Original Article

Volumetric change of calcium silicate-based repair materials in a simulated inflammatory environment: A micro-computed tomography study

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Abstract

Context: An acidic hydrogen potential (pH) in an inflammatory condition in the periapical tissues may affect the properties of repair bioceramic cement.

Aims: The aim of this study was to evaluate the effect of pH on the volumetric change of the ready-to-use bioceramic NeoPUTTY (NP) compared to the powder/liquid MTA Repair HP (MTAHP) after immersion in butyric acid (BA, pH 4.1) or phosphate-buffered saline (PBS, pH 7.35).

Subjects and Methods: Dentin tubes filled with NP or MTAHP were scanned in micro-computed tomography (micro-CT) after 24 h. Then, the specimens were immersed in 1.5 mL of BA: NP/BA, MTAHP/BA or PBS: NP/PBS, MTAHP/PBS. After 7 days, new micro-CT scans were performed. The percentage of volumetric change (extremities and internal part) of the materials was assessed.

Statistical Analysis Used: ANOVA/Tukey and Kruskal–Wallis tests were performed ($\alpha = 0.05$).

Results: All materials showed a volumetric decrease after immersion in BA or PBS at the extremities in contact with the solutions. MTAHP/BA showed the highest volumetric loss. There was no difference in the volumetric change when the internal part of the materials was evaluated.

Conclusions: An acid pH negatively affects the volumetric stability of MTAHP. Low values of volumetric change were demonstrated for NP in both immersion environments.

Keywords: Calcium silicate; endodontics; physical properties; X-ray microtomography

INTRODUCTION

Repair materials are indicated for root-end filling, pulp capping, and root perforation repair.[1] Low solubility and dimensional stability are important properties for an endodontic material since dissolution or contraction can favor leakage, compromising the treatment success.[2] The solubility test recommended by the International Organization for Standardization (ISO 6876).[3] has limitations since it does not consider the loss of mass resulting from the evaporation of liquid present in the material when drying the specimens. Therefore, this methodology, when used to evaluate bioceramic cement, that absorb water from the environment during setting, may not reflect their real solubility.[4]

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Hydrogen potential (pH) of the environment is changed in different clinical conditions to which the bioceramic materials are exposed.[5-7] Immersion in simulated body fluids decreases the solubility for bioceramic materials.[6-9] On the other hand, acidic pH resulting from inflammatory events in the periapical region has been shown to negatively affect the volumetric stability of repair cement,[5,7] as well as promote changes in the chemical structure and interfere with the hydration of these materials,[10] demonstrating greater solubility.[6,7] For better knowledge of the dimensional behavior of bioceramic materials after immersion in different environments, micro-computed tomography (micro-CT) has been used to evaluate the volumetric change of cement under simulated clinical conditions.[4,9,11]

NeoPUTTY (NP; NuSmile, Houston, TX, United States) is a ready-to-use bioactive biomaterial that showed proper cytocompatibility,[12] biocompatibility,[13] and ability to induce mineralization activity.[13] However, regarding the shear bond strength of a resin composite and a resin-modified glass ionomer to four different bioceramic materials, NP had lower values than ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) and NeoMTA2 (Nusmile Inc., Houston, TX; USA).[14] In addition, when comparing in vitro micro-shear bond strength of three different endodontic tricalcium silicate-based materials in contact with a bulk-fill resin-based composite, TheraCal LC (Bisco, Schaumburg, IL, USA) had higher values than NeoMTA2 and NP.[15] According to the manufacturer, NP has high radiopacity, dimensional stability, and low solubility. However, there are no reports in the literature regarding its physicochemical properties.

Specify shear bond strength to what?

MTA Repair HP (MTAHP; Angelus, PR, Brazil) is a calcium silicate-based cement available in powder/liquid form. MTAHP liquid contains water associated with a plasticizer to improve its consistency.[16] MTAHP has a short setting time, radiopacity above 3 mmAl, alkalization ability, and antibacterial activity.[17] The solubility of MTAHP is described as low[17,18] or higher than that recommended by the ISO standards.[16] Furthermore, MTAHP showed greater volumetric change,[4] as well as increased porosity and voids in the material/dentin interface, when compared to Bio-C Repair (Angelus, Brazil) and IRM (Dentsply DeTrey, Konstanz, Germany).[19] In addition, this cement showed a higher percentage of voids and a similar presence of gaps than White MTA (Angelus, Londrina, PR, Brazil) when filling root-end cavities.[20] However, the volumetric behavior of MTAHP after exposure to acidic pH remains unknown in the literature.

