Influence of sodium hypochlorite and ultrasounds on surface features and chemical composition of Biodentine tricalcium silicate-based material

Aleksandra PALATYŃSKA-ULATOWSKA1, Katarzyna BUŁA2 and Leszek KLIMEK3

1 Department of Endodontics, Chair of Conservative Dentistry and Endodontics, Medical University of Lodz, 251 Pomorska street, 92-217 Lodz, Poland
2 Department of Dental Techniques, Chair of Restorative Dentistry, Medical University of Lodz, 251 Pomorska street, 92-217 Lodz, Poland
3 Institute of Materials Science and Technology, Technical University of Lodz, 1/15 Stefanowskiego street, 90-924 Lodz, Poland
Corresponding author, Aleksandra PALATYŃSKA-ULATOWSKA; E-mail: aleksandra.palatynska-ulatowska@umed.lodz.pl

Biomaterials are subjected to various factors during endodontic workflow. The aim was to evaluate the influence of different concentrations of sodium hypochlorite and ultrasound activation on the features and chemical composition of Biodentine. Fifty-four Biodentine samples were divided into 3 groups based on the material setting time. They were subjected to different modes and times of 2% and 5.25% NaOCl irrigation with or without ultrasounds, 12 min (group I), 45 min (group II) and 24 h (group III) after the material mixing. Visual assessment of the sample’s surface was performed using the scanning electron microscope and chemical analysis was made with energy dispersive spectroscopy. Both NaOCl irrigation and ultrasounds affected the surface of the material; however, they did not change its chemical composition. The irrigation enhanced by ultrasounds following the placement of Biodentine should be performed after a longer material setting time. The immediate use of ultrasounds is not recommended.

Keywords: Biodentine, EDS, Irrigation protocols, SEM, Tooth perforation

INTRODUCTION

Since the mid-1990s mineral trioxide aggregate (MTA), a Portland cement-based material, has been recommended for perforation closure procedures in endodontics[1]. Nowadays, new types of bioceramic and bioceramic-like materials are also available. Their chemical composition influences the properties of the material and may also affect clinical outcomes. The hydraulic nature of these cements, their biocompatibility, the release of calcium hydroxide, antimicrobial activity and bonding-to-dentin strength are the features of great importance. Knowledge of the cement type, nature of radiopacifiers, additives and chemical liquid components are important to properly choose, use and manage these materials in different clinical conditions.

In addition to Portland cement-based materials, dicalcium and tricalcium silicate-based cements have also been developed. Biodentine (Septodont, Saint-Maur-des-Fossés, France) also known as a dentin replacement material[2-4] has many indications within the scope of endodontic practice. It can be applied in biological treatment of the vital pulp[5-10], microsurgery[9,11] as well as in repairs of the dentin structures in perforation closures[12,13]. Biodentine is a tricalcium silicate-based material with zirconium oxide as a radiopacifier, calcium carbonate and calcium oxide as fillers, calcium chloride and hydroxysoluble polymer as additives[5,8,14-17]. Its chemical composition supports good biocompatibility, faster setting time, good scalability and color stability.

Root perforation, as a communication between the canal system and the external tooth surface[18], is one of the indications for the use of above materials. Perforations occur as a result of a pathological alteration or an operative procedural accident. In these situations, treatment success depends on early diagnosis and immediate treatment[19-21]. Apart from the size and location of perforation, the key factor in the prognosis of the management of these defects is the time between the occurrence of the perforation and its sealing[21,22]. Therefore, if possible, the sealing of a fresh perforation should be done as quickly as possible to avoid the onset of infection. In most cases, prior to the placement of repairing material a thorough canal debridement is achieved by mechanical and chemical means. However, in large, extensive perforations, when there is a difficulty to maintain the dryness of an operating field due to the excessive bleeding, the early closure of the defect before regular endodontic therapy may be indicated. In some reendo cases with fresh and large perforations of the floor of the chamber, to avoid their contamination and pushing the masses of cement and GP increments being removed from infected canals through the perforation aperture should also be avoided. Thus, these perforations need to be closed prior to completing the root canal procedures. There may also happen that the secondary treatment is needed in the tooth with previously successfully closed perforation with the repairing cement seated on place. In all of the above clinical situations the material placed should withstand the mechanical action of intracanal instruments as well as the chemical action of canal irrigants that may impact on the cements ability to seal the perforation. While mechanical activity is only roughly two-thirds successful in achieving

