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Original article

INFLUENCE OF BLOOD CONTAMINATION ON SURFACE CHANGES OF MINERAL TRIOXIDE AGGREGATE AND BIODENTINE

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ABSTRACT

The biological environment is crucial for the bioactivity of modern calcium-silicate cements. Most of the studies of the materials were performed under ideal conditions, which are difficult to achieve in clinical practice.

The aim of this study was to evaluate the surface characteristics of MTA+ (PPH Cerkamed, Medical company, Polish) and Biodentine in PBS and blood environments with a non-contact 3D optical profilometer

Materials and methods: Retrograde cavities were prepared on the root surfaces of freshly extracted teeth. The cavities were filled with MTA+ or Biodentine, and the samples were immersed in PBS or Blood and placed in an incubator for 28 days. The surface of each specimen was scanned using a 3D optical profilometer at three different time points (1, 3, and 28 days). Statistical calculations were carried out using IBM SPSS Statistics (v.25), and significance was fixed at $p < 0.05$.

Results: Both materials showed that blood contamination affects the roughness of their surface. Both materials placed in blood contaminated conditions show reduced and constant roughness during the reported time periods ($p > 0.05$). This study showed that the surface roughness of MTA+ and Biodentine was adversely affected by blood pollution.

Keywords: MTA, Biodentine, bioactivity, blood contamination,

INTRODUCTION

Recent advances in the field of endodontics have greatly improved the outcome and success rate of dental materials. For the last three decades, there has been great interest in the development of a bioactive dental material with the ability to interact and induce surrounding dental tissues to promote regeneration of pulpal and periradicular tissues.

In general, a bioactive material is defined as a material which has been designed to induce specific biological activities [1]. Based on this broad definition, biologically active materials may include those that promote tissue regeneration by stimulating migration, proliferation, and osteogenic differentiation of the cells. The growth of a layer of apatite on bioactive dental materials is an ideal environment for stem cell and osteoblast differentiation and colonization to support new hard tissue formation [2, 3].

Mineral trioxide aggregate (MTA) and Biodentine are the mostly used calcium silicate-based cements (CSCs) in several endodontic procedures, such as retrograde filling, coronal barrier, pulp capping agent, perforation repair material, and apical barrier for teeth with open apexes. MTA primarily consists of tricalcium silicate (C3S), dicalcium silicate (C2S) and tricalcium aluminate (C3A) with bismuth oxide as a radiopacifier. After hydration of tricalcium silicate, calcium silicate hydrate gel, calcium hydroxide and unreacted tricalcium silicate are formed.

When immersed in different storage media, such as distilled waterand phosphate-containing solutions (e.g. phosphate buffered saline <PBS>), surface precipitates of potential bioactivity were formed on MTA. The precipitation phenomenon was attributed to a possible interaction of the released calcium (Ca^{2+}) with the surrounding fluid environment. In particular, exposure to MTA to PBS resulted in apatite-like crystalline precipitation. It was probable that the formation of apatite-like crystals favorably contributed to the bioactivity of MTA since the ability to induce apatite formation appears to be a common characteristic for silicon-containing biomaterials [4].

Biodentine was introduced to the market in 2011 as a quick-setting bioactive dentine substitute. Biodentine is made up predominantly of highly purified tricalcium silicate as the main core material, calcium carbonate as a filler, and zirconium oxide. The liquid contains water, calcium chloride (used as a setting accelerator), and a hydrosoluble polymer as a water-reducing agent. The absence of bismuth oxide in Biodentine is significant to its properties.

MTA and Biodentine are hydraulic cements which are able to set and harden under water, in blood, plasma, and other fluids [5]. In clinical situations, they may come

into direct contact or even be mixed with blood during or after application. Most of the research on the materials has been done under ideal conditions that are difficult to achieve in clinical practice.

It has been found that different environments can affect the following qualities of materials reduced compressive strength and microhardness, reduced resistance to displacement, sealing ability, including changing the surface characteristics [6, 7].

Surface roughness is an important indicator of the bioactivity of these materials. A rough surface may promote the attachment and proliferation of the cells by increasing material-cell interactions [8], as well as excessive surface roughness might have a negative impact on the strength and sealing of materials [9].

There is little evidence in the literature on the effect of blood contamination on the roughness of MTA and Biodentine and, therefore, on their bioactivity.

This study aims to evaluate the surface characteristics of MTA+ and Biodentine in different environmental conditions with a non-contact 3D optical profilometer.

