefore, the aim of this study was to analyze the effect of distilled water or PBS immersion on the solubility of BCR or MTAHP by using a dentin tube model. The null hypothesis tested was that the different immersion solutions, would not interference the solubility of the materials evaluated.

MATERIAL AND METHOD

The repair cements used in the present study and subdivision of the Experimental Groups are described in Table 1.

Table 1. Experimental groups, materials, their manufacturers, composition, proportions, and immersion solutions used

Experimental Groups	Materials	Manufacturers/Composition	Proportion	Immersion Solution
BCR/DW	Bio-C Repair	Angelus, Londrina, PR, Brasil. Calcium silicate, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agent	Ready to use	Distilled water (DW)
BCR/PBS	Bio-C Repair	Angelus, Londrina, PR, Brasil. Calcium silicate, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agent	Ready to use	Phosphate-buffered saline (PBS)
MTAHP/DW	MTA Repair HP	Angelus, Londrina, PR, Brasil. Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate. Liquid: water and plasticizer	1g powder: 300 µL liquid	Distilled water (DW)
MTAHP/PBS	MTA Repair HP	Angelus, Londrina, PR, Brasil. Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate. Liquid: water and plasticizer	1g powder: 300 µL liquid	Phosphate-buffered saline (PBS)

***BCR**: Bio-C Repair, **MTAHP**: MTA Repair HP.

Sample Calculation

The G* Power 3.1.7 program for Windows (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany) was used for sample calculation. The ANOVA test was used with an Alpha error of 0.05 and a Beta power of 0.95 for all the variables. A previous study[25] was used to determine the specific effect size for solubility, 1.68. A total of 6 specimens per group was indicated as being the ideal size necessary. An n = 7 was used.

Specimen Preparation and Filling

Extracted bovine incisors were used in the study. Radiographic examinations were performed to confirm the absence of anomalies. The roots were cross sectioned in the middle third using carborundum disks (Dentorium, New York, United States), to obtain specimens with a length of 4 mm. Subsequently, each specimen was fixed in the delineator device (Bio-Art, São Carlos, São Paulo, Brazil) and prepared the root canals using Gates-Glidden drills number 6 (Dentsply Maillefer, Ballaigues, Switzerland) coupled to a low-speed motor (Micromotor N270 and Counter-angle; Dabi-Atlante, Ribeirão Preto, São Paulo, Brazil), obtaining a cylindrical tube with an internal diameter of 1.5 mm. The wall thickness of approximately 1.0 mm was made using a cylindrical drill (Maxicut 1503; American Burrs, Palhoça, Santa Catarina, Brazil) being confirmed by digital caliper (Mitutoyo Corporation; São Paulo, SP, Brazil). The manufacturing parameters of the dentin tubes are represented in Figure 1. Throughout the entire preparation procedure root canals were irrigated with 5 mL of distilled water. Final irrigation was performed with 5 mL of 2.5% sodium hypochlorite (Ciclo Farma, Serrana, São Paulo, Brazil) and 5 mL of 17% EDTA (Biodinâmica, Ibiporã, Paraná, Brazil), for 3 minutes, followed by irrigation with 5 mL distilled water.

Figure 1. Parameters for fabrication of dentin tubes models. (A) Specimens with a length of 4 mm; (B) Internal diameter of 1.5 mm and wall thickness of approximately 1.0 mm; (C) Digital radiographic images of the dentin tube to confirm the parameters.

After preparation, the specimens were immersed in 1,5 mL of distilled water and stored in an oven at 37 °C and 95% humidity for 24 hours. After 24 hours, the cavities were filled with BCR or MTAHP (n = 14), using a condenser kit (Ref.: 324501, nºs 2, 3 and 4; Golgran; São Caetano do Sul, SP, Brazil). The samples were kept in an oven at 37 °C and 95% humidity for 24 hours.

Initial Mass Analysis and Immersion of Specimens

After 24 hours, the specimens were weighed on an HM-200 precision balance (A&D Engineering, Inc., Bradford, MA, USA) to determine the initial mass. After this, the specimens were immersed in 7.5 mL distilled water or PBS (n = 7) and kept in an oven at 37°C and 95% humidity for 28 days. Empty dentin tubes (n = 4) were used to calculate the dentin mass loss.

Final Mass Analysis of Specimens

After 28 days of immersion, the samples were weighed again on a precision balance every 24 hour, until the final mass stabilized (0.001g).

The percentage of total mass loss (dentin tube + material) was determined by the difference between the initial and final mass of the samples. Subsequently, the percentage of mass loss of the material was defined by subtracting the percentage of mass loss of empty tubes and the percentage of total mass loss. The methodology used in the present study is represented in Figure 2.

