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BIOACTIVITY AND PH OF NANO-WHITE MTA VERSUS NEOMTA™ PLUS® AND MTA ANGELUS® AS ROOT REPAIR MATERIALS: AN *IN VITRO* STUDY

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Objective: This study compared the bioactivity and pH of Nano-white mineral trioxide aggregate (NWMTA), Neo-mineral trioxide aggregate plus (NeoMTA Plus), and Mineral trioxide aggregate angelus (MTA-A) as root repair materials.

Methods: A total of 60 discs made from the three materials (20 discs each) were prepared according to the manufacturer's instructions. These discs were packed into plastic molds and allowed to set before testing. For bioactivity study, ten discs of each material were immersed in Hanks' Balanced Salt Solution (HBSS) for 28 days, and analyzed with scanning electron microscope with energy dispersive X-ray (SEM/EDX). Ten discs of each material were used to assess the pH changes by the pH meter at 3 h, 24 h, 72 h, and 168 h. All data were statistically analyzed.

Results: After 28 days of immersing in HBSS, the crystals of Nano WMTA, NeoMTA Plus and MTA were covered with calcium phosphate precipitates with no statistically significant difference ($P=0.908$). The three tested materials induced alkalization of the deionized water after 3 h of immersion and started to decrease at 3 days continuing until the last test at 7 days.

Conclusion: The Nano WMTA, NeoMTA Plus and MTA have similar bioactivity and strong alkalizing activity.

Keywords: Apatite-forming ability, MTA angelus, Nano-White MTA, Neo-MTA plus, Root perforations

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Conflicts of interest:
The authors declare no conflicts of interest.

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BIOACTIVITÉ ET PH DU NANO-WHITE MTA VERSUS NEOMTA™ PLUS® ET MTA ANGELUS® EN TANT QUE MATÉRIAUX DE RÉPARATION RADICULAIRE : UNE ÉTUDE IN VITRO

Objectif : Cette étude a comparé la bio-activité et le pH de l'agrégat de trioxyde minéral nano-blanc (NWMTA), de l'agrégat de trioxyde néo-minéral plus (NeoMTA Plus) et de l'agrégat de trioxyde minéral angelus (MTA-A) en tant que matériaux de réparation des racines.

Méthodes : Un total de 60 disques fabriqués à partir des ces trois matériaux (20 disques chacun) ont été préparés selon les instructions du fabricant. Ces disques ont été emballés dans des moules en plastique et laissés durcir avant de les tests. Pour l'étude de la bioactivité, dix disques de chaque matériau ont été immergés dans une solution saline équilibrée de Hanks (HBSS) pendant 28 jours et analysés au microscope électronique à balayage avec rayons X à dispersion d'énergie (SEM/EDX). Dix disques de chaque matériau ont été utilisés pour évaluer les changements de pH à l'aide du pH-mètre à 3 h, 24 h, 72 h et 168 h. Toutes les données ont été analysées statistiquement.

Résultats : Après 28 jours d'immersion dans HBSS, les cristaux de Nano WMTA, NeoMTA Plus et MTA ont été recouverts de précipités de phosphate de calcium sans différence statistiquement significative (P=0,908). Les trois matériaux testés ont induit une alcalinisation de l'eau déminéralisée après 3 h d'immersion et a commencé par diminuer à partir du 3ème jours jusqu'au dernier test au 7ème jours.

Conclusion : Les Nano WMTA, NeoMTA Plus et MTA ont une bioactivité similaire et une forte activité alcalinisante.

Mots clés : Capacité de formation d'apatite, MTA angelus, Nano-White MTA, Neo-MTA plus, Perforations radiculaires

Introduction

Root repair materials in endodontics play an important role in the successful treatment of damaged or perforated root surfaces [1]. These materials seal and restore the integrity of the root structure, prevent the invasion of bacteria and ensure the long-term stability of the tooth [1, 2]. The prognosis of perforation repair depends upon several factors like location and size of the perforation, time delay before repair, previous contamination of the lesion and biocompatibility of the repair material [2, 3].

Mineral trioxide aggregate (MTA) is one of the most widely applied endodontic repair materials due to its biocompatibility, excellent sealing properties and induction of dentin-like mineralized tissue formation at the interface with the dentin [1-4]. MTA has shown promising results in various clinical and experimental situations like pulp capping, root perforations, apical surgeries and apexification procedures [5-7].