MATERIAL AND METHODS

Extracted human single-rooted teeth were used in this study. The crowns were resected from the cement-enamel junction (CEJ) using a high-speed diamond bur under water spray. Each root was cut in two halves, the surfaces of which were polished with abrasive discs. In the root canal of each half, a cavity was prepared using an ultrasound retrotype with a diamond coating (Xpedent, E10D, China) at a depth of 3 mm. Irrigation was performed for 3 min using 2.5% sodium hypochlorite (NaOCl), followed by 1 min of 17% ethylenediaminetetraacetic acid (EDTA) to remove any debris in the cavity. The prepared cavity was dried with paper points and obturated with MTA+ (PPH Cerkamed, Medical Company, Polish) or Biodentine (Septodont, Saint Maur des Fausses, France), prepared according to the manufacturer's instructions. The cavities were slightly overfilled, and the excess material was gently burnished. After that, the specimens were assigned to two groups according to the experimental condition (n=6): wet (Dulbecco's Phosphate Buffered Saline: PBS; Sigma-Aldrich, United Kingdom, pH 7.4) or blood. Human blood was obtained by phlebotomy from the first author and placed in a container containing K2 EDTA 5.4 mg per 3 mL of blood to prevent coagulation. The specimens were then placed in an incubator with 100% humidity at 37°C throughout the experimental period. The media were refreshed three times per week.

The surface of each specimen was scanned using a 3D optical profilometer Zeta-20 (Zeta Instruments, KLA Corporation, USA), with a vertical resolution of 1nm a field of view from 0.006 mm^2 to 15 mm^2 and magnification x5.

The average roughness (Ra-Arithmetic average of deviations from the mean), root mean square (Rq-Root mean squared value of deviations), and maximum peak-to-valley height (Rpv- maximum peak-to-valley difference) were measured.

The measurements for surface roughness analysis were performed for each sample at three different time points (1, 3, and 28 days).

Statistical analysis

Statistical calculations were carried out using IBM SPSS Statistics (v.25), and significance was fixed at $p<0.05$. All indicators were presented as mean and standard deviation (mean ± SD). The mean values of the materials and conditions were compared using a t-test or Mann-Whitney Test for 2 independent samples, depending on the presence or absence of a normal distribution of indicators established by the Kolmogorov-Smirnov test with Lilliefors Significance Correction.The means by the time were compared with the Paired Samples Test or Wilcoxon Signed Ranks Test for 2 dependent samples, depending on the form of distribution.

RESULT

Table 1 shows the mean ± SD of Ra, Rq, and Rpv values of each group. Both materials showed that blood contamination affects the roughness of their surface. The roughness of MTA+, when placed in PBS, increases up to 3 days and, after that, slightly decreases ($p<0.05$). The roughness of Biodentine increases during the first 3 days ($p<0.05$) and stays constant for up to 28 days ($p<0.05$).

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Both materials placed in blood polluted conditions show reduced and constant roughness during the reported time periods ($p>0.05$). Rq of MTA+ in PBS is bigger than that of Biodentine on day 1 ($p<0.05$). On day 3, they became equal ($p<0.05$), and on day 28, MTA again showed greater roughness ($p<0.05$). No statistically significant difference in roughness was found between the two materials in blood condition on days 1, and 3 ($p>0.05$), but the such difference was found in favor of MTA+ on day 28 ($p<0.05$) (Figura 1).

Table 1. Time-dependent surface roughness values (mean ± SD) of MTA+ and Biodentine in different environmental conditions

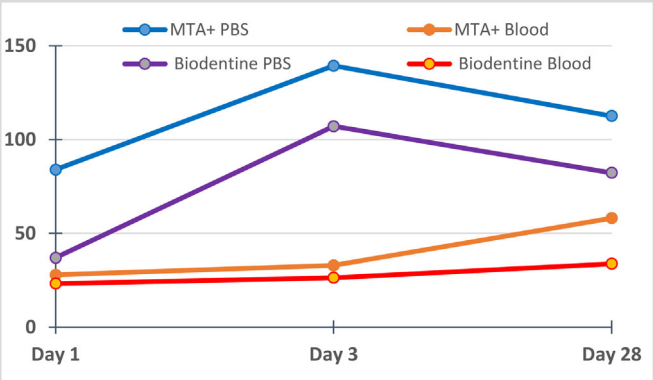
Parameter	Material	Condition	Day 1	Day 3	Day 28
Ra	MTA+	PBS	60.60±6.97 $^{a-?}$	113.24±7.90 $^{c-!}$	87.95±17.26 $^{b-?}$
		Blood	19.35±5.79 $^{a+!}$	24.15±9.09 $^{a+!}$	40.92±9.37 $^{b+?}$
	Biodentine	PBS	27.79±6.06 $^{a-!}$	84.92±30.93 $^{b-!}$	65.34±12.09 $^{b-!}$
		Blood	16.47±3.33 $^{a+!}$	19.04±8.03 $^{a+!}$	25.85±12.97 $^{a+!}$
Rq	MTA+	PBS	84.01±8.02 $^{a-?}$	139.39±8.39 $^{c-!}$	112.58±19.56 $^{b-?}$
		Blood	27.95±8.78 $^{a+!}$	32.91±11.40 $^{a+!}$	53.12±11.90 $^{b+?}$
	Biodentine	PBS	37.02±6.92 $^{a-!}$	107.08±36.05 $^{b-!}$	82.32±14.28 $^{b-!}$
		Blood	23.26±4.63 $^{a+!}$	26.37±10.30 $^{a+!}$	33.83±15.63 $^{a+!}$
Rpv	MTA+	PBS	623.50±7.48 $^{a-?}$	1257.68±95.01 $^{c-?}$	930.50±109.93 $^{b-?}$
		Blood	332.63±110.42 $^{ab+!}$	319.27±73.97 $^{a+!}$	429.72±56.84 $^{b+?}$
	Biodentine	PBS	408.75±39.73 $^{a-!}$	927.16±194.45 $^{c-!}$	684.88±82.62 $^{b-!}$
		Blood	284.49±41.84 $^{a+!}$	260.62±71.59 $^{a+!}$	314.14±96.98 $^{a+!}$

