Evaluation of pH changes produced by dental pulp capping materials: An in vitro study

Ocena zmian pH wywoływanych przez materiały pokrywające miążgę zębową – badania in vitro

Elżbieta Łuczaj-Cepowicz^{1, A–D}, Grażyna Marczuk-Kolada^{1, C–F}, Małgorzata Pawińska^{2, E–F}, Marta Obidzińska^{1, B, D}

¹ Department of Pedodontics, Medical University of Białystok, Białystok, Poland

² Department of Integrated Dentistry, Medical University of Białystok, Białystok, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

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Address for correspondence

Elżbieta Łuczaj-Cepowicz

E-mail: elzbieta.luczaj-cepowicz@umb.edu.pl

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Abstract

Background. Ideal direct pulp capping materials should be biocompatible, have antibacterial and anti-inflammatory properties, promote dental pulp regeneration, provide appropriate sealing, be insoluble in tissue fluids, and have sufficient mechanical properties.

Objectives. The purpose of this study was to observe time-related changes that occur in the pH values of different pulp capping materials.

Material and methods. Nine materials were included in this study: Calcipulpe®, Calcipro®, Biopulp®, Life®, Dycal®, ProRoot MTA Grey®, ProRoot MTA White®, MTA Angelus Grey® and MTA Angelus White®. The pH values were recorded immediately after immersion (baseline) and after 1, 2, 3, 4, 24, 48, 72, 168, 336, and 504 h with a pH-meter, previously calibrated with solutions of known pH levels.

Results. All testing materials had an alkaline pH. Analysis of the pH changes as a function of time showed a gradual rise in the pH of all the materials. Differences in pH values between the first and last experimental period were statistically significant ($p < 0.001$). The highest pH increase was observed for setting and the lowest for non-setting calcium hydroxide preparations. Based on the obtained results, the preparations were divided into groups relating to their alkaline abilities. The highest pH increase was observed for setting and the lowest for non-setting calcium hydroxide preparations.

Conclusions. In conclusion, we can say that from the point of view of alkalizing properties, all the evaluated preparations can be used in vital dental pulp treatment.

Key words: pH, mineral trioxide aggregate (MTA), dental pulp capping materials, calcium hydroxide

Słowa kluczowe: pH, konglomerat trójtlenków metali (MTA), materiały do pokrycia miążgi zęba, wodorotlenek wapnia

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The aim of biological dental pulp treatment is to maintain its vitality and function. Preserving tooth vitality is very important for physiological tooth growth and maintaining the balance of the oral cavity environment. Direct pulp capping and pulpotomy (partial and total) are used nowadays. These methods are indicated in situations when dentin and pulp are altered by caries, treatment procedures, or traumatic injuries. The primary objective of the application materials in vital pulp therapy is to stimulate the dentinogenic capabilities of pulp cells.¹ The success of such therapy is determined by the location and type of injury, the state of tooth development, the kind of capping material used, and the sealing ability of the restoration.

A wide spectrum of dental pulp capping materials has been developed. Over the last few decades different preparations have been investigated. Calcium hydroxide (non-setting and setting) and various MTA products are commonly used in clinical practice.

Ideal direct pulp capping materials should be biocompatible, have antibacterial and anti-inflammatory properties, promote dental pulp regeneration, provide appropriate sealing, be insoluble in tissue fluids and have sufficient mechanical properties.² The biological activity of pulp capping agents is determined by the release of hydroxyl and calcium ions. The presence of hydroxyl ions delivered by the material may alter the environmental pH to levels beneficial for cell division and matrix mineralization.³ High pH values induce the secretion of proteoglycans, growth factors, and metalloproteinases from the dentin. These molecules may indicate undifferentiated cells to migrate to the lesion site, proliferate, differentiate into odontoblast-like cells to release organic extracellular matrix, and activate mineralization.^{4–6} Moreover, high pH contributes to the antibacterial action of pulp capping preparations by having a destructive effect on bacterial cell walls and proteins.^{1,7} For that reason, the effectiveness of the bioactive action is directly proportional to the ability of hydroxide ions to dissociate.