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this goal, the use of an aggressive chemicals such as sodium hypochlorite (NaOCl) has been a benchmark for years. Sodium hypochlorite is widely used in root canal procedures\textsuperscript{23-25} and is a non-specific proteolytic and antimicrobial agent that is able to dissolve pulp tissue remnants and disinfect the root canal system\textsuperscript{26}. It's properties vary depending on concentration used, volume, application or delivery methods, thermic, sonic or ultrasonic activation. However, studies have shown that in its application passive ultrasonic irrigation (PUI) approach is significantly superior when compared to manual irrigation in regard to debris and smear layer removal\textsuperscript{26}.

Many studies have documented and compared the specific features of MTA and Biodentine\textsuperscript{2,10,13,27-31}. According to Torabinejad et al.\textsuperscript{1,10} the MTA cement hardens within 4 h, which delays further treatment. While Biodentine cement is claimed to set in 12 min, and it is structurally and clinically stable after mixing according to the manufacturer's manual, there is no data concerning any possible impact of sodium hypochlorite along with ultrasonic applications on Biodentine. The aim of the study was to evaluate the influence of different concentrations of sodium hypochlorite and ultrasound activation on the surface features and chemical composition of Biodentine, based on its setting time and the time of the rinsing procedure.

**MATERIALS AND METHODS**

Fifty-four standardized Biodentine discs were prepared following the manufacturer's instructions in terms of proportion, time and mixing method (Rotating Capsule Mixing Device RotoMix, 3M ESPE, St. Paul, MN, USA). Prepared cement was inserted into a polyvinyl tube (PVC, polivinyl chloride; Cellfast, Stalowa Wola, Poland) and compacted with a fitted plugger for 5 s on a smooth glass plate. The size of the cylindrical samples was 8 mm in diameter and 3 mm in height as shown in Fig. 1A. The specimens were then left to set. They were removed from the PVC forms with the help of a customized plugger after three different setting times that were measured from the end of the mixing process: 12 min (according to Biodentine Active Biosilicate Technology Scientific File), 45 min\textsuperscript{30,32} and 24 h, respectively. The Biodentine samples were randomly divided into 3 investigated groups based on the material setting time. The exact distribution of experimental samples regarding the time of irrigation protocol and concentration of rinsing solution is presented in Fig. 2. The surface of the evaluated items under the magnification of 1,000× was uneven and unequal in random examined locations. Therefore, in order to unify their surface, 36 evaluated items were polished with rotating sandpapers (following grits of 600, 800, 1,000 and 1,200). Eighteen Biodentine samples were found impossible to polish due to instability of the material after the shortest 12-min setting time. Despite the soft consistency of those specimens, further experimental steps were made.

Examined Biodentine specimens were immersed in 10 mL of 2% or 5.25% concentration of NaOCl for an exact time of 5 and 20 min and were subjected to no further activity or were subjected to ultrasound application according to a planned protocol (Fig. 2). An ultrasonic device Sonic-0.5 (50 Hz frequency, Polsonic, Warsaw, Poland) was used to activate the rinsing solutions. Subsequently and in order to avoid any precipitates on the evaluated surfaces, each sample was removed from the container, suspended in demineralized water for 30 s and dried with air. The control group comprised of 6 control samples prepared after corresponding 12 min, 45-min and 24-h setting times and was not subjected to any irrigation protocol.

Scanning electron microscope (SEM) investigation and elemental analysis of 36 samples were performed using a SEM (S–3000N, HITACHI, Tokyo, Japan) with magnifications of 1.0 k with 15 kV accelerating voltage. The chemical composition analysis of the Biodentine surface was conducted in the SEM using the X-ray microanalysis with the energy dispersive spectroscopy (EDS) method and using Vantage software (Thermo Fisher Scientific, Waltham, MA, USA).

**RESULTS**

**Setting time**

The 12-min Biodentine samples were impossible to polish due to the instability of the material. Furthermore, when immersed in both NaOCl concentrations with and without ultrasonic activation, they became severely

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**Fig. 1** Biodentine samples (8×3 mm) removed from the PVC form (A). Destruction of 12-min-set Biodentine sample immersed in 2% NaOCl for 5 min without ultrasonic activation (B) and 5.25% NaOCl for 5 min with ultrasonic activation (C).
Fig. 2 SEM images of the Biodentine control samples without any rinsing procedures (A) and the studied samples after 45-min (group II) (B) and 24-h (group III) (C) setting time subjected to different irrigation protocols.