In each row, the different letters (a, b, c) indicate significant differences ($p < 0.05$) in time.

In each column, different symbols (+, -) indicate significant differences ($p < 0.05$) under different conditions and the same material.

In each column, the different symbols (!,?) Indicate significant differences ($p < 0.05$) under different materials and under the same conditions.

Fig. 1. Surfaceroughness of MTA+and Biodentine placedin PBS or blood on 1, 3 and 28 day



DISCUSSION

Bioactivity reflects the ability of the material to release calcium ions, produce calcium hydroxide and form an interfacial layer between the material and dentinal wall leading to the deposition of apatite crystals over the surface of the material when it is placed in a synthetic tissue fluid environment such as phosphate buffer saline [10].

Mineral trioxide aggregate was the first bioactive material used in endodontics, which demonstrated excellent clinical success due to its ability to stimulate tissue repair. As described by previous studies, calcium-silicate-based cements, including MTA and Biodentin, are capable of releasing their major cationic components, resulting in the precipitation of a hydroxyapatite-like mineral on their surface. The formation of a mineral attachment to the inorganic content of dentine is a desirable property among endodontic cements.

The bioactivity of MTA has been proved in several studies. Sarkar et al. [11] showed the formation of hydroxyapatite crystals in phosphate buffered saline (PBS) on MTA surface and MTA–dentine interface by using Scanning Electron Microscope (SEM) and X-ray diffraction (XRD) analysis.They suggested that calcium ions released by MTA react with phosphate in PBS, yielding a hydroxyapatite interfacial layer in the MTA-dentin interface. However, recently it was shown that hardened Portland cement, an active ingredient in MTA, releases calcium hydroxide, which interacts with a phosphate-containing fluid to produce calcium-deficient apatite via an amorphous calcium phosphate phase [12].

In vitro studies by Atmeh et al. [13] and Han and Okiji [14] showed the bioactivity of Biodentine in distilled water and PBS.Although there is less research on biodentine, different methods have shown that the formation of an interfacial layer and a tag-like structure in the Biodentine-dentine interface [15].

In this study, a 3D optical profilometer was used to compare the effects of PBS and blood on the surface roughness of MTA+ and Biodentine over time. The optical profilometer is a non-contact 3D surface topography measuring system. It allows repeatable analyses at different time points without any sample preparation and damage. In most in vitro studies, materials are tested under ideal conditions, which are often lacking in clinical practice.

Whole, fresh human blood was chosen to more closely replicate the clinical situation. However, experiments involving whole, fresh human blood present difficulties such as ethical considerations, biohazard problems, and obtaining sufficient blood volumes over a long period of time.

In our study, it was found a trend of increased surface roughness of both materials in wet conditions. Similarly,the study measured the surface roughness using atomic force microscopy and reported similar values for MTA+ and Biodentine [8]. The increased roughness in wet conditions is a result of the material properties. Both materials develop a bone-like hydroxyapatite layer on the surface when immersed in saline solution; this is a common characteristic observed in calcium-silicate based materials [11, 16]. I.e. both cements demonstrated bioactivity proved by increasing the roughness.

While in Biodentine, the level of roughness at 3 and 28 days is maintained the same, in MTA+, we observed a decrease in roughness. It seems that the bioactive potential of cements based on calcium silicates is a result of the solubility of these materials even after setting. When the material completed setting in 3 days, the excess water might have prolonged the dissolution of the biomaterials, leaving a more porous outer surface.

In blood conditions, the surface roughness of both materials had low values. During clinical application, exposure of the material to blood might affect its setting reaction. This might negatively affect the biological and physical properties of the material.A number of studies have proven the harmful effects of blood on the hardening process and the properties of MTA and Biodentine [17, 18]. Reduced roughness of the samples in blood conditions compared to PBS is attributed to the decreased hydration [19] as a result of the adhesion of blood proteins to the sites of crystal nucleation, as explained by Nekoofar et al. [20]. Another reason for the reduced roughness could be the air entrainment properties of blood proteins that affected the porous microstructure of cements.

CONCLUSION

This study showed that the surface roughness of MTA and Biodentine was adversely affected by blood pollution. Further research is needed to clarify the specific mechanisms and their significance.

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