Thus, this study aimed to evaluate by micro-CT using a dentin tube model the effect of pH on the volumetric change of the ready-to-use bioceramic NP compared to the powder/liquid MTAHP. The null hypothesis was that the pH of the different immersion environments would not influence the volumetric change of the evaluated cements.

SUBJECTS AND METHODS

Sample size calculation

The G*Power 3.1.7 for the Windows program (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany) was used for the sample size calculation. ANOVA test was used with an alpha-type error of 0.05 and a Beta power of 0.95. A previous study was used to determine the specific effect size for the volumetric change property.[9,10] A total of 4 specimens for groups were indicated as being the ideal size needed.

Preparation and filling of the specimens

Incisive extracted bovine teeth were used in the study (*n* = 20). Digital radiographs (Kodak RVG 6100; Marne-la-Vallée, France) were performed to confirm the absence of anomalies. The middle third of each root was positioned in an IsoMet 1000 precision cutting machine (Buehler Ltd, Lake Bluff, Illinois, United States) and was transversely sectioned, obtaining specimens of a length of 4 mm. After the fixation of each specimen on the delineator device (Bio-Art, São Carlos, São Paulo, Brazil), the root canal preparation was performed using Gates Glidden 6 (Dentsply Maillefer, Ballaigues, Switzerland), obtaining an internal diameter of 1.5 mm. The wall's thickness was determined to be approximately 1.0 mm using a cylindrical drill (Maxicut 1503; American Burrs, Palhoça, Santa Catarina, Brazil), which was confirmed by a digital caliper (Mitutoyo Corporation; São Paulo, SP, Brazil). During the entire preparation, the 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 min, followed by 5 mL of distilled water. After preparation, the specimens were immersed in distilled water and stored in an oven at 37 °C for 24 h. After that period, the dentin tubes were filled with NP or MTAHP (*n* = 10), according to instructions from the manufacturers, using a condenser kit (Ref.: 324501, n° 2, 3, and 4; Golgran; São Caetano do Sul, São Paulo, Brazil). The samples were kept in an oven at 37 °C for 24 h to allow complete setting of the repair materials, according to previous studies.[17,18]

Micro-computed tomography scanning and specimen immersion

After 24 h in an oven, the specimens were scanned by micro-CT (SkyScan 1176, Bruker, Kontich, Belgium) using the following parameters: copper and aluminum filter, 80 kV

X-ray tube voltages, 310 uA anode current, exposure time of 1900 ms, rotation angle 180°, rotation step of 0.5, frame average of 4, and isotropic voxel of 8.74 μm. After initial scanning, each specimen was inserted individually in the Eppendorf tube with 1.5 mL of butyric acid (Sigma Aldrich, Barueri, SP, Brazil, pH 4.2) or PBS (Sigma Aldrich, pH 7.35) ($n = 5$) for 7 days and kept in an oven at 37 °C. The butyric acid solution was changed every 24 h to ensure pH stability. New micro-CT scans were performed after 7 days using the same parameters described above.

Micro-computed tomography analysis

Image reconstructions were performed by NRecon software (v. 1.6.4.7; Bruker micro-CT) using individual parameters for each material. The three-dimensional reconstructed images were registered before and after immersion through DataViewer software (v. 1.5.1; Bruker micro-CT). The registered images were analyzed quantitatively by CTAn software (v. 1.15.4.0; Bruker micro-CT). For volumetric change analysis, each specimen was divided: 1 mm for the upper extremity and 1 mm for the lower extremity (extremities) and 2 mm in the center of the sample (internal part) as represented in Figure 1, allowing the calculation of the difference to the total volume of materials, in mm^3, before and after immersion in butyric acid or PBS. The grayscale range needed to recognize each object under study was determined with a density histogram using adaptive thresholding. Representative images were created by CTVox software (v. 3.2; Bruker micro-CT).

Statistical analysis

The data were submitted to the Shapiro–Wilk normality test and the values of the percentage of volumetric change in the extremities showed normal distribution, and the internal part did not present normal distribution. Therefore, ANOVA/Tukey tests were used to compare the extremities of the materials after 7 days in butyric acid or PBS, and the Kruskal–Wallis test was used to evaluate the data of the internal part. The level of significance was 5% for all analyses.

RESULTS

At the extremities of the samples, all groups showed similar volumetric loss when immersed in butyric acid or PBS ($P > 0.05$). However, MTAHP showed a greater volumetric decrease compared to the other groups when immersed in butyric acid ($P < 0.05$) [Table 1]. In the internal part of the samples, there was no statistical difference in the volumetric change between the evaluated materials ($P > 0.05$) [Table 2]. Figure 2 represents the volumetric change of the cements in different immersion environments.

