YAMAKIN

From Production Skills to Scientific Knowledge,
and Onward to Fusion with Medical Science

TMR-MTA cement Mielle
Product Report

Feature of TMR-MTA cement Mielle and key point of treatment

Made in Japan

Bismuth Free

TMR MTA cement
Mielle

Edited by YAMAKIN Ph.D. Group

1 Introduction

We have been actively engaging in industry-academia collaborations with universities and research institutions to advance the research and development of dental materials. This "industry-academia collaboration" has allowed for innovation in advanced technology to be achieved through technological interactions with universities and other research institutions.

In 2011, the YAMAKIN Ph.D. Group, was established as an internal expert organization, consisting of doctoral degree-holders in dentistry, pharmacy, engineering, science, agriculture, and Entrepreneurial Engineering. Regardless of the members' company history or field of work, the Group is the driving force to continually generate innovation by integrating knowledge, experience, and technology across multiple disciplines rather than working in isolated specialist fields.

The story of the MTA Cement Project:

Prof. Kazuhiko Endo Ph.D. (Division of Biomaterials and Bioengineering, Department of Oral Rehabilitation, School of Dentistry, Health Sciences University of Hokkaido) and Prof. Masato Saito Ph.D. (Division of Pediatric Dentistry, School of Dentistry, Health Sciences University of Hokkaido) were conducting basic studies on MTA cement., The YAMAKIN agreed with the ideas of Professors Endo and Saito, and launched the MTA Cement Project in spring 2012. The fundamental research is very much done by the experts at Health Sciences University of Hokkaido. It has progressed at a rapid pace, and the knowledge base has expanded continuously.

As a result, we succeeded in finding an outstanding composition in 2014.

However, MTA cement is not a simple material that can be completed on an as-is basis when its constituent powders are mixed. Differences in manufacturing conditions greatly affect the performance underlying MTA cement , such as setting time and compressive strength.

It is also necessary to strictly control production environment factors such as humidity and cleanliness, given the product's direct contact with the pulp. Each of these challenges has been overcome, and in July 2017, we were able to release our conventional product, TMR-MTA Cement, for commercialization.

TMR-MTA cement Mielle, a product that responds to the opinions of clinical practitioners

Our conventional product, TMR-MTA Cement has been well received for its superior operating, physical, chemical, biological, and aesthetic properties, but there have been requests from clinical practitioners to improve the X-ray visibility without sacrificing the merits of the conventional product. In order to respond to such opinions, a new project was started immediately, leading to the launch of TMR-MTA cement Mielle two years after the release of the conventional product.

This report covers the characteristics and usage of the TMR-MTA cement Mielle and offers access to video for areas that are difficult to image with text and photographs alone. Please read the QR code attached. Dental treatment continues to change tremendously in its technologies and systems. We will continue to strive for the development of the dental industry by creating new products that meet the needs of the times through further innovation.

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What is YAMAKIN Ph.D. group?

This is an expert group from various specialties in YAMAKIN, and it is the driving force to continually generate innovation by integrating diverse knowledge, experience, and technology.

2 What is MTA cement?

If the pulp is exposed due to caries treatment or other such intervention, pulp protection is performed by sealing the pulp with dental material to protect the nerve (Figure 2-1). MTA cement is a dental pulp-capping material used in this treatment. The MTA in the name stands for "Mineral Trioxide Aggregate" and, in MTA cement, materials that confer X-ray contrast, such as bismuth oxide, are added to the mineral trioxide aggregate. Generally speaking, “cement” in dentistry is strongly associated with glass ionomer cement and adhesive resin cement, so there are some people who misunderstand MTA cement as being used for coalescence and adhesion. However, the term "cement" is used because the calcium silicate-based raw materials that are the main component of MTA cement are close to Portland cement, used for building materials.

The launch of MTA cement in the Japanese market began in April 2007 with "PROROOT MTA" sold by Dentsply Sankin Co., Ltd. (now Dentsply Sirona). In Japan, it is approved only for use as a pulp-capping material for dental use, but overseas it is applied not only for indirect or direct pulp capping, but also in various clinical applications, such as closure of perforations, root canal filling, and reverse root canal filling material for root-end resection.

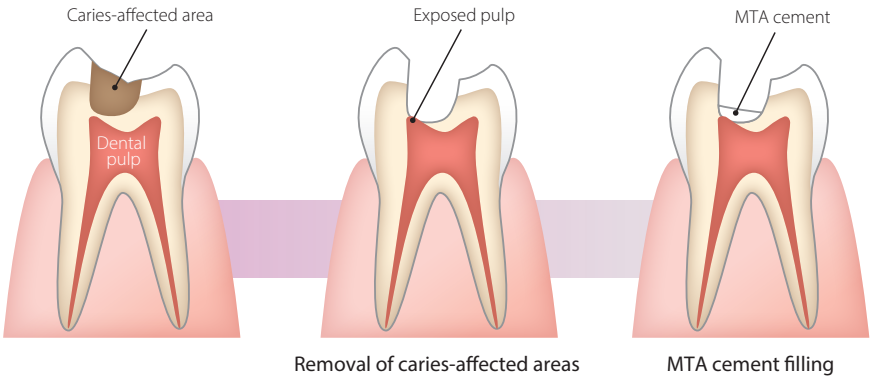

Figure 2-1 Schematic illustration of the use of MTA cement (pulp-capping)

2-1 Characteristics of MTA cement

The merits of treatment using MTA cement are summarized in Table 2-1. The operational method is very easy: simply make the material paste by mixing powder and water, and cap the exposed pulp.

Table 1 The Merits of Treatment Using MTA cement

Setting by Mixing with Water
MTA cement is set by being mixed with water, making it suitable for use in the intraoral area.

Sealing Ability
As MTA cement expands slightly while it is set, restrains leakages at the microscopic level are inhibited, preventing bacterial infection.

Biocompatibility
(Effect of Promoting Hard Tissue Formation)
The cytotoxicity of MTA cement is lower than that of other dental cements, and MTA cement promotes the formation of new dentine.

Disinfectant Ability
MTA cement shows strong alkalinity (pH 12) during the setting process, it disinfects the adhering and surrounding bacteria.

Regenerating Tooth Material
MTA cement promotes the formation of hydroxyapatite, which is a constituent of teeth, by releasing CA^{2+} at the dentinal tubules.

Preserving Dental Nerves
MTA cement enables treatment to proceed leaving nerves undisturbed, extending the life of teeth.

After setting, MTA cement prevents the recurrence of caries by its sealing ability; moreover, it deters bacteria and demonstrates antibacterial ability caused by strong alkalinity of calcium hydroxide, which is generated by reacting with water. (Figure 2-2) Also, calcium ion in the component is released then hydroxyapatite is formed around it; moreover, the formation of new dentine will be promoted by the high biocompatibility.

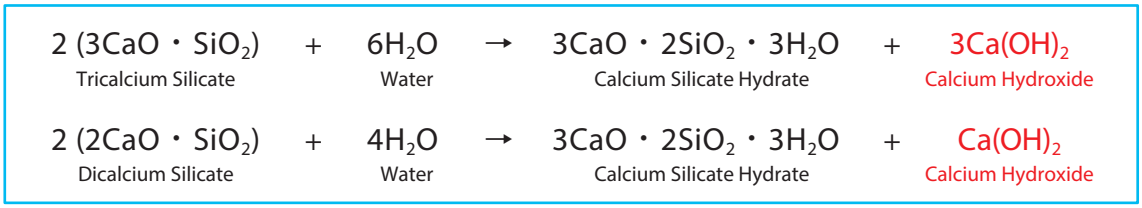

Figure 2-2 Hydration Reaction of MTA cement

Thanks to these effects, nerves can be protected even with pulp exposure; as a result, treatment can be done preserving the pulp as much as possible. Especially in the field of pediatric dentistry, there is a strong need for MTA cement as pulpectomy will affect the formation of permanent tooth substance.

2-2 Mechanism of Setting Reaction

The mechanism of setting reaction by mixing MTA cement and water is as follows:

1) Hydration reaction occurs on surfaces of cement particles and hydrate crystals are extracted on the surfaces.
2) Setting reaction starts slowly as hydrate crystals on the surfaces of cement and surrounding hydrate crystals become interlocked.

Regarding the hydration reaction which is shown in Figure2-3, if the amount of water is insufficient, the density of hydrate crystals becomes low and coupling between particles will be weaker; as a result, setting will be insufficient [1)]. However, if too much water is added, the excess water, which will not affect the hydration reaction, will lower the compressive strength, because the number of spaces inside the cement will increase; therefore, avoid adding too much water [1)]. Also, the particle diameter and surface area differ depending on the product; so, the moisture ratio has to be adjusted in each case, based on the recommended moisture ratio given by the manufacturer.

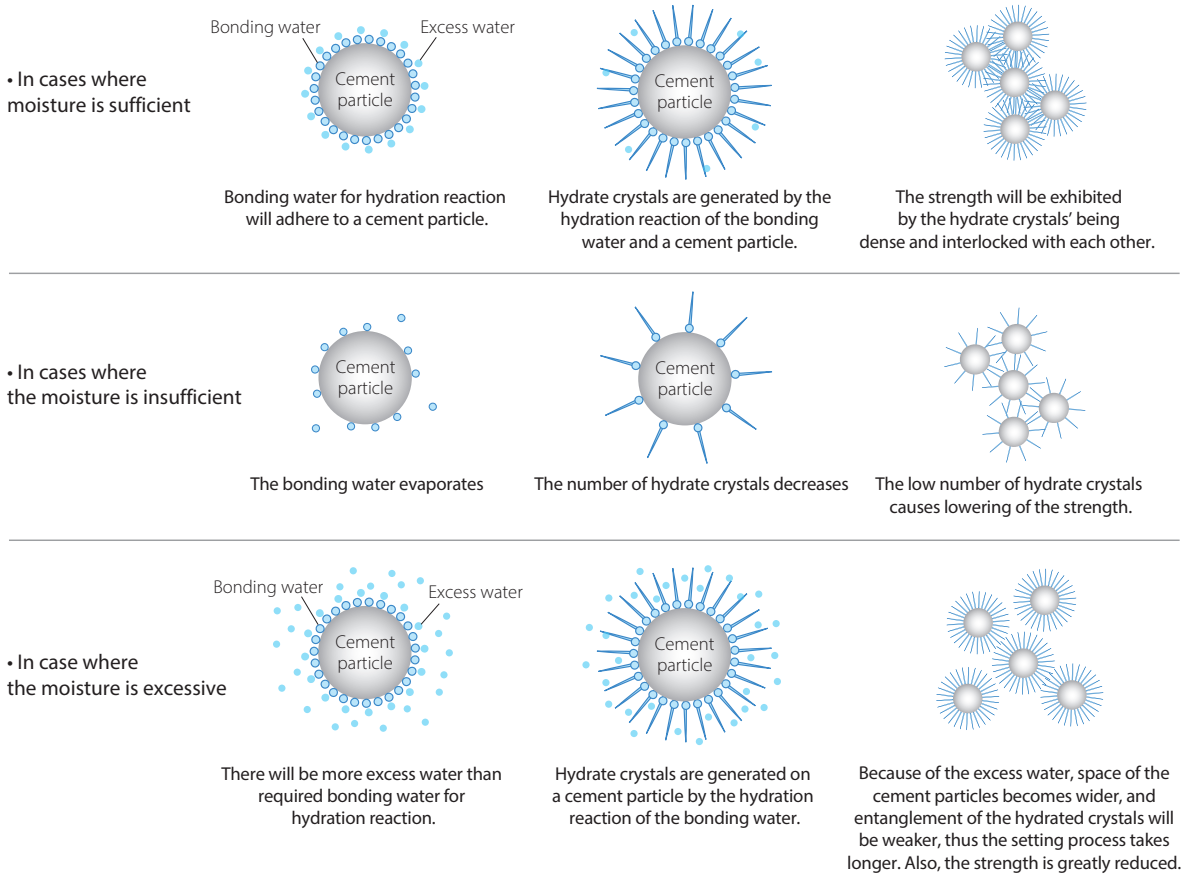

Figure 2-3 The Difference in Hydration Reaction Caused by the Amount of Water

2-3 Comparison with calcium hydroxide preparations

Generally, calcium hydroxide preparation is used for pulp protection procedures. It exhibits hard-tissue-inducing action and disinfectant ability caused by its strong alkalinity; moreover, the material cost is low. Therefore, it is widely used in various endodontic treatments. However, unlike MTA cement, calcium hydroxide preparations do not harden (set), so that the strength of the sealing is weak [4], and the components are easily eluted. Its elution further reduces sealing ability, and thus, it can be said that there is a high risk of bacterial infection in the long term. However, the lack of ability to set is a merit when the purpose is to sterilize the root canal because it needs to be removed at the time of re-examination.