In recent years, novel root repair materials such as NeoMTA Plus and Nano WMTA have been introduced to the market, offering improved handling properties and enhanced bioactivity [4, 8].

NeoMTA Plus is a modified form of MTA that contains tricalcium silicate, dicalcium silicate, calcium aluminate, calcium sulfate, calcium hydroxide, and zirconium oxide instead of tricalcium silicate, tricalcium aluminate, tricalcium oxide, and bismuth oxide at MTA. This difference in composition of NeoMTA Plus enhances its handling properties and overall performance [4, 8, 9]. NeoMTA Plus has a faster setting time (30 to 40 minutes) compared to MTA. This shorter setting time can improve the efficiency of the procedure and decrease chair time for the patient [9]. Although NeoMTA Plus and MTA have some similarities in terms of biocompatibility and tissue response, NeoMTA Plus has several advantages in terms of handling, setting time, resistance to washout, and improved formulation [4, 8, 9]. These differences make NeoMTA Plus a preferred choice for many clinicians in endodontic procedures where these characteristics are necessary for successful results [4, 10, 11].

Nano WMTA is an advanced form of MTA which has introduced as promising cement for root perforation repair. Its nanoscale particle size provides unique characteristics which enhance the repair of root perforations. This nanoscale particle size allows for better adaptation to irregularities in the perforation site, ensuring a tight and impermeable seal [12, 13]. Moreover, nanoscale particles enable better interaction with cells and tissues, promoting the formation of a biologically active dentin bridge and enhancing the natural regenerative process [13].

These materials continue to undergo research and development to optimize their characteristics and expand their applications in endodontic treatment. Therefore, this study compared the bioactivity and pH of Nano WMTA versus NeoMTA Plus and MTA-A root repair materials.

Materials and Methods

Ethical approval

This study was approved by the Ethical Committee of Faculty of Dentistry, Ain Shams University, Egypt (Approval Number: FDASU-RecID041906).

Preparation of the samples

A total of 60 discs made from three different root repair materials: Nano WMTA® (Tooth-colored MTA, DENTSPLY, Tulsa Dental, Tulsa, OK, USA), NeoMTA Plus® (Avalon Biomed Inc. Bradenton, FL, USA) USA), and MTA Angelus® (Londrina, Brazil) were tested. The discs were prepared by mixing each material according to the manufacturer's instructions and then packed into plastic molds [14]. Mechanical vibration was applied to create a flat and even surface.

The discs had an exposed surface area of approximately 8.0 ± 0.1 mm in diameter and a thickness of around 1.6 ± 0.1 mm. After setting, the discs were stored in an incubator at 37°C and 99% relative humidity for 24 hours [14]. The prepared samples were randomly classified into two groups (30 discs each) for assessment of the bioactivity and pH as follows:

Bioactivity

This test was performed for evaluation of calcium phosphate deposits formation on the materials' surface (Apatite forming ability) [14, 15]. Immediately after sample preparation, 10 discs of each tested material were individually immersed in 20 mL of HBSS which was used as simulated body fluid and stored at 37°C for 28 days. The HBSS was renewed weekly.

The surface of each disc was examined in dried status by Environmental Scanning Electron Microscope that was connected to a secondary electron detector for energy dispersive X-ray (EDX) analysis with computer-controlled software at low vacuum (100 Pascal) accelerating voltage of (20 kV), working distance 8.5 mm, 0.5 wt% detection level, 133 eV resolution, magnification time 100 microseconds, measuring time: 600s for element mapping and 60s for spectra. Elemental Xray microanalysis provided micro-chemical spectra, element mapping, and semi-quantitative compositional tables (weight% and atomic% of the elemental composition). The Ca/P ratio was calculated from the obtained data according to Shokouhinejad *et al.* [16].

pH changes

Ten discs of each tested material were immediately immersed in ten test glass tubes containing 10 mL deionized water (pH 6.8) and stored at 37°C. The pH was measured with a pH meter previously calibrated with solutions of known pH (4, 7, 10). The pH of the deionized water (6.8) was verified before the immersion of specimens. The measurements were taken using the pH meter connected with a multi parameter laboratory meter at a constant temperature of 25 ± 2°C, which was kept using an air conditioner. The measurements were taken at 3 h, 24 h, 72 h, and 168 h [17]. Samples were maintained at 37°C in an incubator during all periods between measurements.