DISCUSSION

Repair bioceramic materials may have physicochemical

Table 1: Means and standard deviations of the percentage of volumetric change observed in the extremities for NeoPUTTY or MTA repair HP after immersion in butyric acid or phosphate-buffered saline for 7 days

Test/materials	NP-BA	NP-PBS	MTAHP-BA	MTAHP-PBS
Volumetric change (%)	-0.56 ± 1.22^b	-0.44 ± 1.54^b	-3.86 ± 1.26^a	-0.60 ± 1.33^b

Different lowercase letters in the same line indicate statistical differences between the experimental groups (ANOVA/Tukey tests, $P<0.05$). NP: NeoPUTTY, BA: Butyric acid, PBS: Phosphate-buffered saline, MTAHP: MTA Repair HP

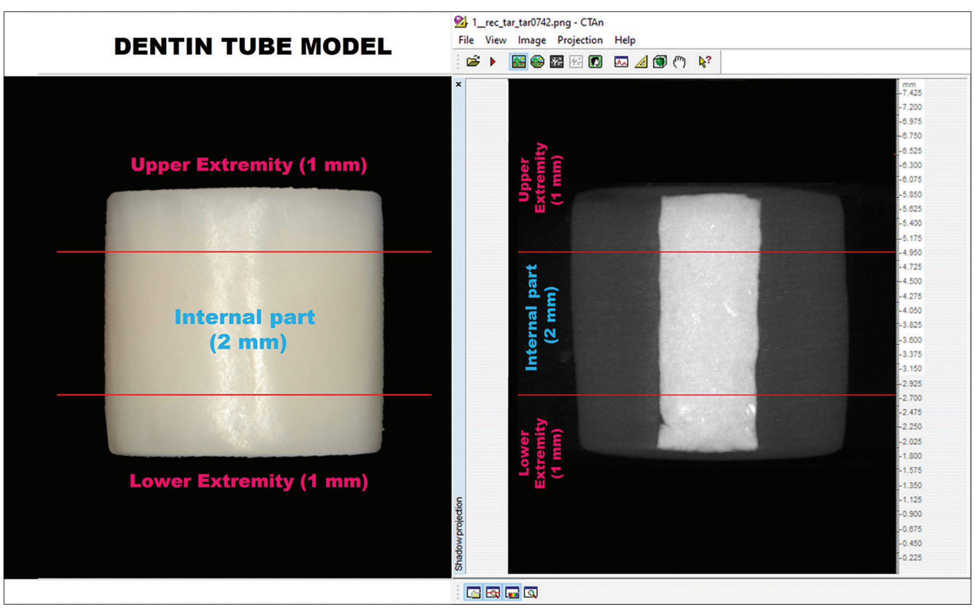

Figure 1: Photograph of dentin tube model (left) illustrating the division for analysis of volumetric change using CTAn software (right)

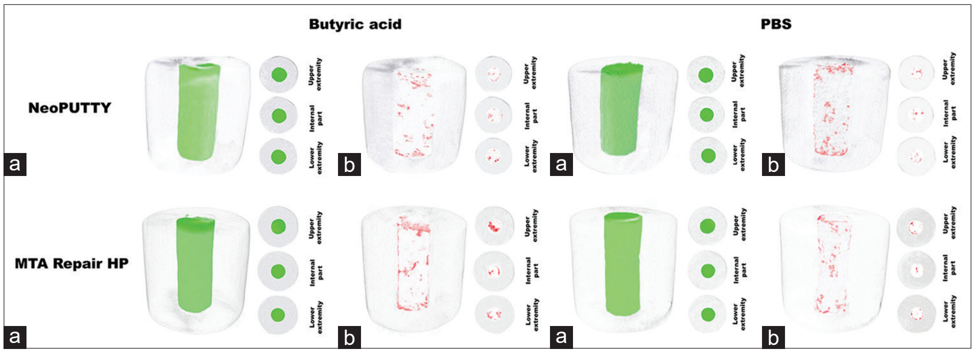

Figure 2: Three-dimensional reconstructions and cross-sectional views of two dentin tubes filled with NeoPUTTY or MTA Repair HP showing initial volume (green) and volumetric loss (red) before, (a) and after, (b) immersion in butyric acid or phosphate-buffered saline for 7 days. PBS: Phosphate-buffered saline