In the comparisons of the long-term outcomes of pulp protection with MTA cement and calcium hydroxide preparations [5], the MTA cement success rate* was approximately 80% or more after three years, whereas the success rate after one year with calcium hydroxide preparations was approximately 75%, which is roughly equal to MTA cement. However, the success rate falls to 55% after two years and to about 45% after three years. *"Success rate" is evaluated from clinical findings on the presence or absence of pain, (e.g., spontaneous pain, occlusal pain, percussion pain, cold/warm water pain), tooth sway, periodontal pocket confirmation, and radiographic findings of the root canal segments at re-examination.

In conservative treatment, long-term stability is considered essential. Therefore, MTA cement is a valuable material for patients.

References

1) Mahmoud Torabinejad (edited), Yoshitsugu TERAUCHI (inspected): complete MTA. Quintessence Publishing, 2017.

2) Eldeniz AU, Hadimli HH, Ataoglu H, Orstavik D: Antibacterial Effect of Selected Root-End Filling Materials. J Endod, 32(4), 345-349, 2006.

3) Takashi OKIJI (Edited): MTA its basics and clinics. Hyoron Publishers, Tokyo, 2016.

4) Horsted-Bindslev P, Lovshall H: Treatment outcome of vital pulp treatment. Endod Topics, 2, 24-34, 2002.

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3 Features of TMR-MTA cement Mielle

3-1 Usability

TMR-MTA cement Mielle is characterized by the fact that when water comes into contact with the cement, it is quickly absorbed and turns into cement mud that can be easily kneaded (see Video 2). This is because the presence of zirconia particles and spherical silica particles between the calcium silicate particles creates small spaces between the calcium silicate particles, and water is absorbed by the capillary phenomenon. Besides, the bearing effect (Figure 3-1) of the spherical-silica fine particles improves the product's fluidity. This enables users to make an even paste easily with a small amount of water in a short time.

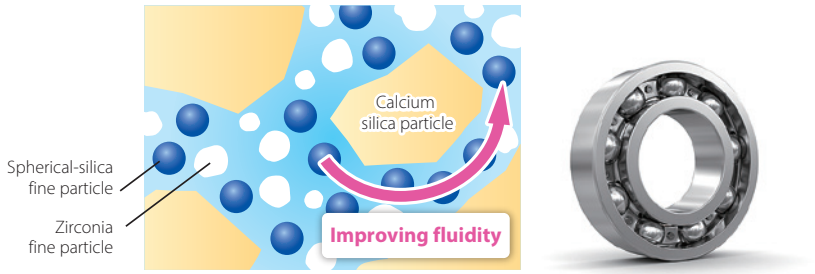


Figure 3-1 Image of Bearing Effect

3-2 Setting Time and Self-Setting Ability

3-2-1 Initial setting time

As discussed above, TMR-MTA cement Mielle mixes well to an even paste with a small amount of water. Since it can be mixed with a small amount of water, its designed setting time is short, so that the initial setting is completed in 15 to 30 min. when the moisture ratio is 20%. In this section, the effect of moisture ratio during mixing and initial setting time of MTA cement are evaluated.

Materials and Methods

The initial setting time was measured in accordance with the measurement of setting time in ISO 6876:2012 and JIS T 6522:2015 (Dental Root Canal Sealing Materials). That is, paste-like samples obtained by mixing MTA powder and distilled water were filled into molds with a diameter of 10 mm and a depth of 2 mm (Figure 3-2), and a load was applied by a Vicat needle (mass: 100.0 ± 0.5 g, tip diameter: 2.0 ± 0.1 mm plane, Figure 3-3) while measuring the time from the time of mixing, and the setting time required to obtain hardness at which no trace of the Vicat needle appeared on the surface was taken as the initial setting time.

The moisture ratio at the time of mixing the MTA powder and the distilled water was adjusted using a micropipette.

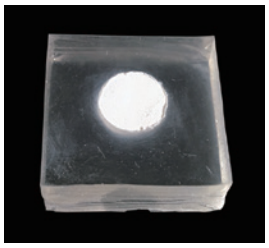


Figure 3-2 Sclerotic test sample

Figure 3-3 Vicat needle tester

Results and Discussion

As shown in Figure 3-4, the time required for setting tended to increase with increasing water ratio. When the moisture ratio is 35% and 40% by mass (about 0.108 g and 0.133 g of distilled water to 0.2 g of MTA powder, respectively), the paste form cannot be maintained after mixing because the water amount is excessive.

In this case as well, although it is set until finally no trace of the needle appears, the compressive strength described below is lowered, so that it is necessary to remove excessive moisture.

By adjusting the appropriate amount of moisture with dry cotton or the like, the initial setting time can be shortened.

However, this initial setting time is the time taken until the cement component sets to some extent, and corresponds to the time called solidification time in industrial cement [1)], not to the time when the cement component completely sets due to the hydration reaction. Since the hydration reaction progresses fully after initial setting, and the strength increases, the measurement of compressive strength was measured 1-7 days after adequate setting had progressed.

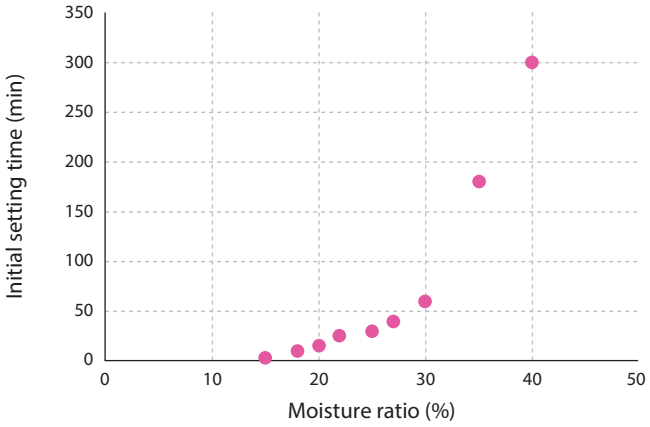


Figure 3-4 Relationship between moisture ratio and initial setting time

3-2-2 Collapsibility in water before initial setting

If MTA cement is dissolved or eluted before initial setting by contact with body fluids, there are concerns that performance of MTA cement will be impaired. Therefore, in this section, the collapsibility of Mielle in water before initial setting was evaluated.

Materials and Methods

Pasted samples of Mielle and distilled water mixed at 20% moisture ratio were filled into a 1 mm-diameter acrylic hole of 2 mm depth and immersed in 37°C distilled water immediately after filling (Figure 3-5). As a control sample, calcium hydroxide powder was used mixed with distilled water at a moisture ratio of 50% so as to have a paste viscosity equivalent to that of Mielle. The samples were removed from the water after 24 h to check the surface condition. In addition, the amount of decrease (mm) from the exposed surface of the sample before immersion was measured using a micrometer, and the amount of dissolution/outflow was determined.

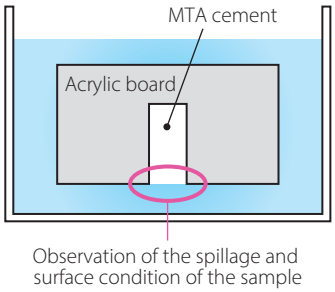


Figure 3-5 Diagram of disintegration test

Results and Discussion

As shown in Figure 3-6 and Figure 3-7, no elution or collapse of the surface was observed in Mielle. On the other hand, in calcium hydroxide, dents were observed on the surface, and elution and collapse of the sample were observed.

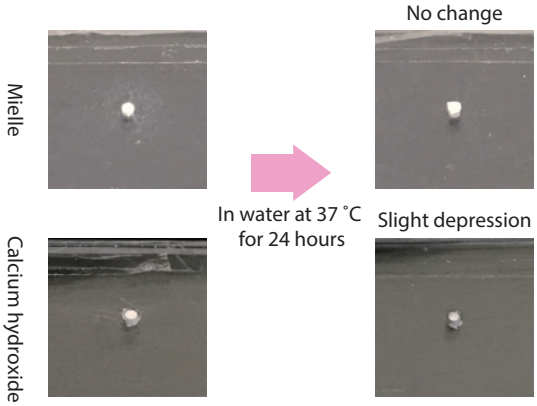


Figure 3-6 Changes in appearance before and after immersion in water

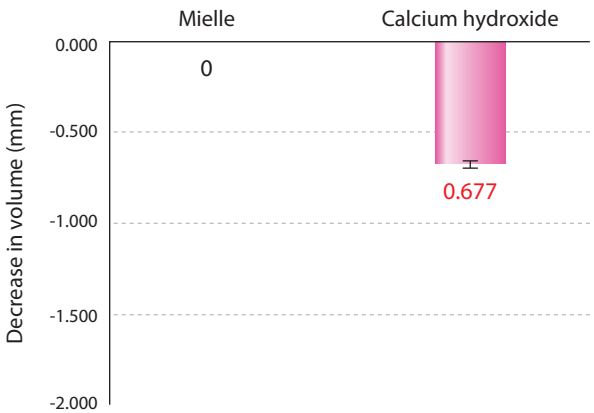


Figure 3-7 Elution of Samples in Water Immersion

As a result of this test, it was confirmed that if water pressure for washing with a three-way syringe, etc. is not applied to Mielle, it will not dissolve or flow out in water even immediately after filling.

3-3 Compressive strength

The strength of MTA cement after setting is assessed by compressive strength. In this section, the compressive strength of Mielle was tested in accordance with JIS T 6609-1:2005 (Dental Water-Based Cement-Part 1: Powder/liquid Acid-base Cements) to determine the effect of setting conditions on compressive strength.

MATERIALS AND METHODS

Columnar samples (Figure 3-8) were prepared by filling stainless-steel split jigs with internal dimensions of 4 mm in diameter and 6 mm in height, with samples obtained by mixing MTA powder samples with 1) distilled water at a moisture rate of 20% by mass (0.1 g of distilled water to 0.4 g of powder) and 2) water ratio of 18, 20, 23, 25, 30, and 35% by mass.

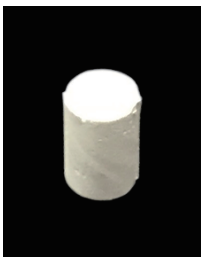


Figure 3-8 Compressed specimen

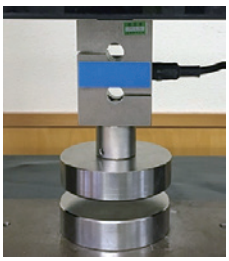


Figure 3-9 Compact Universal Testing Machine

Thereafter, a compressive load was applied using a small universal tester (EZ-Graph: Shimadzu Corporation, Figure 3-9). (1) a sample stored for 24 hours in water at 37°C, and a sample stored in air with a humidity of 90% or more for 24 hours. This test shows the difference between the condition in water and in air.

(2) a sample stored in water at 37°C for 24 hours and a sample stored in water at 37°C for 168 hours (7 days). This test shows the difference between the condition in water after one day and after seven days.

The compressive strength of each above samples were calculated from the load when the sample was broken.

Results and Discussion

Figure 3-10 shows the compressive strength of Mielle when it is mixed at a moisture ratio of 20 mass%. MTA cement is commonly used for treatment in which a wet cotton swab is placed after filling MTA cement and then a composite resin is filled on the next day or later.

Mielle stored in air demonstrates compressive strength equivalent to that of a sample stored in water (assuming a wet cotton swab after MTA cement is filled). In this way, Mielle is sufficiently set in the same way as the conventional product, with no need for moisture replenishment by moist cotton swabs, etc. Thus, it enables a one-off filling of the composite after MTA cement Mielle has been used. Both procedures are performed on the same day, which reduces the burden on patients.

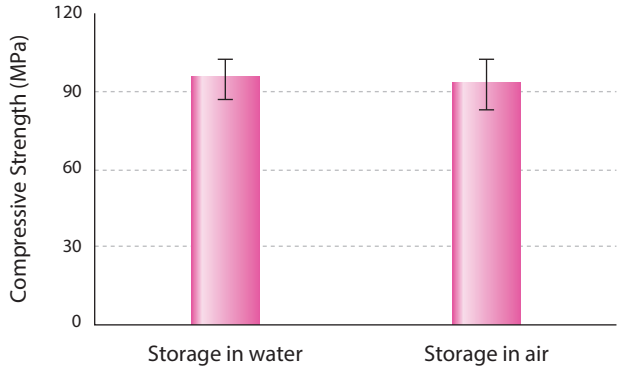


Figure 3-10 Compressive strength of Mielle
(1 day after filling; water ratio of 20 mass%)

In addition, the results for compressive strength compared to the conventional product are shown in Figure 3-11. Since setting time of Mielle is as fast as that of the conventional product, it shows high compressive strength (about 90 MPa) at 20 mass% moisture ratio after one day of settlement, and it increases to about 140 MPa after one week. This is a compressive strength comparable to that of glass ionomer cement, which is sufficient as the strength required for pulp protection.