Statistical analysis

All data were expressed as mean and standard deviation values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two non-related samples. Repeated measure ANOVA followed by Paired sample t-test was used to compare between more than two related samples. Independent sample t-test was used to compare between two non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

Bioactivity findings

The EDX assessment of tested materials provided the qualitative and semi quantitative elemental composition. The elemental analysis of the three tested root repair materials revealed that they were composed mainly of carbon (C), oxygen (O), calcium (Ca), and silicon (Si). The percentage of each element varied between them. Traces of aluminum (Al), sodium (Na), phosphorous (P), and sulfur (S) were also present. Tantalum (Ta) was only detected in NeoMTA Plus.

After 28 days of immersing in HBSS, the crystals of Nano WMTA, NeoMTA Plus and MTA were covered with calcium phosphate precipitates (Figure 1).

There was no statistically significant difference in the apatite forming ability between Nano-WMTA, NeoMTA Plus and MTA (P=0.908). The highest mean Ca/P ratio was found in the Nano-WMTA samples while the least mean value was found in the MTA samples (Table 1).

The elemental analysis revealed significant changes in the composition of the tested root repair materials compared to their original composition. There was a significant reduction in C and Si contents of all tested materials. In contrast, the P content of all tested materials showed a significant increase. The Ta content of NeoMTA Plus was significantly reduced.

pH findings

The effect of time periods on pH changes

The three tested materials induced the alkalization of the deionized water after 3 h of immersion. The pH decreased starting at 72 h and continuing until the last test at 168 h.

There were no statistically significant differences between the pH measurements at 3 h, 24 h, 72 h and 168 h (P=0.300). The highest mean pH values were found at 24 h while the least mean values were recorded at 168 h in all tested materials (Table 2).

The effect of materials on pH changes

There were no statistically significant difference between Nano-WMTA, NeoMTA Plus and MTA (P=0.496). The highest mean value was found in Nano-WMTA samples while the least mean value was found in MTA samples (Table 2).

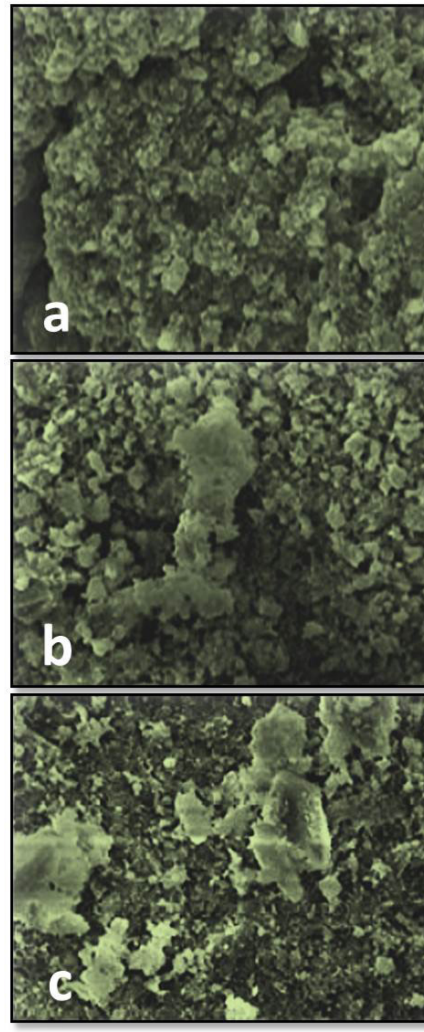

Fig. 1: SEM images showing apatite-like structures formed on the surfaces of Nano WMTA (a), NeoMTA plus (b) and MTA (c).

Table 1: The mean and standard deviation (SD) values of apatite forming ability (Ca/P ratio) of the tested materials

Tested materials	Apatite forming ability (Ca/P ratio)	
	Mean	SD
Nano-WMTA	1.747[a]	0.358
NeoMTA Plus	1.730[a]	0.120
MTA	1.697[a]	0.240
***P*-value**	**0.908 ns**	

Mean with different small letters in the same column indicate statistically significance difference. ns: non-significant (P>0.05).