The purpose of this study was to observe time-related changes that occur in the pH values of different pulp capping materials.

Material and methods

Sample preparation

The following 9 materials were tested in this study:

1. Non-setting calcium hydroxide pastes: Calcipulpe® (CP) (Septodont, Saint Maur des Fosses, France), Calcipro® (CR) (Igea artis Pharma GmbH + Co, Dettenhausen, Germany), Biopulp® (B) (Chema-Elektromet, Rzeszow, Poland).

2. Setting calcium hydroxide pastes: Life® (L) (Kerr Italia S.r.l., Salerno, Italy), Dycal® (D) (Dentsply DeTrey GmbH, Konstanz, Germany).

3. Mineral trioxide cements (MTA): ProRoot MTA Grey® (GPMTA), ProRoot MTA White® (WPMTA) (Dentsply Tulsa Dental Specialties, Dentsply International Inc., Johnson City, USA), MTA Angelus Grey® (GAMTA), MTA Angelus White® (WAMTA) (Angelus Industria de Produtos Odontologicos LTDA, Londrina-Parana, Brasil).

The materials were mixed immediately before the test, according to the manufacturer's instructions under aseptic conditions, except for Calcipulpe which was packaged in a syringe, with no preparation needed pH assay.

Shortly after preparation, 0.1 g of each material was placed into dialysis tubes (Sigma Aldrich Chemie, Steinheim, Germany) and transferred into separate plastic vials containing 20 mL of deionized water (pH 6.6). A total of 6 samples were used for each material. The vials were hermetically sealed and kept in an incubator at 37°C.

Before each measurement, the vials were shaken for 5 s to ensure uniform hydroxyl ion distribution. The pH values were recorded immediately after immersion (baseline) and after 1, 2, 3, 4, 24, 48, 72, 168, 336, and 504 h with a pH-meter (ISE 710A, Orion Research Inc., Boston, USA), previously calibrated with solutions of known pH levels.^{8–10} Each sample was measured twice, and the mean value was recorded. The experiment was performed in static conditions (without changing the deionized water).¹⁰

Statistical analysis

All statistical analyses were performed using the STATISTICA 8.0 (StatSoft, Inc., Tulsa, USA) software package. One-way analysis of variance, ANOVA, was applied to compare the pH levels of the materials at each time point. If the difference was significant, individual comparisons were performed using Tukey's multiple comparisons test. The level of significance was set at $p < 0.05$.

Results

Table 1 presents the means and standard deviations of hydroxyl ion release from the materials at different periods of time. All testing materials used in our study had alkaline pH. Most of the tested materials, besides those marked at the bottom of Table 1, showed statistically significant differences in terms of pH values ($p < 0.05$). Analysis of pH changes as a function of time showed a gradual rise in the pH of all materials. Differences in pH values between the first and last experimental period were statistically significant ($p < 0.001$).

Based on the results obtained, the preparations were divided into groups relating to their alkaline abilities (Fig. 1). The greatest similarity in pH was found in the following groups: the first consisted of the most alkaline materials

Table 1. Mean and standard deviation pH values evaluated over time periods of the experiment