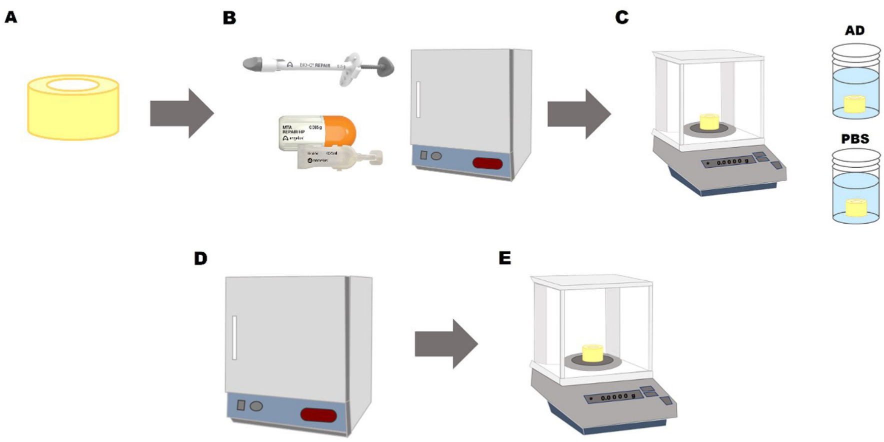

Figure 2. Schematic figure representing the methodology. (A) Bovine dentin tube; (B) Filling the dentin tubes with Bio-C Repair or MTA Repair HP (n=14) and kept in an oven at 37°C and 95% humidity for 24h; (C) Determination of the initial mass of the specimens on a precision balance and immersion in distilled water (DW) or PBS (n=7) for 28 days; (D) Storing of the samples in an oven at 37°C; (E) Determination of the final mass of the specimens on a precision balance after 28 days.

Statistical Analysis

All data were submitted to the Shapiro-Wilk test and were shown to be normally distributed. ANOVA and Tukey tests were used. The level of significance was 5% for all analyses.

RESULT

Bio-C Repair and MTA Repair HP showed gain mass in distilled water and mass loss in PBS ($p < 0.05$) (Table 2).

Table 2. Means and ±standard deviation of solubility (%) of the Bio-C Repair or MTA Repair HP after immersion in distilled water or phosphate-buffered saline (PBS) for 28 days

	Bio-C Repair		MTA Repair HP	
	Distilled water	PBS	Distilled water	PBS
Solubility (% mass loss of material)	-0.32 ± 1.31[b]	1.93 ± 0.88[a]	-1.57 ± 1.24[b]	2.63 ± 0.90[a]

Positive values: mass loss, **Negative values:** mass increase. *Different lowercase letters in the same line indicate statistically significant differences between the experimental groups ($p<0.05$).

DISCUSSION

A dentin tube model and the use of the solution PBS were proposed in the present study, as bioceramic cements in PBS solution provide deposition of calcium phosphate at the dentin/material interface due to the bioactivity of calcium silicate-based cements[13,26]. Furthermore, the interaction with dentin and material may have taken to results closer to clinical reality, observing in this study a gain mass in distilled water and mass loss in PBS. Thus, the null hypothesis was rejected since the solubility of the materials showed differences when evaluated in different immersion environments.

According to ISO 6876 standards, solubility is evaluated in cement specimens after immersion in distilled water for 24 hours. However, high solubility has been reported for bioceramic materials when the conventional test was used[5,6,27]. The present study showed an increase in mass for BCR and MTAHP when immersed in distilled water. This result could be related to the hydrophilic nature of bioceramic cements, capturing water from the environment even after their setting time[28]. Furthermore, during the hydration reaction, mass may increase due to the incorporation of water from the environment[19,29]. The increase in mass of MTAHP has been reported after immersion in distilled water[4]. In contrast, our results showed mass loss for BCR and MTAHP after immersion in PBS, with values lower than 3%. The use of PBS reduced the solubility of calcium silicate-based materials[11,26,30]. However, this is the first study that has evaluated the solubility of bioceramic repair materials by using dentin tubes model after immersion in distilled water or PBS for a period of 28 days.

Although the conventional solubility test is used to assess changes in mass after 24 hours of immersion in distilled water, longer periods of analysis have been used for the purpose of observing the longitudinal behavior of the materials[27,28,30]. Furthermore, the hydration process of calcium silicate cements remains even after final setting[26]. Therefore, in the present study, the solubility of the materials was evaluated after 28 days of immersion in PSB or distilled water. Moreover, in the conventional ISO 6876 solubility test, the specimens come into greater contact with the immersion solution[10]. In the present study, the material came into contact with the immersion solution and interacted with the bioceramic/dentin at the extremities of the dentin tubes, providing a condition closer to that which occurs in the clinical situation.

The development of methodologies alternative to ISO standards allows a better understanding of the behavior of bioceramic materials, considering new solutions, interaction with dentin and longer periods. Therefore, the use of PBS in a dentin tube model could be an alternative to ISO standards for testing the solubility of bioceramic cements.

CONCLUSION

It was concluded that the immersion solutions influenced the solubility of BCR and MTAHP after 28 days using a dentin tube model. The use of PBS as an immersion solution and dentin as a filling model may represent important methodological alternatives for the correct assessment of the solubility of bioceramic repair cements.

AUTHOR CONTRIBUTIONS

Giovanna da Cunha Mendonça: data curation, formal analysis, methodology, validation, investigation, visualization, writing – original draft.

Karina Ines Medina Carita Tavares: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft.

Airton Oliveira Santos-Junior: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft.

Jáder Camilo Pinto: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft.

Juliane Maria Guerreiro-Tanomaru: Conceptualization, funding acquisition, investigation, project administration, supervision, writing – original draft, writing - review & editing.

Mário Tanomaru-Filho: Conceptualization, funding acquisition, investigation, project administration, supervision, writing – original draft, writing - review & editing.

*All authors read and approved the final version of the manuscript as submitted and are qualified for authorship. Furthermore, the authors believe that this submission represents honest work and take full responsibility for the reported findings.

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CONFLICTS OF INTERESTS

The authors declare there are no conflicts of interest regarding this study.

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