The Table also shows the distribution of all groups and evaluated specimens in the study based on the setting time of the material (12 min, 45 min and 24 h), time of irrigation protocol (5 and 20 min), concentration of NaOCl (2 and 5.25%) and the use of ultrasounds (+US).
softened and/or totally degraded (Figs. 1B, C). Therefore, neither SEM analysis nor EDS method were possible in the exact time of 12 min and those samples were excluded from further investigation. Two control samples after 12-min setting time were also excluded from the study due to soft consistency of the material and the impossibility of conducting SEM analysis at the exact time. The samples (group II and III) were sufficiently set to be polished and examined after longer setting time.

**Irrigation protocol**

SEM images of the II and III sample groups were compared to the images of the control group not subjected to any irrigation protocol. The comparisons showed that both concentrations of sodium hypochlorite alone and those enhanced with ultrasonic activation, visibly affected the Biodentine surface (Fig. 2). All of the surfaces in groups II and III were altered and became more irregular, compared to the regular surface of control specimens.

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**Fig. 3** Examples of energy dispersive spectrograms of Biodentine control and utmost evaluated samples.

**Table 1** Chemical composition (atom percentage) of Biodentine surface after 45-min (group II) and 24-h (group III) setting time

| Element | Untreated (control group) | 2% NaOCl; 5 min | 5.25% NaOCl; 5 min | 2% NaOCl+US; 5 min | 5.25% NaOCl+US; 5 min | 2% NaOCl; 20 min | 5.25% NaOCl; 20 min | 2% NaOCl+US; 20 min | 5.25% NaOCl+US; 20 min |
|---------|---------------------------|-----------------|--------------------|---------------------|-----------------------|------------------|---------------------|---------------------|-----------------------|
| C       | 11.35                     | 15.49           | 17.75              | 17.79               | 17.96                 | 14.23            | 16.53               | 15.17               | 16.04                 |
| O       | 49.61                     | 55.38           | 58.64              | 57.3                | 57.4                  | 59.48            | 61.72               | 59.62               | 57.44                 |
| Si      | 5.96                      | 3.38            | 1.69               | 1.69                | 1.94                  | 0.57             | 1.52                | 1.87                | 2.2                   |
| Zr      | 0.56                      | 0.27            | 0.07               | 0.18                | 0.12                  | 0.12             | 0.12                | 0.14                | 0.1                   |
| Cl      | 3.64                      | 1.43            | 1.23               | 0.92                | 2.49                  | 0.57             | 0.41                | 1.94                | 3.75                  |
| Ca      | 28.87                     | 24.04           | 20.62              | 22.12               | 20.09                 | 25.15            | 19.71               | 21.26               | 20.48                 |

**Group II** —atom percentage (%)

**Group III** —atom percentage (%)

| C       | 40.41                     | 16.11           | 13.46              | 14.1                | 14.31                 | 19.3             | 12.99               | 16.97               | 16.83                 |
| O       | 48.93                     | 54.02           | 53.95              | 57.54               | 61.58                 | 59.55            | 58.13               | 57.9                | 57.75                 |
| Si      | 9.09                      | 3.64            | 7.61               | 5.69                | 4.04                  | 3.75             | 6.81                | 2.69                | 2.28                  |
| Zr      | 0.74                      | 0.13            | 0.34               | 0.19                | 0.11                  | 0.11             | 0.48                | 0.16                | 0.19                  |
| Cl      | 2.09                      | 0.57            | 1.16               | 0.77                | 0.61                  | 0.43             | 1.43                | 0.04                | 0.25                  |
| Ca      | 27.74                     | 25.53           | 23.47              | 21.71               | 19.35                 | 16.85            | 20.16               | 22.25               | 22.68                 |
In the subgroups of the second (II) investigated group of 45-min-set specimens there are only minor changes observed between the surfaces of samples subjected and not subjected to ultrasound (vertical comparison of images between subgroups in Fig. 2C). However, the time of irrigation and NaOCl concentration seemed to have influenced the surfaces (horizontal comparison of images in Fig. 2C).