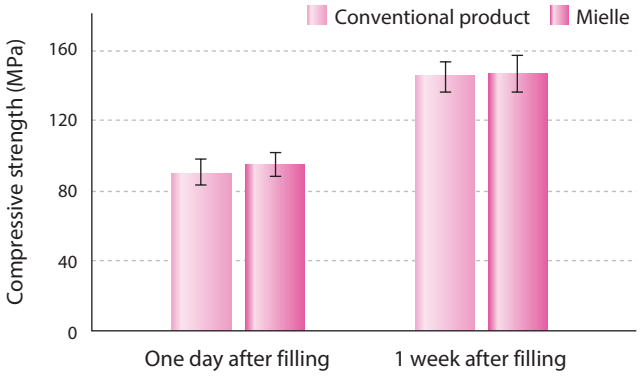


Figure 3-11 Compressive Strength of the Conventional Product and Mielle

Figure 3-12 shows the compressive strength of Mielle when it is mixed at various moisture rates. The material properties of MTA cement are known to become higher when the moisture ratio decreases and lower when the moisture ratio increases [3]. The result of this test similarly showed that its compressive strength of Mielle tended to be higher when moisture ratio was low and lower when water ratio was high 1 day and 1 week after filling, respectively [4].

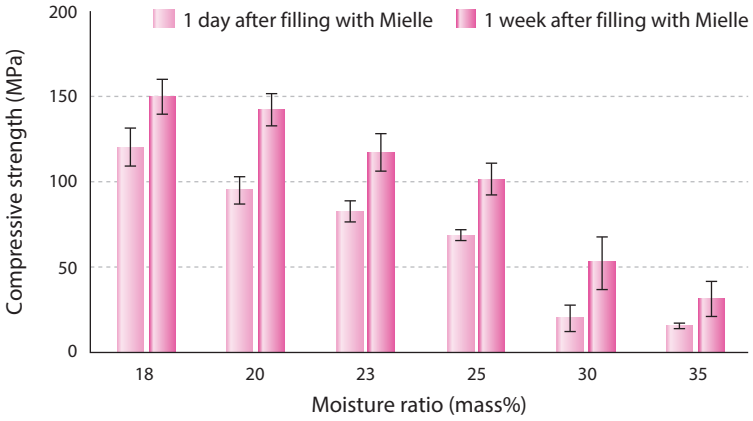


Figure 3-12 Compressive Strength of Mielle by Moisture Ratio

When the water amount at the time of mixing was 30% by mass or more (0.085 g or more of water to 0.2 g of powder), the water ratio of the mixed product became excessive and the strength after setting was insufficient. This may be attributed to the fact that, as shown in Figure 3-13, the gap between the cement particles increases in the presence of excessive moisture, so that although hydrated crystals grow and harden over time, but sufficient adhesion strength cannot be obtained.

Since Mielle can achieve a uniform paste even with a small amount of water due to the bearing effect of spherical particles, water should be added little by little in small amounts while mixing, so that the amount of water is appropriate. If the moisture becomes excessive, it is necessary to absorb the excess moisture with dry cotton or the like before filling.

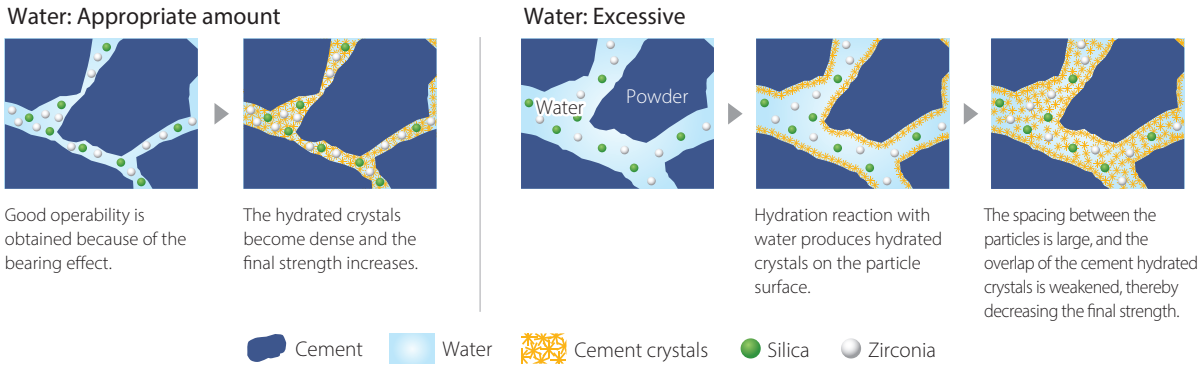


Figure 3-13 Schematic diagram of Mielle setting with appropriate and excessive water

3-4 Bismuth-free

General MTA cement contains Bismuth oxide as an X-ray contrast agent; however, the bismuth oxide sometimes turns black, due to exposure to light. On the other hand, Mielle contains zirconia, which has been successfully used as an X-ray contrast agent in various biological applications, such as in dental materials and joint prosthesis. Zirconia is a material which is chemically stable and is not prone to discoloring. Therefore, color change of Mielle is unlikely to happen. The effect of different X-ray contrast agents on biological safety is explained in the following sections.

3-5 Effect of different X-ray contrast agents on cells

As described above, in typical MTA cement compositions, oxides such as bismuth oxide and zirconia are added to Portland cement as an X-ray contrast agent [5]. This section presents a review of studies examining the effects of each of the oxide powders used as an X-ray contrast agent on cells [6].

Materials and Methods

As samples, bismuth oxide (Bi_2O_3) and zirconia oxide (ZrO_2) powders were used. Murine macrophage-like cells (RAW264.7) and murine osteoblast-like cells (MC3T3-E1) were used for the cells. D-MEM supplemented with 10% FBS was used as a culture medium. Cells were seeded at 5×10^4 cells/mL on culture plates, then cultured in a carbon dioxide incubator (37°C, 5 % CO_2) for one day.

・Cell activity test

Oxide powders whose mean particle size was adjusted to about 1 μm were added to the culture medium to 0.01, 0.1, 1.0 and 10.0 0 mM, and after culturing for an additional 3 days, the activity of the cells was evaluated using WST-8 [7, 8] (Figure 3-14).

WST-8 is reduced to orange WST-8 formazan by dehydrogenases (NAD+, NAD(P)+ dehydrogenase) that metabolize in living cells. By measuring the intensity of this orange shade as absorbance, it is possible to assess the effect of the sample on the metabolic activity of the cell.

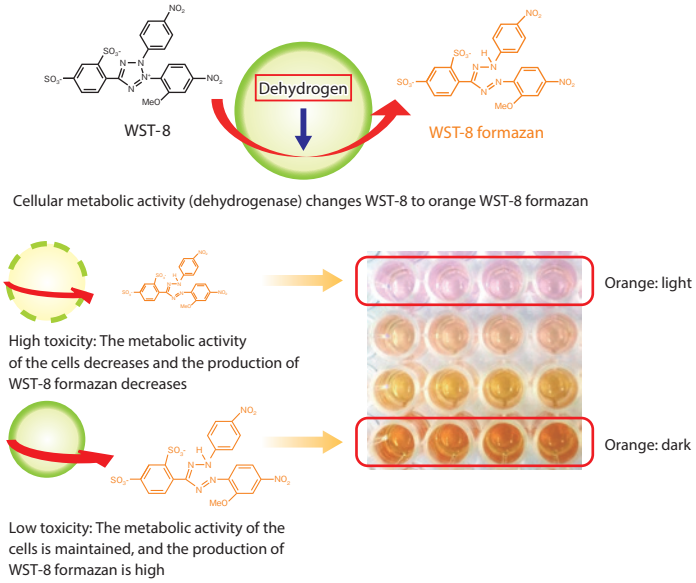


Figure 3-14 Principle WST-8 test

That is, the darker the orange color (the higher the absorbance), the less the sample has an effect on the metabolic activity of the cell.

・Transmission electron microscopy (TEM) observation

Cells were seeded, and powders with an average particle size of approximately 1 μm were added. The cells were cultured for 3 days, and then pre-fixed with 2.5% glutaraldehyde diluted with PBS. Thereafter, those post-fixed with osmium tetraoxide were dehydrated with ethanol series, and embedded in epoxy resin, to produce an ultrathin section with a thickness of 90 nm. Ultrathin sections were stained electronically with uranyl acetate and lead citrate, and the cells were observed by TEM.

Results

In the cellular activity test, when powder with an average particle size of approximately 1 μm was added to RAW264.7 cells, the absorbance indicative of metabolic activity decreased significantly to less than 10% in the group supplemented with 0.1 mM or more of Bi_2O_3 powder against RAW264.7 compared with the control group. On the other hand, when zirconia powder was added, a decrease of about 50% was observed in the 10 mM addition group, but no decrease in absorbance was observed in the 1 mM or less addition group (Figure 3-15).

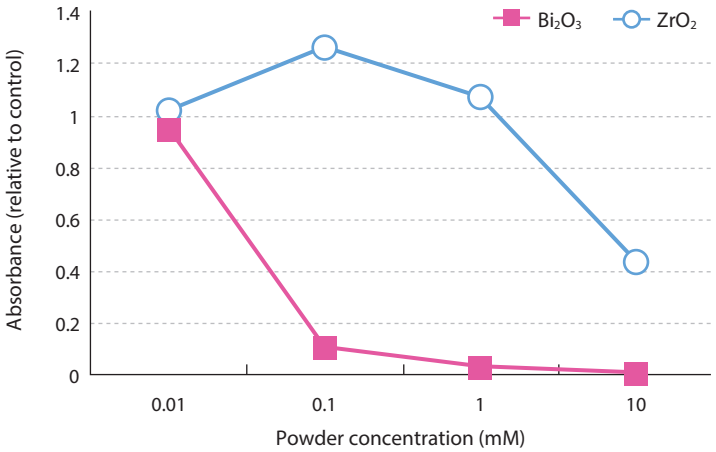


Figure 3-15 Cellular activity upon adding sample powder to RAW264.7 cells

Subsequently, when the sample powder was added to MC3T3-E1 cells, the group supplemented with bismuth oxide powder in an amount of 0.1 mM or more showed an absorbance of 20% or less compared with the control group, indicating a significant decrease in cellular activity. On the other hand, in the group supplemented with zirconia powder, no decrease in absorbance was observed even in the group supplemented with 10 mM (Figure 3-16).

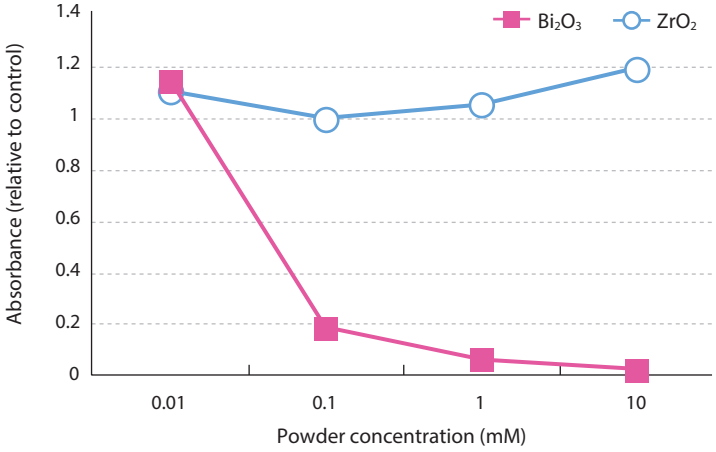
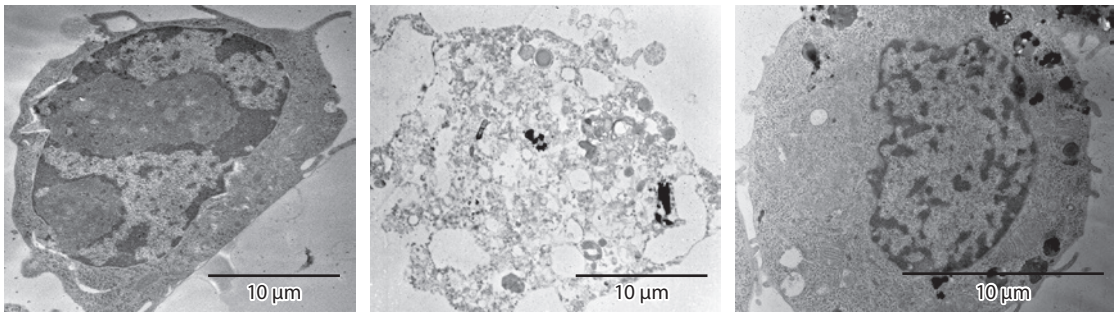


Figure 3-16 Cellular activity upon adding the sample powder to MC3T3-E1 cells

Figure 3-17 shows TEM images of each oxide powder added to RAW264.7 cells. Particles of each oxide powder incorporated into the cytoplasm of RAW264.7 were observed. No obvious differences in cell morphology were observed in the group supplemented with zirconia powder compared with the control group, and no disruption images of the cell membrane or nucleus were observed. On the other hand, in the group supplemented with bismuth oxide powder, ruptured and swollen cells and organelles were observed, with obvious cell destruction.