Table 2: The mean and standard deviation (SD) values of pH changes in the tested materials

Time	pH changes						
	NeoMTA Plus		Nano-WMTA		MTA		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
After 3 hours	11.18[aA]	0.67	11.39[aA]	1.11	10.90[aA]	0.92	0.496 ns
After 24 hours	11.50[aA]	0.91	11.76[aA]	1.00	11.43[aA]	1.21	0.698 ns
After 72 hours	11.09[aA]	0.86	11.30[aA]	1.33	10.63[aA]	1.06	0.393 ns
After 168 hours	10.38[aA]	0.69	10.80[aA]	1.05	10.35[aA]	0.73	0.417 ns
P-value	0.300 ns		0.373 ns		0.147 ns		

Mean with different small letters in the same column indicate statistically significant difference. Mean with different capital letters in the same row indicate statistically significant difference. ns; non-significant (P>0.05)

Discussion

Prompt and effective repairing of root perforations plays a crucial role in maintaining the tooth health [18]. Root repair materials are used in endodontic therapy to seal the accidental or intentional perforations that may occur during root canal procedures [18-21]. These materials seal and stabilize the perforation site, prevent further damage, and promot tissue regeneration [20-22]. These materials should have specific characteristics like bioactivity which can positively impact the outcome of the treatment and the long-term success of the affected tooth [23, 24].

Apatite is a mineral compound that is a major component of both bone and tooth enamel. Therefore, the apatite-forming ability of a perforation repair material is a necessary feature that relates to its bioactivity and ability to promote tissue healing and regeneration [4, 14, 16]. The bioactive materials like calcium silicate-based cements form a surface layer of an appetite-like material in the presence of an inorganic phosphate solution [25]. When calcium silicate-based cements contact fluids, they rapidly release calcium and hydroxyl ions and form an alkaline pH on its external surface, leading to the formation of apatite on the materials' surface [26-28]. Similar to the findings of the present study, several studies have been proved that calcium silicate-based materials have the ability to form apatite deposits (calcium phosphate crystals) on their surface when in contact with body fluids [29-31]. These calcium and phosphate apatite crystals enable regeneration and remineralization of the adjacent hard tissues when a calcium silicate - based material is applied as a perforation repair material [32].

The results of EDX analysis showed that all tested materials develop a calcium phosphate rich layer that contains carbonate ions after immersion in a simulated physiological solution. HBSS solution was used in the present study as a storage solution to simulate the clinical environment. In this study, the results revealed the capacity of MTA to induce apatite deposits formation when immersed in simulated body fluid. These results are in accordance with several previous studies [33-35].

Regarding NeoMTA plus, EDX revealed an increase in the Ca/P ratio. This high Ca/P ratio indicated that the formed precipitates could be a mixture of hydroxyapatite and may be calcium carbonate (calcite). The presence of these precipitates indicates that the tested materials are bioactive [35]. Moreover, the aluminum disappeared from EDX analysis of the tested materials. This could be related to the presence of calcium phosphate precipitates on the surface of materials hiding the trace elements present in the materials' core.

Nano WMTA showed thick coating of Ca/P after 28 days in simulated body fluid. This could be attributed to the use of finer powder particles. This finding was also detected by several previous studies which indicated the bioactive property of this material [36-38].

In the present study, the pH was measured in deionized water rather than simulated body fluid. This is in agreement with the international regulations for the measurements of the chemical-physical properties in order to standardize the test conditions [39].

The tested materials demonstrated strong alkalization of the soaking medium in the first three hours, reaching a maximum level after 24 hours, then decreasing. Similar findings were reported by earlier workers [40]. The alkaline pH induces an antibacterial activity and favors apatite formation [41, 42]. At 7 days, all tested materials showed a pH > 9. The alkaline environment created by the tested materials is attributed to the release of calcium and hydroxyl ions upon contact with fluids. These values suggest that the tested materials may preserve their properties in the long term, providing support to peri radicular healing processes.

Future studies on the biocompatibility of the Nano-White MTA versus NeoMTA™ Plus® and MTA Angelus® root repair materials are recommended.

Conclusion

The Nano-White MTA, NeoMTA™ Plus® and MTA Angelus® have similar bioactivity and strong alkalizing activity.

Clinical Relevance

Nano-White MTA can alternate both NeoMTA™ Plus® and MTA Angelus® when used as a root repair material.

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