Table 2: Median, minimum, and maximum of the percentage of volumetric change observed in the internal part for NeoPUTTY or MTA Repair HP after immersion in butyric acid or phosphate-buffered saline for 7 days

Test/materials	NP-BA	NP-PBS	MTAHP-BA	MTAHP-PBS
Volumetric change (%)	−0.41 (−1.15–0.25)	−0.47 (−1.66–0.20)	−1.74 (−2.04–−0.74)	−1.27 (−1.99–−0.04)

There was no significant difference between the groups (Kruskal–Wallis test, $P>0.05$). NP: NeoPUTTY, BA: Butyric acid, PBS: Phosphate-buffered saline, MTAHP: MTA Repair HP

changes at an acidic pH.[5] Therefore, in this study, a ready-to-use bioceramic cement, NP was compared to the powder/liquid MTAHP regarding their volumetric change. A clinical environment was simulated in an acidic or neutral environment using a dentin tube model. Significantly different results were observed for these materials, rejecting our null hypothesis.

The evaluation of the volumetric behavior keeping only the ends of the materials in contact with solutions, simulating physiological environments, has been proposed.[11] The present study showed a greater volumetric decrease for the extremities of MTAHP after immersion in butyric acid. Repair cement can be modified by direct contact with an acidic pH environment,[5,7,21] promoting greater solubility of the materials. MTAHP demonstrates solubility above that recommended by the ISO standards,[16] besides a greater volumetric decrease when compared to Bio-C Repair after immersion in distilled water.[4] A previous study showed a considerable percentage of porosity and voids in the interface between MTAHP and a human dentin tube model.[19] In addition, an acidic environment negatively influenced the volumetric stability of ProRoot MTA, MTA Angelus, BioAggregate (Innovative Bioceramix, Vancouver, Canada), and Biodentine (Septodont, Saint-Maur-des-Fossés, France).[5] The plasticizer present in the liquid of MTAHP can promote a surfactant effect that allows the dispersion of cement particles[22] and increases its solubility,[16,22] which may explain the volume loss in the extremities of the samples filled with MTAHP after immersion in butyric acid in this study.

In the present study, NP showed low volumetric change values regardless of the immersion solution. Although this is the first study evaluating the volumetric change of NP, our results corroborate a previous study that observed a lower volumetric change for a ready-to-use repair cement when compared to MTAHP.[4] On the other hand, the premixed sealer EndoSequence BC (Brasseler USA, Savannah, GA, USA) showed higher solubility than AH Plus Jet (Dentsply De Trey, Konstanz, Germany) after immersion in an acid environment.[6] Furthermore, EndoSequence BC demonstrated greater volumetric loss than AH Plus Jet after implantation in the subcutaneous tissue of rats or immersion in PBS.[23] Thus, the findings of this study can serve as a starting point for future investigations regarding the physical behavior of NP under different pH environments.

When evaluating the volumetric change in the internal part of the samples, we did not find significant differences between the materials in both immersion environments. We can suggest that this result is related to the limited contact of the different immersion solutions in the present study with the internal part of the dentin tube and consequently with the materials, approaching a real clinical situation. The long-term volumetric stability of repair materials increases their sealing ability and prevents bacterial microleakage.[11] Furthermore, the interaction of bioceramic cement with dentin and PBS allows the biomineralization process, increasing the bond strength of the material to the dentin.[24]

There are no standardized tests for the assessment of physicochemical properties of repair cement.[7] Thus, tests employing micro-CT to analyze the volumetric change of endodontic and repair cements after immersion in different solutions provide important information.[4,7,11]

Our study used butyric acid to simulate the presence of an inflammatory reaction since bioceramic materials can get in touch with periapical tissues during endodontic procedures.[5,7] PBS was used to mimic a physiological condition, serving as a comparison parameter for the results obtained after an acid challenge. Besides that the dentin tube model employed in our methodology can allow a more reliable physical assessment of bioceramic materials, considering that these cement require moisture and dentin interaction to adequately perform their properties.[19,25]

The volumetric behavior of bioceramic materials in different pH environments and contact with the dentin can approach a real-life scenario and provide important information. The present study contributes to the selection of the most appropriate bioceramic repair material for different clinical applications. Additional studies should be performed to evaluate other physicochemical and biological properties *in vitro* and *in vivo* for these biomaterials.

CONCLUSIONS

The ready-to-use bioceramic cement NeoPUTTY showed volumetric stability after immersion in butyric acid and PBS.

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Conflicts of interest
There are no conflicts of interest.

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