Control　　Bismuth oxide　　Zirconia

Figure 3-17 Incorporation of each oxide powder by RAW246.7cells

In conclusion, the effects of bismuth oxide and zirconia, which are contained as X-ray contrast agents in MTA cement, on macrophage-like cells and osteoblast-like cells were investigated. The use of chemically stable zirconia as an X-ray contrast agent may lead to an improvement in the biological safety of MTA cement because bismuth oxide is stronger than zirconia for all cells in reducing cell activity.

Next, the effects of MTA cements containing bismuth oxide or zirconia on osteoblast-like MC3T3-E1 cells (Figure 3-18) were examined.

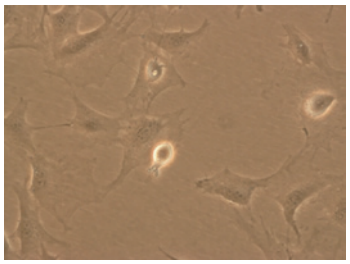


Figure 3-18 Murine osteoblast-like cells MC3T3-E1 cells

Materials and Methods

As test materials, TMR-MTA cement Mielle, containing about 30% by mass zirconia, TMR-MTA CEMENT (the conventional product), containing about 20% by mass zirconia, and prototype MTA (Bis20, Bis30) containing 20% or 30% by mass bismuth oxide, were prepared. The sample powders were kneaded with distilled water at a moisture ratio of 20% by mass and set for 24 hours at 37℃ and 100% relative humidity. To each test piece, 1 mL cell culture medium (MEMα with 10% FBS) was added per 1 cm^2 of surface area, and the cell culture medium was soaked in the dark at 37℃ for 24 hours. This immersion liquid was collected and filtered through a 0.22 μm filter to be used as a test liquid, which was subjected to a cell proliferation test.

・Cell proliferation study

MC3T3-E1 cells were seeded in 96-well culture plates at 1000 cells/well and cultured for 24 hours (5% CO_2, 37℃). It was confirmed that cells adhered to the bottom surface of the wells, and the medium was replaced with each test solution, and the cells in the wells were further cultured for 48 hours. Adding 10 μL of Cell Counting Kit-8 solution (Dojindo Laboratories) to each well and coloring for 2 hours to determine the absorbance at 450 nm [7, 8].

Results and Discussion

Observation of the test specimens after immersion in the cell-culture medium revealed no change in the appearance of the zirconia-containing Mielle and the conventional product, but black discoloration was observed in Bis20 and Bis30, the bismuth oxide-containing products (Figure 3-19). Since the cell culture medium used as the immersion medium contained components such as sugars and amino acids, it was inferred that the blackening observed in Bis20 and Bis30 was caused by the reactions between bismuth oxide and one or some of the components in the cell culture medium.

The cell proliferation test found that absorbance was less in the order Mielle to the conventional product to Bis20 to Bis30 (Figure 3-20). Higher absorbance was observed for zirconia-containing MTA cement versus bismuth-oxide-containing MTA cement. Although it has been reported that MTA cement exhibits antimicrobial properties due to the strong alkalinity that occurs during the setting process, the pH of the sample solutions was equivalent, and therefore, it was inferred that the difference in eluted components affected the cell proliferation.

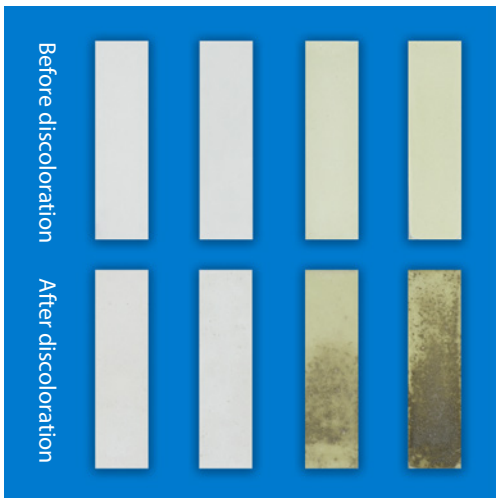


Figure 3-19 Test specimens after immersion in cell culture medium (Conventional, Mielle, Bis20, Bis30 from left)

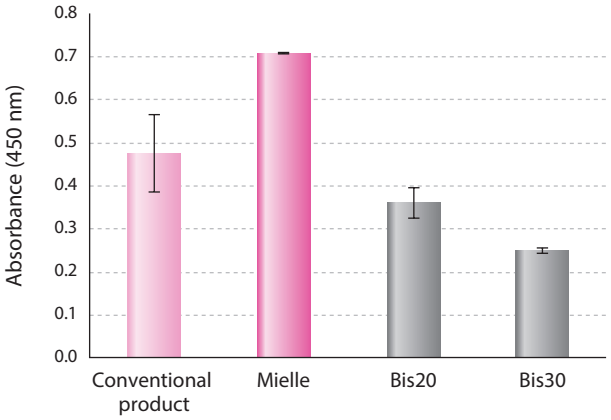


Figure 3-20 Cell proliferation in each test solution

3-6 X-ray contrast

By increasing the ratio of zirconia, an X-ray contrast agent component contained in the composition, the X-ray contrast has been greatly improved compared to the conventional product. The X-ray contrast of dentine is said to be comparable to that of aluminum 1 mm thick, so the X-ray contrast of dental materials is generally compared with that of aluminum based on JIS standard testing. In this section, X-ray contrast of Mielle was compared with the conventional product.

Materials and Methods

Conventional and Mielle powder samples were kneaded with distilled water to 20 mass% moisture, and a 12 mm diameter and 1 mm thickness pellet-type sample was prepared using stainless steel braces. X-ray imaging was then performed of a stepped wedge of aluminum with a stair-like structure of 1, 2, 3, 4,... mm in thickness (Figure 3-21), and the optical density of the X-ray contrast was calculated by comparing the optical density of the X-ray image of the sample with the optical density of the image of the aluminum step of each thickness (Figure 3-22).

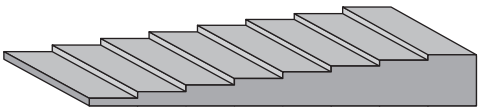


Figure 3-21 Schematic diagram of aluminum step wedge

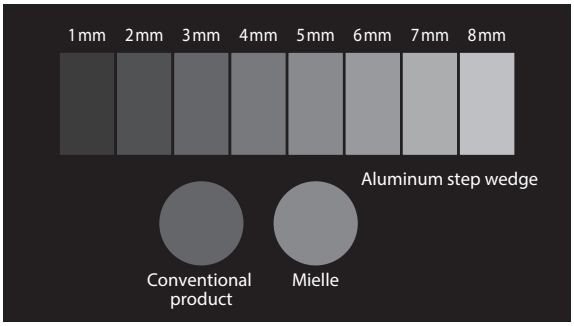


Figure 3-22 X-ray image schematic

Results and Discussion

The conventional product has an X-ray contrast of 3.4 mm in aluminum thickness, while Mielle has an X-ray contrast of 5.4 mm in aluminum thickness, an increase of about 1.6 times (Figure 3-23).

The X-ray photograph taken by dental X-ray apparatus (Arm-type X-ray CT diagnostic apparatus AUGE: Asahi Roentgen Ind. Co., Ltd.) is shown below (Figure 3-24). As is clear from the figure, the visibility of the Mielle is greatly improved over the conventional product.

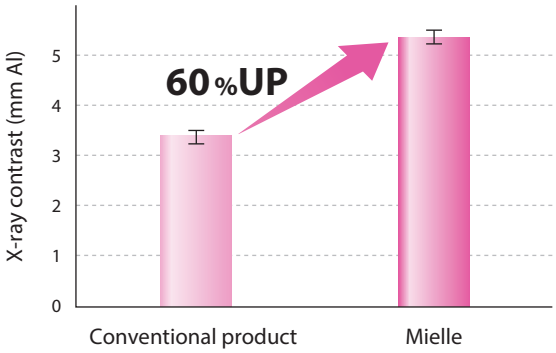


Figure 3-23 X-ray Contrast

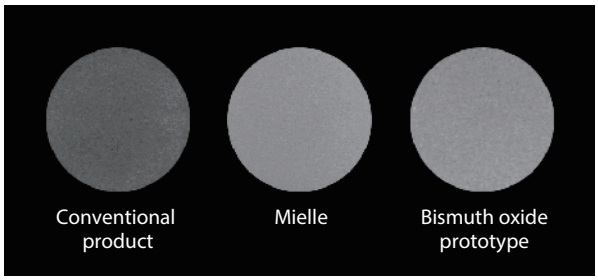


Figure 3-24 X-ray photographs of 1-mm-thick pellets of MTA cement

In actual clinical practice, the affected area may be small and the amount of MTA cement used may be very small. Therefore, in order to confirm the visibility of MTA cement on X-rays when applying small amounts in patches in the clinic, a dental model (Figure 3-25) was filled with the conventional product and Mielle and an X-ray was performed.

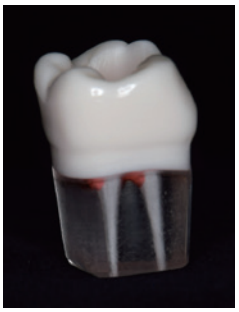


Figure 3-25 Dental model filled with MTA cement (Left: Conventional product, Right: Mielle)

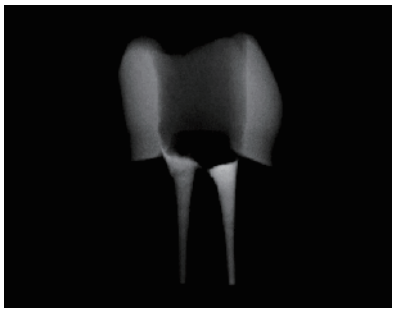


Figure 3-26 Confirmation by X-ray photography (Left: Conventional product, Right: Mielle)

Even where very small amounts of MTA cement Mielle are used, it is possible to see the MTA filled area more easily than conventional product. (Figure 3-26).

3-7 Strong alkalinity and sustained release of calcium ions

As described in the previous section, the X-ray contrast of Mielle has been improved by increasing the ratio of zirconia compared with the conventional product.

In this section, the following experiments were conducted to verify the strong alkaline and calcium sustained release levels and compare them with the conventional product.

Materials and Methods

The conventional product and the powder sample of Mielle were kneaded with distilled water at a moisture ratio of 20% by mass to prepare a pellet-type sample (for pH test) having a diameter of 6mm and a thickness of 1 mm, and a rectangular parallelepiped sample (for sustained calcium ion release

test) having a length of 33 mm, a width of 13 mm and a thickness of 2 mm. Thereafter, the samples were set in an environment of 37°C and 100% relative humidity for 24 hours, then immersed in 10 mL of distilled water, and the pH and the amount of calcium ion released in the immersion solution after one day were measured.

Results and Discussion

Figure 3-28 shows the results of measuring the pH of immersion solutions obtained by separately immersing pellets of the conventional product and Mielle in distilled water for one day using a pH meter (F-55: Horiba, Ltd., Figure 3-27).

Figure 3-27 pH meters

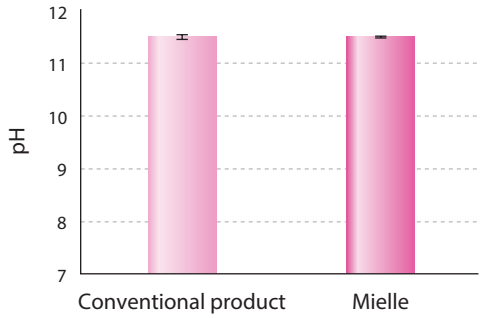


Figure 3-28 pH after 1 day in immersion solution

The immersion solution exhibited a high pH of 11.5 for both the conventional product and the Mielle and an equivalent strong alkalinity.