Different surface features of 24-h-set Biodentine material were observed in all the SEM images of the third (III) group (comparing the Fig. 2D images in both vertical and horizontal directions). Only the surfaces of these specimens subjected to ultrasound looked almost the same regardless of the time and concentration of the irrigant. When compared to the control group, the surfaces of the 24-h-set samples subjected to ultrasound rinsing appeared to be affected the most and in a similar fashion. Due to numerous hollow pits and round-shaped defects they resembled etched dentin with open tubules, being concave, contrary to the convex unevenness of the control samples.

Chemical composition
On the basis of the data obtained from EDS, spectrograms were generated (Fig. 3) and the percentage composition of the evaluated material was calculated (Table 1). EDS showed no significant differences in chemical composition of Biodentine samples after any kind of analyzed irrigation protocol. Neither the setting time of the material nor the concentration of the rinsing solution, the ultrasound usage or the time of their action influenced the percentage composition of the evaluated Biodentine specimens.

DISCUSSION
Biocompatibility and the sealing properties of the materials used for perforation closure are crucial, however, proper selection and management of cement are even more important. Compared to MTA, Biodentine serves as good quality perforation repair cement after being exposed to various intracanal irrigants.

According to the in vitro results, the 12-min setting time of Biodentine recommended by the manufacturer is too short for the cement to withstand the immediate irrigation protocol using ultrasound. Even though the present protocol did not influence the chemical composition of the cement, its degradation or destruction may affect the treatment outcome.

The Biodentine samples maintained their form and remained dimensionally stable after removing them from the PVC form. While the setting time purported for Biodentine may be sufficient for proper management of carious defects in conservative dentistry, the results of this study showed that rinsing procedures with NaOCl, including ultrasound application may disturb surface features, degrade the three-dimensional form and diminish the stability of the material. Biodentine may simply be rinsed away by the flow of irrigating liquid. Although a push-out bond strength of the material that affects dentin might stay relatively stable after different irrigation protocols, it is the uncovered, non-bonded surface that seems to suffer more. Thus, the overall quality of the sealed perforation site sealing may deteriorate, thereby affecting the success rate of the overall procedure.

The 45-min setting time for Biodentine given by Grech et al., was taken into account in this study. The present study showed lack of chemical changes to the set cement after the examined irrigation protocols. Visual assessment of the microscopic images showed slight modifications of the Biodentine surface. The material seemed to be incompletely set, as the inter-grain area remained slightly runny. This may help to “protect” the material from external chemical and physical forces. It could be described as flowable material adaptation preventing single zirconium oxide and tricalcium crystals (grains) anchored in the inter-grain gel matrix from being ripped out of the cement.

The longest 24-h setting time made a difference, as the calcium silicate Biodentine matrix became solid. However, sodium hypochlorite irrigation and ultrasound appeared to pluck the crystalline forms from their matrix, leaving empty holes, visible on the SEM images. However, these microscopic differences in the surface texture of the material set 45 min or 24 h before, require further investigation. However, one possibility may be that the development of the surface defects may have an impact on the push-out bond strength of Biodentine and traditional final restoration materials. An additional consideration for the presence of these defects may be in the manner in which the Biodentine was mixed and manipulated, as studies have shown that triturated Biodentine may provide a better seal as opposed to hand mixing of the material.

In the light of current studies and present results it seems that Biodentine should be given more time to set. According to Hashem et al., Biodentine is a weak restorative material in its early setting phase. The cited authors concluded that composite resin placement as part of the layered definitive restoration should be delayed for 2 weeks to allow sufficient intrinsic maturation of Biodentine to withstand contraction forces from the resin composite. The results of the present study also confirm the importance of the setting time and assumed inner maturation of the material used for perforation repairs, where additional chemical and mechanical factors may take place. The more the cement is set, the less susceptible it is to any external damage. Therefore, sodium hypochlorite irrigation or PUI should be performed during the next appointment, preferably after 24 h.

This study confirmed that Biodentine is composed of tricalcium silicate, calcium carbonate, zirconium oxide and calcium hydroxide. The EDS analysis clearly showed peaks of calcium, silica, carbon, zirconium and oxygen. The chlorine peak indicates the presence of calcium chloride used as an activator in Biodentine liquid. Neither rinsing with sodium hypochlorite nor its passive ultrasonic activation influenced the given
composition of the properly set material.

In the era of biocompatible bioceramic materials, the knowledge of their indications, proper management and application modes are important. For a long-term success, especially in challenging and compromised cases of chamber or root perforations, it is highly, clinically relevant.

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