Material		Time (h)										Statistical analysis 1 h–504 h
		1	2	3	4	24	48	72	168	336	504	
CP	mean	11.75	11.90	11.98	12.05	12.21	12.23	12.28	12.24	12.25	12.31	$p < 0.0001$
(1)	(SD)	(0.10)	(0.08)	(0.07)	(0.06)	(0.04)	(0.03)	(0.01)	(0.01)	(0.02)	(0.01)	
CR	mean	11.68	11.81	11.89	11.96	12.31	12.47	12.32	12.27	12.27	12.24	$p < 0.0001$
(2)	(SD)	(0.14)	(0.18)	(0.19)	(0.19)	(0.11)	(0.06)	(0.05)	(0.01)	(0.01)	(0.02)	
B	mean	11.60	11.74	11.87	11.94	12.25	12.35	12.20	12.15	12.15	12.15	$p < 0.0001$
(3)	(SD)	(0.06)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.01)	(0.01)	
GPMTA	mean	11.50	11.63	11.69	11.70	11.86	11.91	11.93	12.00	12.05	12.17	$p < 0.0001$
(4)	(SD)	(0.13)	(0.10)	(0.15)	(0.15)	(0.13)	(0.12)	(0.12)	(0.11)	(0.06)	(0.01)	
WPMTA	mean	11.31	11.48	11.58	11.62	11.76	11.78	11.84	11.88	11.95	12.06	$p < 0.0001$
(5)	(SD)	(0.12)	(0.13)	(0.15)	(0.14)	(0.14)	(0.12)	(0.12)	(0.10)	(0.09)	(0.09)	
GAMTA	mean	11.14	11.29	11.40	11.44	11.67	11.70	11.75	11.81	11.82	11.95	$p < 0.0001$
(6)	(SD)	(0.20)	(0.19)	(0.18)	(0.18)	(0.13)	(0.09)	(0.09)	(0.10)	(0.09)	(0.10)	
WAMTA	mean	10.94	11.12	11.20	11.27	11.50	11.57	11.69	11.86	12.00	12.15	$p < 0.0001$
(7)	(SD)	(0.21)	(0.19)	(0.18)	(0.20)	(0.16)	(0.15)	(0.12)	(0.08)	(0.07)	(0.07)	
L	mean	10.15	10.35	10.47	10.55	11.09	11.20	11.15	11.21	11.35	11.42	$p < 0.0001$
(8)	(SD)	(0.15)	(0.20)	(0.24)	(0.23)	(0.13)	(0.12)	(0.12)	(0.12)	(0.12)	(0.12)	
D	mean	9.83	10.03	10.15	10.25	10.88	11.17	11.11	11.25	11.55	11.73	$p < 0.0001$
(9)	(SD)	(0.31)	(0.33)	(0.32)	(0.31)	(0.25)	(0.20)	(0.24)	(0.15)	(0.08)	(0.09)	
Statistical analysis		1–2, 3, 4;	1–2, 3, 4;	1–2, 3, 4;	1–2, 3;	1–2, 3;	1–3;	1–2, 3;	1–2, 3;	1–2, 3;	1–2;	no statistically significant differences $p > 0.05$
		2–4;	2–4, 5;	2–4, 5;	2–4, 5;	2–3;	2–3;	3–2;	2–3;	2–3;	2–3, 4, 7;	
		3–2, 4, 5;	3–2, 4, 5;	3–2, 4, 5;	3–2, 4, 5;	4–5, 6;	4–5, 6;	4–5, 6;	3–4;	3–4;	3–4, 5, 7;	
		4–5;	4–5;	4–5, 6;	4–5, 6;	5–6;	5–6, 7;	5–6, 7;	4–5, 7;	4–5, 7;	4–5, 7;	
		5–6;	5–6;	5–6;	5–6;	7–5, 6;	6–7;	6–7;	5–6, 7;	5–6, 7;	5–6, 7;	
		6–7;	6–7;	6–5, 7;	6–7;	8–9	8–9	8–9	6–7;	6–7;	6–7;	
		8–9	8–9	8–9	8–9				8–9			

such as CP, CR and B; the second was composed of 2 MTA groups: GPMTA, WPMTA and GAMTA, WAMTA. The third group contained setting calcium hydroxide preparations (L and D), which were the least alkaline.

The highest pH increase was observed for setting and the lowest for non-setting calcium hydroxide preparations.