Next, Figure 3-29 shows the results of measuring the sustained release of calcium ions in the immersion solution using an ICP-emission spectrometer (SPS3500DD: Hitachi High Technology Science Co., Ltd.) after separately immersing rectangular parallelepiped samples of the conventional product and Mielle in distilled water for one day.

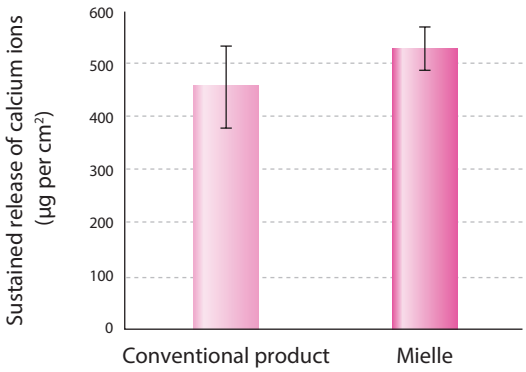


Figure 3-29 Calcium ion sustained release after 1 day in immersion solution

Calcium ion sustained release of the conventional-product and Mielle were comparable.
The weight ratio of zirconia to the conventional product was increased in Mielle, and this composition change was found to improve the X-ray contrast while not affecting the strong alkalinity and calcium sustained release.

3-8 Enhancement of hard tissue formation

MTA cement has been reported to promote the formation of hard tissues in surrounding tissues when it acts on the exposed pulp. In addition, hard tissue formation just under the pulp exposure has been previously confirmed by direct pulp capping for rat molars [1]. The details will be discussed later, but the volume ratio of the cement component involved in hard tissue formation is the same as that of the conventional product. Accordingly, it is expected that Mielle has the same effect as the conventional product in promoting hard tissue formation. Therefore, in this section, the following tests were performed using Mielle (Figure 3-30).

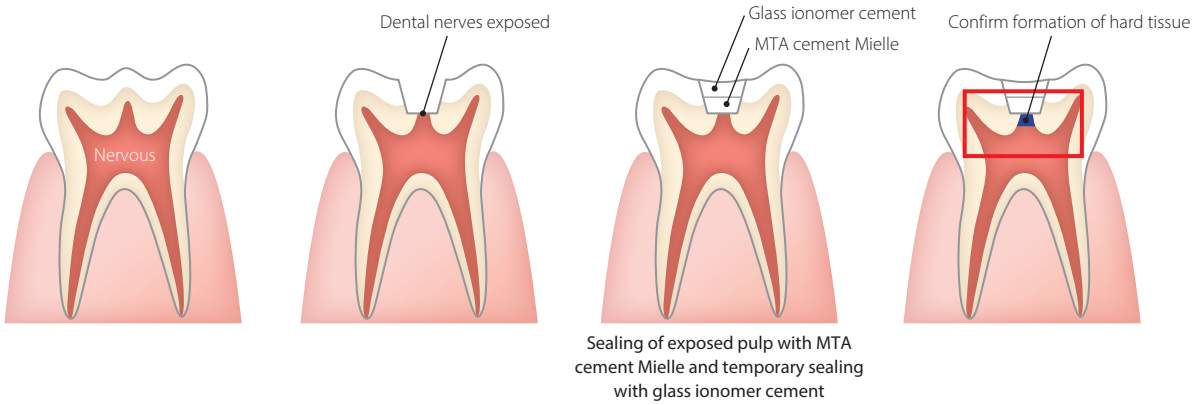


Figure 3-30 Schematic representation of the rat pulp capping test

After pulpotomy in 8-week-old female rats, Mielle was patched on and temporarily sealed with glass ionomer cement. Two weeks later, the surrounding tissue including the patch site was fixed in 4% paraformaldehyde-containing PBS for 24 hours. Sections were prepared by degreasing and decalcification, followed by Hematoxylin-Eosin staining and observation by light microscopy.

As shown in Figure 3-31, 2 weeks after the patch application, the formation of hard tissue with sufficient thickness to completely cover the exposed pulp surface was found under the treated lesion.

From the above, it has been clarified that Mielle has an effect of promoting the formation of a hard tissue of the surrounding tissue, similarly to the conventional product.

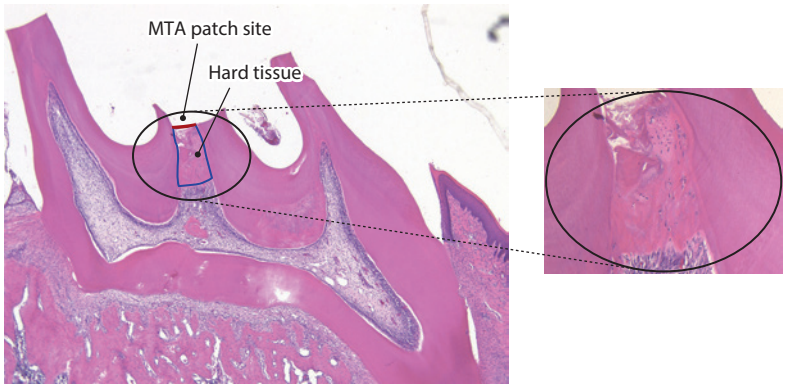


Figure 3-31 Hard tissue formation after direct pulp capping (rat molars, 2 weeks after application)
Image courtesy of Health Sciences University of Hokkaido

3-9 Enhancement of X-ray Contrast and Maintenance of the Strengths of the Conventional Product

The increased zirconia ratio which distinguishes Mielle from the conventional product is associated with a decrease in cement content (calcium silicate). Calcium silicate is an ingredient directly linked to the important properties of MTA cement i.e., setting, being strongly alkaline, and exhibiting sustained release of calcium ions. Therefore, the pH of the immersion solution and the sustained release amount of calcium ions have been confirmed to be equivalent to those of the conventional product, as shown in Section 3-7. This section discusses the reasons why the physical properties have not changed.

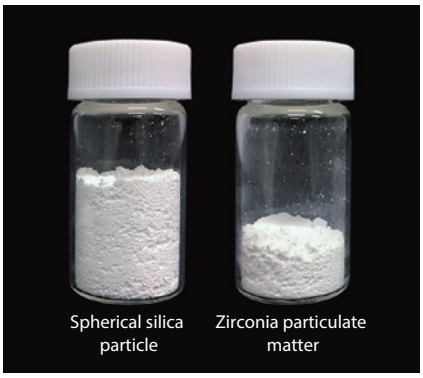


Figure 3-32 Appearance of each micro particle at the same weight (5 g)

In Mielle, the zirconia ratio is increased by replacing some of the spherical silica fine particles which are included mainly for the purpose of improving operability with zirconia fine particles.
Silica and zirconia have densities of about 2.2 g/cm^3 and about 5.5 g/cm^3, respectively, and so the volume of silica is about 2.5 times larger than that of zirconia when the weights are the same (Figure 3-32).
Due to this difference in density, the volume ratio of calcium silicate in Mielle was equivalent to that of the conventional product (Figure 3-33). In the comparative verification of physical properties as shown in Sections 3-2, 3-3, and 3-7, reflecting these facts, Mielle and the conventional product were considered to show equivalent physical properties.

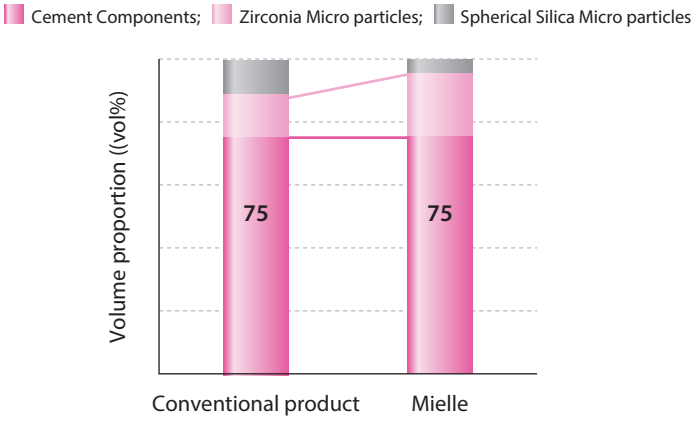


Figure 3-33 Volume ratio of ingredients of the conventional product and Mielle

3-10 Adhesion Strength with CR System

The adhesion strength with the CR system was measured assuming composite restoration after pulp protection by Mielle. The test sample was prepared by using Mielle and TMR-AQUABOND 0 (YAMAKIN CO., LTD.) as adhesive material, and TMR-Z Fill 10. (YAMAKIN CO., LTD.) as composite resin.
First, hardened Mielle was set into a silicon mold, and a smooth surface was formed using water-resistant abrasive paper.

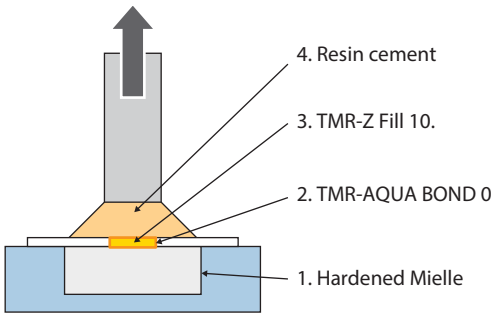


Figure 3-34 Structural Schematic Diagram of Adhesive Test Specimens

The surface was masked with tape containing holes with a diameter of 5 mm to limit the adhesive surface. Next, TMR-AQUABOND 0 was applied to the adhesive surface of Mielle and processed as usual.

TMR-Z Fill 10. was then filled and photopolymerized using LED light irradiator, and a stainless steel rod was attached using resin cement to make the test body. Samples were stored in water at 37℃ for 1 day and 7 days. After that, a test was carried out using a small universal tester (EZ-Graph: Shimadzu Corporation) to pull the stainless steel rod perpendicularly to the bonding surface at a rate of 1 mm per minute, and the stress at break was defined as the tensile adhesion strength.(Figure 3-34).
The results of the adhesion test are shown in Figure 3-35.

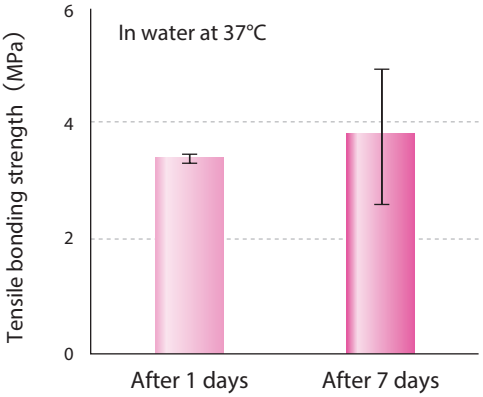
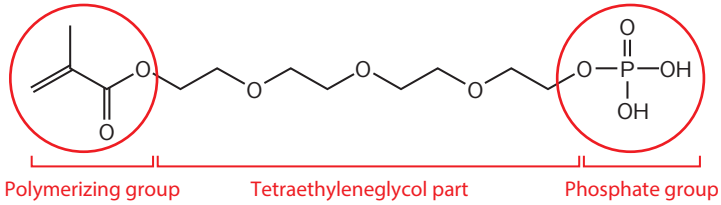


Figure 3-35 Tensile adhesive strength between Mielle and TMR-AQUABOND 0

As a result of this study, it was confirmed that TMR-AQUABOND 0 showed constant adhesive strength between MTA cement and CR. This study was limited to adhesion between MTA cement surfaces of 5 mm diameter and CRs, but in the clinic, the surfaces of teeth other than MTA cement in the cavity also become bonding surfaces, so it is considered that more firm adhesion can be obtained.
TMR-AQUABOND 0 uses M-TEG-P (Figure 3-36), which is an amphiphilic monomer.

M-TEG-P® is a registered trademark of YAMAKIN CO., LTD.



Characteristic properties
- Demineralization effect by phosphate group can be controlled by organic group
- Higher detergency than phosphoric acid
- Improvement of compatibility

Figure 3-36 Chemical structures and properties of M-TEG-P

The use of M-TEG-P allows TMR-AQUA BOND 0 to exhibit hydrophilicity, so it is considered that it has excellent compatibility with water-containing Mielle surfaces and adheres closely to the unevenness of cement surfaces (Figure 3-37). It is also known that phosphoric monomers adhere well to zirconia [9-11], and it is thought that the zirconia contained in Mielle and M-TEG-P phosphate group bind to each other for effective adhesion (Figure 3-38).

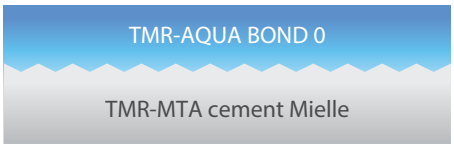


Figure 3-37 Image of interface

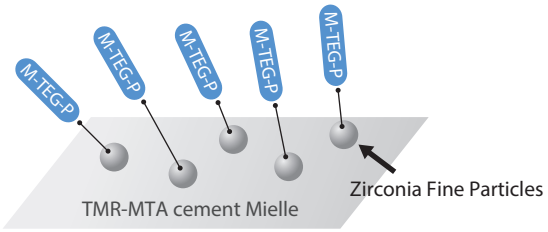


Figure 3-38 Binding between M-TEG-P and Zirconia

After treatment of pulp protection by excellent setting property TMR-MTA cement Mielle, TMR-AQUA BOND 0, which provide stable adhesion even in moist conditions can be used. Moreover, there is direct composite TMR-Z Fill 10. which can expect strengthening of tooth and preventing bacteria to stay on tooth by releasing fluoride. These products achieve a complete system from pulp capping to filling as TMR Series.

Figure 3-39 Innovation through the TMR Series

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4 MTA cement biological safety evaluation

Table 4-1 presents the biological safety considerations for medical devices for dental use. Medical devices for dental use in contact with the human body should be classified according to contact site (non-contact devices , surface contact devices , internal and external devices, implanted devices) and duration of contact (temporary contact, short-and medium-term contact, long-term (permanent) contact) and evaluated for biological safety as appropriate. “Evaluation of biological safety” refers to the risk assessment of factors (e.g., cytotoxicity, sensitization, genotoxicity) that may adversely affect human health, and sale of medical devices for dental use is permitted after such assessment of biological safety.

Section 3-5 demonstrated that zirconia (ZrO_2) has less effect on cells than bismuth oxide (Bi_2O_3) as an X-ray contrast agent. This section presents examples of biological safety evaluation studies of cytotoxicity, sensitization, and intradermal reactions that require risk assessment for all medical devices for dental use in contact with the body to assess the biosafety of MTA cement using zirconia as an X-ray contrast agent.

Table 4-1 Biological safety considerations in medical devices for dental use

Category of Medical device for dental use	Contact period		Biological tests						
	A: Temporary (Within 24 hours) B: Short-and medium-term (24 hours to 30 days) C: Long-term (permanent) (Beyond 30 days)		Cytotoxicity	Delayed-type hypersensitivity (sensitization)	Skin irritation and intradermal reactions	Acute systemic toxicity	Subacute (or subchronic) systemic toxicity	Genotoxicity	Implantation
Non-contact devices									
Surface contact devices	Skin	A	○	○	○				
		B	○	○	○				
		C	○	○	○				
	Oral tissue (mucosa)	A	○	○	○				
		B	○	○	○				
		C	○	○	○		○	○	
	Damaged surface	A	○	○	○				
		B	○	○	○				
		C	○	○	○		○	○	
Device that connects the body with the outside	(Tissue/bone/teeth)	A	○	○	○				
		B	○	○	○	○	○	○	○
		C	○	○	○	○	○	○	○
Implanted device	(Tissue/bone)	A	○	○	○				
		B	○	○	○	○	○	○	○
		C	○	○	○	○	○	○	○

4-1 Cytotoxicity test [1)]

“Cytotoxicity” refers to the ability of cells to inhibit functions such as proliferation or metabolic inhibition, or to cause cell death. Cytotoxicity has the potential to lead to toxicity to tissues, organs, and thus organisms. Therefore, evaluation of cytotoxicity is required for medical devices for dental use in any contact, even minor contact, with the human body.

Various methods exist for the evaluation of cytotoxicity, but here we performed a cytotoxicity study

using V79 cells (Chinese hamster lung-derived fibroblasts) according to ISO 10993-5: 2009, Biological evaluation of medical devices-Part 5: Tests for in vitro cytotoxicity.
Extracts were prepared by immersing MTA cement in cell culture medium MO5.
After culturing the V79 cells in this extraction solution, cell colonies (cell groups composed of 50 or more cells) that are formed were counted. As shown in Figure 4-1, even the 100% concentration showed a high colonization rate of approximately 80%. When the colonization rate of the extract at 100% is 70% or more, it is assessed as not cytotoxic.

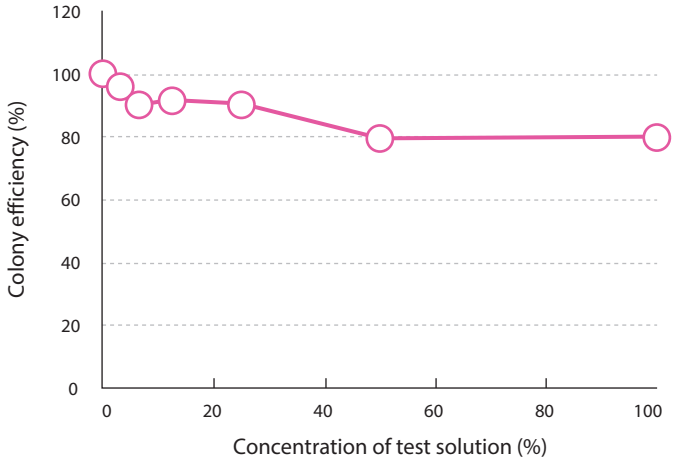

Figure 4-1 Colonizing V79 Cells in MTA cement

4-2 Sensitization test[2)]

Some chemicals cause allergic inflammation when in contact with biological tissues. As an allergy related to the dental material, the delayed type allergy represented by the metal allergy can be mentioned. Sensitization tests were conducted using guinea pigs in accordance with ISO 10993-10: 2010, Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization, a test to evaluate the delayed allergenicity of dental materials.
First, a test solution is prepared by extraction with physiological saline from MTA cement at 121°C for 1 hour. The test solution and Freund's complete adjuvant, a sensitization potentiator, are injected intradermally into guinea pigs to induce primary sensitization, and the test solution is applied (open or closed) to the guinea pig intradermal injection site to induce secondary sensitization. Following the induction of secondary sensitization, the test solution is used to induce sensitization and observe and numerically evaluate the skin changes (erythema and oedema) for the sensitization potential of the test material (Table 4-2).

Table 4-2 Skin changes

Findings	Score
No apparent response	0
Scattered or patchy erythematous plaques	1
Moderate erythema covering the entire application site	2
Marked erythema and edema	3

When the test solution was applied closed, no skin reactions were observed at any of the application sites (all scores: 0). Based on the above, the saline extract of MTA cement was not considered to be sensitizing under the conditions of this study.

4-3 Intradermal response testing [2)]

The irritation test (intradermal response test) is a test to evaluate the injurious, pro-inflammatory, and irritant properties of medical devices for dental use in biological tissues. Appropriate test methods should be selected and evaluated based on the site of medical devices for dental use contact, including intradermal test (contact with tissues in vivo), skin irritation test (contact with skin), and eye irritation test (contact with eyes). This section presents an intradermal test in rabbits.

Intradermal response tests were performed according to ISO 10993-10:2010, Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization.

Extract solution (test solution) was obtained from MTA cement by extraction with physiological saline or sesame oil at 121°C for 1 hour. This was administered intradermally to the test animals (rabbits) as the test solution. Skin reactions were observed immediately after administration and 24, 48, and 72 hours after administration to verify the formation of erythema and edema at the administration site of the test solution.

No skin reaction was observed in the saline extract at any observation time. On the other hand, mild erythema was observed in the sesame oil extract, but similar erythema was observed in blank extract, and the differences between the extract and blank extract were 0. Based on the above, MTA cement was considered to have acceptable risks for intradermal reactions.

This paper presents biological safety assessment of MTA cement in actual use, as required by the law and legal system (the Pharmaceutical and Medical Device Act). In addition to the biological safety tests introduced in this study, only materials that have been evaluated for biological safety in consideration of the manner of use of the materials and confirmed to have no potential problem in any of the evaluation items are actually approved as products.

References

1）ISO 10993-5:2009, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity

2）ISO 10993-10:2010, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization

5 Discoloration of MTA cement by Exposure to Light

MTA cement has excellent functions such as antimicrobial action, sealing ability, biocompatibility, and hard tissue inducibility and is used in applications such as direct pulp capping. X-ray contrast agents have been added to MTA cement for prognostic follow-up. Various types of X-ray contrast agents have been used in different products, including bismuth oxide, zirconia, and tantalum oxide. Because the world's first MTA cement used bismuth oxide, bismuth oxide has been added in many MTA cement products.

Although MTA cement has various functionalities as described above, problems of discoloration by blackening over time have been reported in recent years [1]. The cause of this is that bismuth oxide changes to black due to a reduction reaction (oxygen-deficient state) due to adhesion of blood when hemostasis is insufficient in treatment, reaction with sulfur in proteins, exposure to ultraviolet rays under oxygen blocking conditions, and the like.

Instead of bismuth oxide, in Mielle, zirconia is added as an X-ray contrast agent to prevent blackening due to this exposure.

In this section, we examine the blackening of MTA cement caused by exposure to ultraviolet light. Examination of such blackening was undertaken for X-ray contrast agents (zirconia and bismuth oxide) as simple substances , the prototype which contains bismuth oxide, and MTA cement Mielle, containing zirconia instead of bismuth oxide. In addition, the biological safety effects of this blackening [2] are also verified. XXX

5-1 Discoloration verification of zirconia and bismuth oxide

Materials and Methods

Zirconia powder and bismuth oxide powder were filled as simple substances into a mold made of silicon. Each powder was photoirradiated using an ultraviolet lamp (UV light: Hi-STRON N-900L, Kyowa Irika Co., Ltd.) with and without application of glycerin, and the color tone was evaluated visually.

Results and Discussion

Figure 5-1 shows photographs of zirconia powder and bismuth oxide powder as simple substances before and after exposure to light with ultraviolet lamp. Zirconia was not altered by exposure to light. On the other hand, bismuth oxide showed no color change due to exposure without glycerin coating, but blackening was observed with glycerin coating. It is presumed that the bismuth oxide became oxygen-deficient by reduction reaction by being exposed under conditions in which oxygen was blocked by the glycerin coating, and blackening occurred.

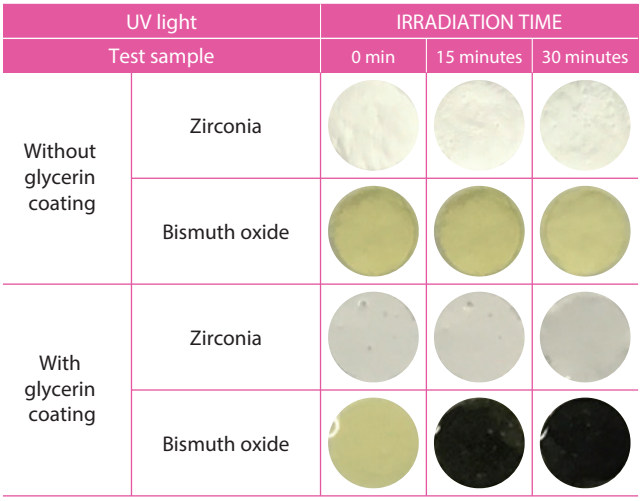

Figure 5-1 Color changes induced by ultraviolet irradiation of zirconia powder and bismuth oxide powder as simple substances

5-2 Effects of X-ray Contrast Agent on Color Change in MTA cement Composition

Materials and Methods

Mielle mixed with 30% by mass of zirconia and a prototype MTA cement mixed with 20% by mass of bismuth oxide was kneaded at a moisture ratio of 20% by mass and filled into a silicone mold. Glycerin was applied immediately after filling, and changes in color tone were assessed visually after photoirradiation with LED light irradiators (LED light: G-Light Prima II, GC Corporation).

Results and Discussion

Figure 5-2 shows the change in appearance over time of Mielle, containing zirconia, coated with glycerin, and the prototype MTA cement, containing bismuth oxide, coated with glycerin, when illuminated by an LED-illuminator. No color change due to light irradiation was observed in Mielle. On the other hand, blackening was observed in the prototype MTA cement, and the discoloration tended to be stronger as the duration of irradiation increased.