Discussion

Release of hydroxyl ions from different dental materials has been investigated by numerous authors.^{2,10–13} There are no uniform standards for pH assays. Two methods have been described for the evaluation of pH changes: potentiometry and titration curves.^{2,11–14} The investigators have also used 2 experimental models: with or without medium replacement after each time period measurement.^{2,3,10,14–17} We applied the second method in the present study. Only Liberman et al.¹⁸ used both methods simultaneously. Additionally, the data obtained in the studies was different and not directly comparable because of the various other methodological approaches employed (quantity of sample, time intervals, type of acceptor medium, method of extraction).

The main purpose of vital pulp therapy is to preserve pulp integrity and functionality. Previous reports have suggested that ionic dissociation of hydroxyl ions from contemporary preparations used in dental pulp protec-

Fig. 1. pH changes of materials tested during the course of time of the experiment

tion is a major factor in their therapeutic action. Authors emphasize the importance of the vehicle in calcium hydroxide dissociation. Aqueous solutions enable better dissociation and thus an increase in pH.^{10,13}

In this study, we evaluated the long-term pH variations of materials used in vital pulp therapy. The materials tested were able to alkalize deionized water at all the tested time periods and reached the maximum rate of hydroxyl ion release at the final reading. The greatest increase in pH was observed in setting calcium hydroxide materials.

Just a few authors have examined different kinds of pulp capping agents under similar experimental conditions as those we applied in this study.^{10,15,19} However, there are no papers comparing non-setting and setting calcium hydroxide materials as well as MTA cements in a single trial.

We showed that the highest pH level throughout the whole observation period was achieved for non-setting calcium hydroxide preparations. Zmener et al.¹⁹ demonstrated similar results in relation to that group of materials, which had yielded a permanent increase in pH for up to 30 days. In contrast, Tamburic et al. found maximum release of hydroxyl ions at 8 h of the experiment.²⁰ Moreover, studies in other experimental conditions confirmed an increase in the pH of these materials.^{12,21}

In the present study, all 4 MTA materials showed alkalizing abilities. Unfortunately, there are no reports comparing these identical MTA products simultaneously. Our results revealed no variations in hydroxyl ion release between white and gray MTA from the same commercial brand throughout the duration of the whole experiment. In contrast, Chng et al. demonstrated a significantly higher increase in the pH of white MTA in comparison with gray MTA after 70 min.¹⁶ In addition, our findings revealed significant differences between GPMTA and GAMTA as well as between WPMTA and WAMTA. This can probably be attributed to variations in the composition of different commercial MTA brands.

Setting calcium hydroxide cements exhibited continuous hydroxyl ion release resulting in a rise in pH until the end of our experiment. Liberman et al.¹⁸ and Gençay et al.²² obtained similar results in shorter 2-hour and 7-day studies. In contrast, Tamburič et al. indicated maximum pH of Dycal at 24 h with subsequent declines at 48 and 72 h.²⁰ Liberman et al. examined the short-release rate of OH⁻ ions into aqueous solutions of 7 calcium hydroxide-containing lining materials, with and without replacing the medium.¹⁸ In the first group, a decline in pH values was recorded, and in the second one an increase in pH values was observed, which caused accumulation of OH ions in the unchanged medium. Maintaining an alkaline pH of setting calcium hydroxide preparations, despite replacement of the medium in long-term evaluations, was also noted by other authors.^{2,3,12,23}

It should be remembered that extrapolation of in vitro investigations to a clinical situation has some limitations.

It is essential to emphasize the fact that there is a permanent exchange of tissue fluids at the material interface that will reduce pH.³ The buffering effect of dentin also has clinical significance.²⁴ However, we can assume that the use of materials with a prolonged alkaline effect may be more advantageous in terms of anti-inflammatory and mineralization activity.

Conclusions

In conclusion, we can say that, from the point of view of alkalizing properties, all of the preparations evaluated can be used in vital dental pulp treatment, but in view of the limitations of this study, further investigation into the conditions that mimic clinical situations is required.

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