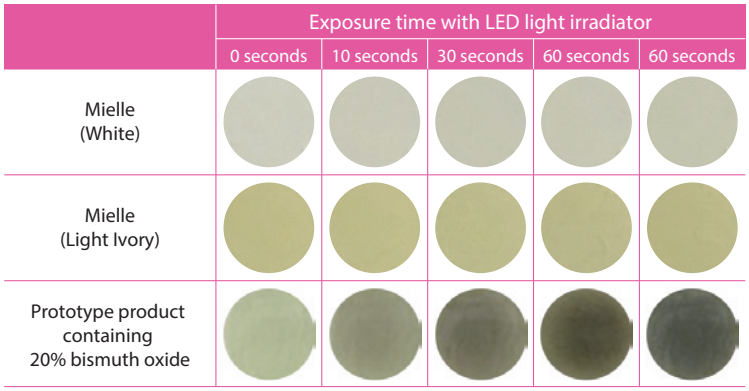

Figure 5-2 Light exposure test with LED light irradiator of Mielle and prototype containing bismuth oxide

5-3 Effect of blackening on biosafety

As described above, the zirconia-containing MTA cement does not undergo discoloration due to light exposure, while the bismuth oxide-containing MTA cement does . It has also been shown in Section 3-5 that fine powdered bismuth oxide with an average particle size of 1 μm is taken up by cells and causes damage to cells. If the blackening is caused by the conversion of bismuth oxide to a more chemically unstable state, the blackened MTA cement may have some effect on the biological safety. Therefore, the effects of blackening in bismuth oxide-containing MTA cements on 1) the elution of components and 2) the cytotoxicity to human monocytic leukemic cells and human mucosal epithelial cells are investigated in this section [2].

Materials and Methods

MTA cement (hereinafter referred to as “Zr-n”) containing 20% by mass of zirconia and a prototype of MTA cement (hereinafter referred to as “Bi-n”) containing 20% by mass of bismuth oxide were kneaded at a moisture ratio of 20% by mass, filled in a mold made of silicone, and set for 24 hours. After coating the surface of Bi-n sample with glycerin, the surface was irradiated with a photopolymerization device (LED CURE Master, marketed by YAMAKIN CO., LTD.) for 180 seconds to prepare a blackened sample (hereinafter referred to as “Bi-black”) (Figure 5-3). It has been confirmed that Zr-n does not blacken with the same treatment.

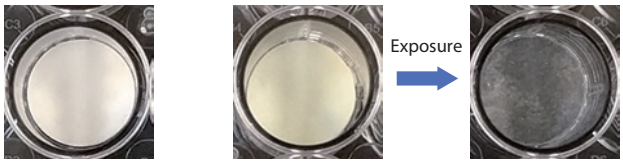

Figure 5-3 Prototype MTA cement containing bismuth oxide (before and after light exposure) and Zr-n

1) Elution test

The samples were immersed in a mixed solution of 0.1 M lactic acid and 0.1 M sodium chloride (1 mL/cm^2) and allowed to stand at 37°C for 7 days. The eluted components in the immersion liquid were measured using an ICP-emission spectrometer (SPS3500DD, Hitachi High-Tech Science Corporation).

2) Trypan blue dye exclusion test [3)]

A sample of 15 mm in diameter and 1 mm in thickness was placed in the wells of 24-well culture plates, where a human monocytic leukemia cell line (THP.1 cell) was seeded (10.0×10^4 cells/well). After 72 hours of incubation in a carbon dioxide incubator, the cells were mixed with trypan blue, and living and dead cells were counted separately on a hemocytometer (Figure 5-4).

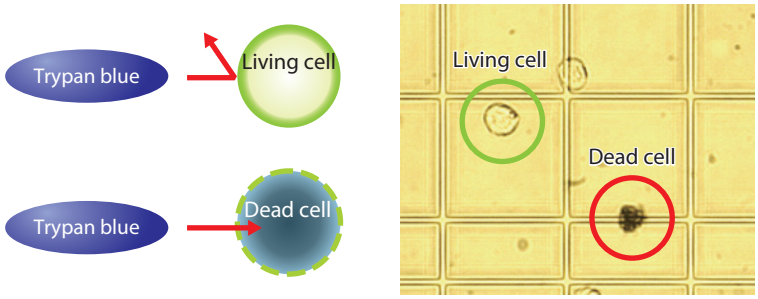

Figure 5-4 Principle of trypan blue dye exclusion test

3) WST cell proliferation test [4, 5)]

Samples were immersed (1 mL/3 cm^2) in serum-free culture medium for keratinocytes for 72 h. Wells of 96-well culture plates were seeded (1.0×10^4 cells/well) with human mucosal epithelial cells (RT-7 cells) and cultured in a carbon dioxide incubator for 24 h. The culture medium of the cell was changed to immersion solution and cultured for another 48 hours. 10 μL of Cell Counting Kit-8 (Dojindo Chemical Laboratories, Inc.) agent was added. The cells were then colored for 2 hours, and absorbance at 450 nm was measured.

Results

1) Elution test

The elution of Mg, Sr, Al, Si, Ba, B, K, and Ca was observed from all samples. Among these eluates, the elution of Ca was remarkable at >1200 ppm in all samples. There was no elution of Zr from Zr-n for the elution derived from the X-ray contrast medium contained in the samples. On the other hand, Bi was eluted from Bi-n and Bi-black. In addition, the amount of Bi eluted was in the order Bi-black> Bi-n, confirming the increase of Bi elution by blackening in MTA cements containing bismuth oxide(Figure 5-5). It was presumed that this was because bismuth oxide caused oxygen deficiency due to light exposure under oxygen deprivation, and bismuth was generated.

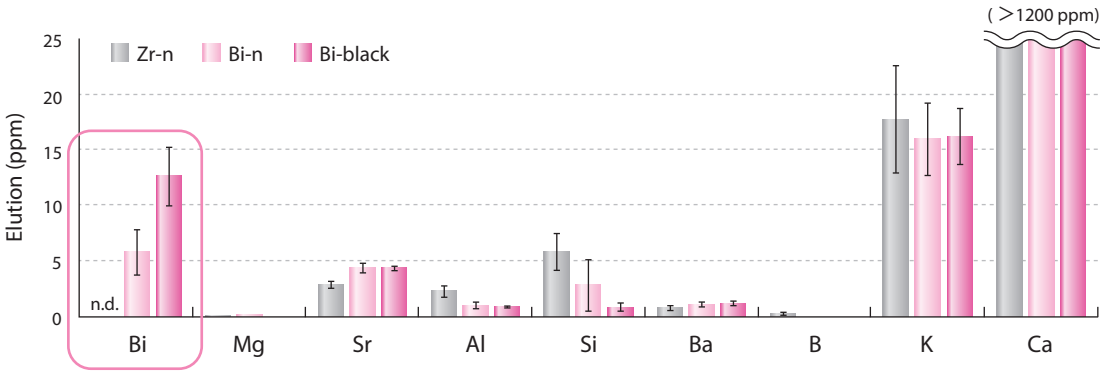

Figure 5-5 Eluted components of each MTA cement

2) Trypan blue dye exclusion test

A prototype MTA cement containing silica in Portland Cement was used as a control sample(PC). When THP.1 cells were cultured on the samples and subjected to the trypan blue dye exclusion test, the increase in cell number was in the order PC ≧ Zr-n > Bi-n ≧ Bi-black (Figure 5-6). Cell numbers were greatly increased on PC and Zr-n from the number of seeded cells (10×10^4 cells/mL), while only a slight increase was observed in Bi-n and Bi-black, with no significant difference between Bi-n and Bi-black. In addition, cell viability was more than 90% in all samples, and there was no cytotoxicity that resulted in cell death (Figure 5-7).

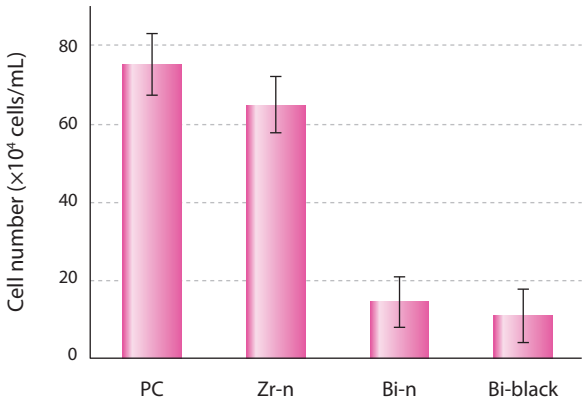

Figure 5-6 Growth of THP.1 cells cultured on MTA cement

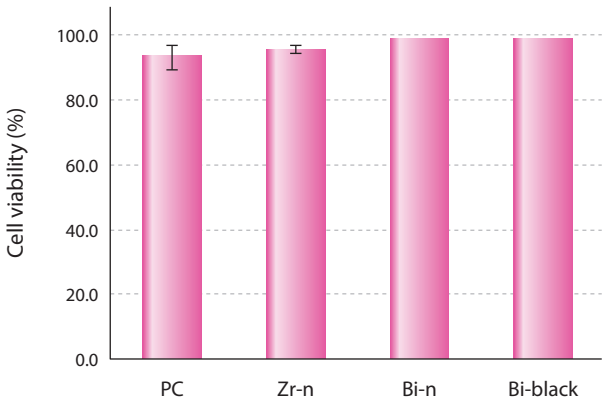

Figure 5-7 Cellular viability of THP.1 cells cultured on MTA cement

3) WST cell proliferation test

The absorbances based on the metabolic activity of the cells were in the order Zr-n > PC > Bi-black ≧ Bi-n (Figure 5-8). Similar to the trypan blue dye exclusion test, no significant differences were found between Bi-n and Bi-black in this method.

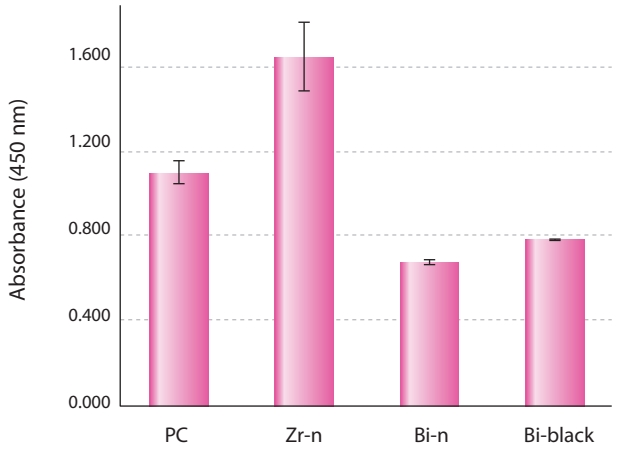

Figure 5-8 Metabolic activity of RT-7 cells cultured on MTA cement

In conclusion, the elution test for the X-ray contrast material used for each cement sample showed that zirconia was not eluted from Zr-n containing zirconia, but Bi was eluted from Bi-n and Bi-black containing bismuth oxide. Cell testing showed good cell proliferation in zirconia-containing cement compared to bismuth oxide-containing cement.

An increase in the amount of Bi eluted was observed in bismuth oxide-containing cements when blackening was produced by exposure under oxygen deprivation. On the other hand, there were no significant differences between Bi-n and Bi-black in any of the cellular tests, and no effect of bismuth oxide on cytotoxicity was observed.

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1) Fridland M, Rosado R: Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. J Endod, 29, 814-817, 2003.

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5) Tominaga H, Ishiyama M, Ohseto F, Sasamoto K, Hamamoto T, Suzuki K, Watanabe M: A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Anal Commun, 36, 47-50, 1999.

6 How to operate TMR-MTA cement Mielle

6-1 Operation Procedure

Adjustment method

The kneading (mixing) of the powder and liquid is adjusted according to the case, based on the standard moisture ratio. Since the material comes into direct contact with nerves, the equipment used should be sterilized and purified water should be used. Caution should be exercised when using metal instruments, as strong forces can cause the metals to shave and darken. The procedure is shown in Figure 6-1.

1. Indications

・Uninfected pulp is indicated if cavity formation or trauma accidentally leads to exposure within 2 mm of the pulp.

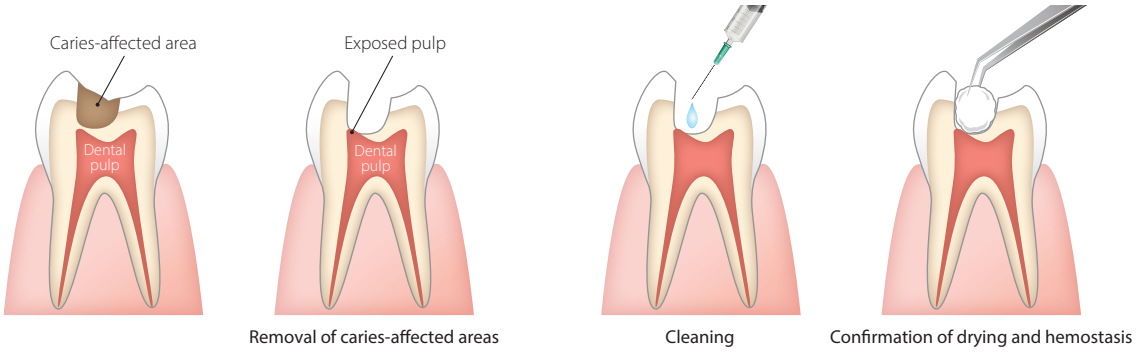
※ Infected pulp is not indicated.

※ During cavity formation, treat with sterile steel bars or the like.

2. Cleaning

1. Clean the exposed pulp surface with sterile saline or the like. When disinfecting the exposed surface, disinfect with 3 to 10% aqueous sodium hypochlorite before washing.
2. Dry with sterile cotton balls and check for hemostasis.

※ If hemostasis is difficult, intense inflammation may be present in the pulp, and pulpotomy or pulpectomy is indicated.



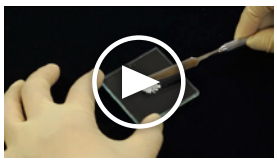
3. Pulp capping by TMR-MTA cement Mielle

Figure 6-1 How to Use Mielle

Movie

How to use TMR-MTA cement Mielle and Moisture Ratio

Summary for mixing procedure.

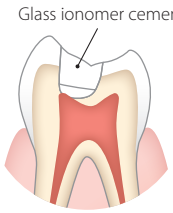


Pulp Capping method

Since this product can be kneaded with a small amount of water and sets quickly, it is possible to temporarily seal the filled portion with glass ionomer cement immediately after filling the exposed pulp portion with paste and removing excess paste and moisture with a cotton ball or the like.

4. Temporary sealing

1. After pulp capping the exposure site with MTA cement, remove excess MTA cement and water with a sterile cotton swab.
2. Temporary seal by glass ionomer cement. It can be temporarily sealed immediately after pulp capping.



5. Final restoration

1. After follow-up, remove some of the glass ionomer cement to the extent that MTA cement does not peel off.
2. After bonding, a final restoration procedure is performed with composite resin.

※Filling and crown formation after direct pulp capping should be done after at least one month of follow-up.

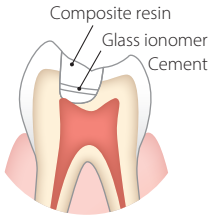
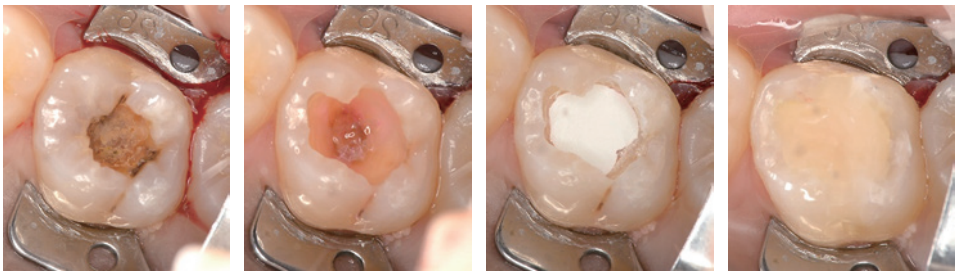
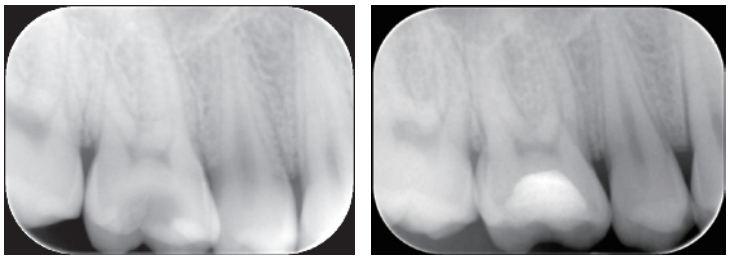


Figure 6-2 Flow of final restoration from temporary sealing



1. Preoperative
2. Removal of softened dentine (pseudo-exposed) pulp
3. Application of MTA
4. Temporary sealing



5. Preoperative dental
6. Postoperative dental

Figure 6-3 Clinical case of direct capping 1

* Images are from a patient whose tooth was temporarily sealed with glass ionomer cement after pulp capping with TMR-MTA cement Mielle.
Photographs courtesy of Professor Masato Saito, Health Sciences University of Hokkaido

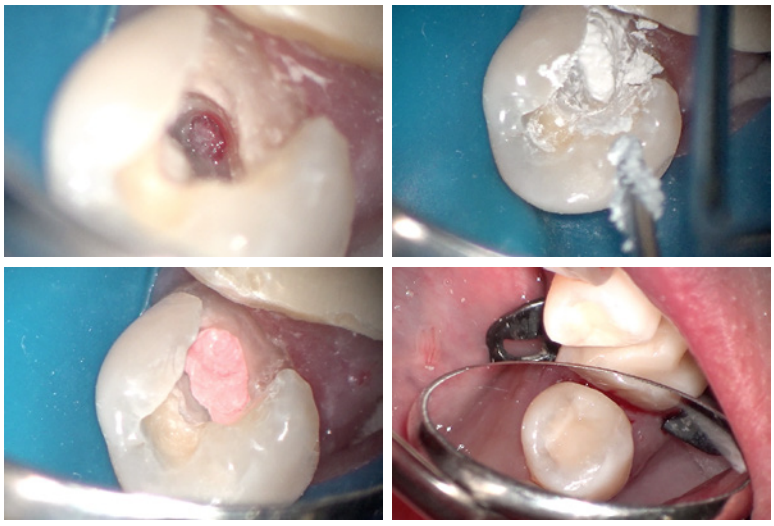


Figure 6-4 Clinical case of direct pulp capping 2

* Images are from a patient whose tooth pulp was capped with TMR-MTA cement Mielle, and after that gutta-percha covering the upper part of the MTA, and bonding followed by restoration with composite resin.
Image courtesy of Professor Susumu Sudo, Nankodai Dental Clinic, Izumi-ku, Sendai City

In addition, the Mielle lineup consists of two colors (white and light ivory) for each color tone of the glass ionomer cement used for temporary sealing, and we recommend that it be used separately, with white for the crown color if the glass ionomer cement is a color such as A3, and light ivory if it is white.

6-2 Operating time and initial setting time by moisture percentage

The typical moisture ratio of the Mielle is 20 mass%, but if the paste is hard to operate or is dried and difficult to operate, it is easy to adjust the viscosity of the paste by adding water prior to starting the setting. It is also possible to adjust the viscosity and duration of operation of the paste by increasing the moisture ratio to 25-30 mass% as shown in Table 6-1.

Table 6-1 Operating time and initial setting time by moisture ratio

Moisture ratio (powder : water)	Paste property	Operating time	Initial setting time
20 mass% (powder 0.2 g : water 0.05 g)	Standard	About 3 minutes	15-30 min
25 mass% (powder 0.2 g : water 0.07 g)	Soft	About 6 minutes	30-40 min
30 mass% (powder 0.2 g : water 0.09 g)	Very soft	About 9 minutes	40-60 min

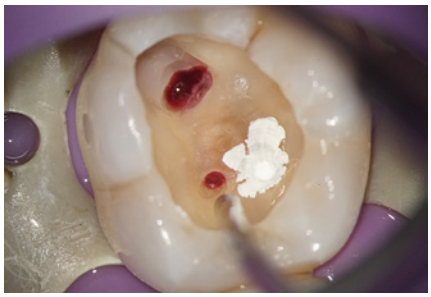
However, since the initial setting time tends to be longer when the paste is filled in a soft state, as shown in Table 6-2, it is possible to shorten the initial setting time by removing excessive moisture with a dry cotton swab or the like after the paste is filled.

Table 6-2 Moisture percentage and initial setting time with and without wiping

Moisture ratio	No wiping	With wiping
20 mass%	15 minutes	5 minutes
25 mass%	30 minutes	5 minutes
30 mass%	60 minutes	10 minutes

Special Feature

Points of precision therapy with MTA

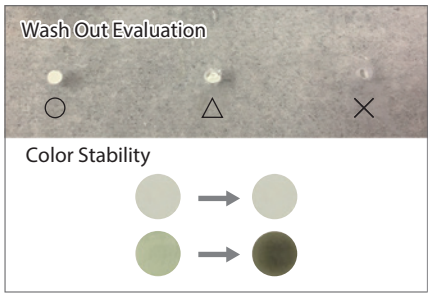


1 Rubber dam moisture protection to prevent pulp infection

It is imperative to use rubber dams in pulp preservation treatments. When removing restorations, a rubber dam is placed in advance to prevent accidental ingestion and aspiration of dislodged restorations, and it also facilitates transfer to direct pulp capping in the event of exposure of the pulp.
When using a variety of chemical solutions, a rubber dam can be used for safety.

2 Thorough sterilization of equipment

Complete cleaning, disinfection, and sterilization of the instruments used are required in pulp-preservation treatment. When using MTA cement, spatulas, kneading plates, filling tools, etc. must be washed and sterilized.



3 MTA cement selection

MTA cement often needs to be used unexpectedly for direct pulp coverage.
For this reason, the speed of setting is important in clinical practice.
Unset cement may be discharged by contact with tissue fluid (washout) and it may not achieve full performance.
In addition, considering aesthetics, it is important to select products that are not prone to discoloration after pulp capping.

4 Use of a microscope to increase treatment accuracy

The use of a microscope ensures a bright surgical field that has been enlarged tens of times. This will ensure removal of infected dentine and minimize invasion, thereby significantly reducing patient burden. Microscopes can also record the treatment content in photographs and videos, allowing patients access to a detailed description of the condition of the affected teeth, which increases patient understanding and satisfaction.

5 Diagnosis by Dental Cone Beam CT

Three-dimensional images of CT can inform the dentist of the structure and the shape of the teeth and what problems are occurring in the teeth. Dental x-rays alone provide only two-dimensional planar information, so treatment must rely on the practitioner's experience and intuition in many areas, whereas CT can anatomically identify the internal condition of the tooth, allowing for proper treatment planning and shortening of the treatment time.

Although MTA cement is a material with good sealability and excellent performance, it is important to use rubber dams and microscopes and to use it correctly, while take appropriate measures against infection. In other words, it should be noted that the use of MTA cement does not increase the rate of successful pulp preservation treatment. For more information on using MTA cement , please refer to Shinai iryoho no sanshu no shinki ("The Three Sacred Treasures of Endodontic Therapy"; Dental Diamond Co.) pp.138-141.

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7 Conclusion

Mielle is an MTA cement with superior usability and biocompatibility. Like the conventional product, it is bismuth-free, and the X-ray contrast has been enhanced by 60% over the conventional product. Therefore, since the filling quantity can be confirmed by X-ray and CT even in small quantities, follow-up is easy, and it can be applicable in a wide range of cases. Together, the improvement of productivity realized by these enhancements has made MTA cement more efficient and economical than ever before.

These enhancements have been in response to the desire of practitioners using the conventional product for an MTA cement that is more user-friendly and more broadly applicable, and from YAMAKIN's desire for more patients to benefit from the wonderful material that is MTA cement.

We believe that this new Mielle can be used not only by specialists in endodontics, but also by practitioners who have not used MTA cement before to protect the pulp of numerous patients. We hope that this book will lead to the correct and widespread use of MTA cement.

The authors

Acknowledgements

The MTA cement project, which led from "TMR-MTA CEMENT" to "Mielle", was started through the enthusiastic commitment of the professors of Health Sciences University of Hokkaido, including Professor Kazuhiko Endo and Professor Masahito Saito. Subsequent joint research has led to the realization of TMR-MTA CEMENT by conducting compositional studies and evaluations as a biomaterial. Let us take this opportunity to express our heartfelt appreciation to everyone involved.

In order to respond to our clients' desire to use MTA cement for more patients, we will continue to publicize and widely disseminate information on the correct use of MTA cement through channels such as this publication.

We also thank many medical professionals, including Professor Testuya Yamamoto, Department of Oral and Maxillofacial Surgery, Kochi Medical School, Kochi University, Dr Mitsuhiko Takata, Takata Dental Clinic, and Dr Susumu Sudo, Nankodai Dental Clinic, who worked in a variety of ways, including advice on biological safety assessment, comments from a clinician perspective, and providing case photographs.

Finally, we would like to express our thanks and heartfelt gratitude to all parties involved in the editing of this publication.

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