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Comparative X-Ray Diffraction Analysis of Conventional MTA and Bacterial Cellulose-Reinforced MTA- An in Vitro Study

[1]Dr.V.Lalitha Priya, III[rd] Year Post Graduate, Department of Pediatric and Preventive Dentistry, SRM Kattankulathur Dental College and Hospital, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India

[2]Dr.Kavitha Ramar, Professor and HOD, Department of Pediatric and Preventive Dentistry, SRM Kattankulathur Dental College and Hospital, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India

Corresponding Author: Dr.Kavitha Ramar, Professor and HOD, Department of Pediatric and Preventive Dentistry, SRM Kattankulathur Dental College and Hospital, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India

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Abstract

Purpose: Bacterial cellulose (BC) has garnered attention in dental materials due to its moldability, cost-effectiveness, high water retention, biocompatibility, and biodegradability. Incorporating bacterial cellulose nanocrystals (BCNC) into Mineral Trioxide Aggregate (MTA) has been shown to accelerate hardening and reduce setting time, thereby enhancing clinical efficiency and patient satisfaction. This study aimed to evaluate and compare the crystalline structures of conventional MTA and bacterial cellulose-reinforced MTA (BC-MTA) using X-ray diffraction (XRD) analysis.

Materials and Methods: Cylindrical samples (6 mm × 4 mm) of conventional MTA (MTA Plus™) and BC-MTA (Vedayukt India Pvt. Ltd.) were prepared and incubated under saturated conditions at 37°C for 24 hours. XRD analysis was performed using a Philips diffractometer with CuKα radiation over a 2θ range of 4° to 70°, at 40 mA and 45 kV. Phase identification was carried out using DIFFRAC.EVA software supported by the ICDD database.

Results: Both materials exhibited similar primary constituents—carbon (C), oxygen (O), calcium (Ca), and chloride (Cl). Conventional MTA showed bismuth oxide as a radiopacifier, whereas BC-MTA contained tungsten, indicating a compositional shift. BC-MTA also exhibited broader and less intense diffraction peaks, suggesting

reduced crystallinity, potentially due to the influence of the cellulose matrix on crystal lattice formation.

Conclusion: BC-MTA and conventional MTA share similar elemental compositions but differ in radiopacifiers and crystallinity. The integration of bacterial cellulose alters the structural properties of MTA, potentially influencing its setting characteristics and clinical performance.

Keywords: Mineral trioxide aggregate, bacterial cellulose, X-ray diffraction, bacterial cellulose reinforced MTA, elemental analysis.

Introduction

Dental materials have progressed alongside advancements in pediatric dentistry, with technological innovations enhancing their physical, chemical, and biological characteristics. Recent progress in dental materials aims to enhance oral health care for young patients, including infants, children, and adolescents. Notably, calcium silicate-based materials have gained recognition as a breakthrough category, providing distinctive benefits and potential applications that may transform pediatric dental treatments. This advancement represents a critical move toward more biocompatible, efficient, and safer restorative solutions for younger populations [1].

Mineral trioxide aggregate (MTA) was first introduced in 1990s by lee et al [2]. MTA is a calcium silicate-based bioactive material primarily composed of Portland cement and bismuth oxide, forming a fine hydrophilic powder that sets upon hydration [3-5]. The superior property of this material is due to its excellent sealing ability, biocompatibility, bioactivity, hydrophilicity, and hard-tissue forming capacity [3,6-9]. It also exhibits desirable characteristics, including antimicrobial activity, dimensional stability, radiopacity, and resistance to moisture[9]. With its versatile properties, MTA is extensively applied in clinical dentistry for procedures like vital pulp therapy, pulp capping, pulpotomies, apexification, apexogenesis, as well as in the repair of root perforations and root-end fillings [8-9].

Although MTA is regarded as a material with ideal clinical properties, its widespread use has been restricted by several drawbacks, including high cost, difficult handling characteristics, prolonged setting time, low compressive strength, and the potential for tooth discoloration [8]. These limitations have driven continuous efforts to develop modified formulations. One of the earliest modifications was made to the original gray formulation (ProRoot MTA) by the manufacturer (Dentsply Tulsa Dental, Tulsa, OK, USA), resulting in a tooth-colored variant known as White MTA, which addressed discoloration by eliminating iron from its composition [9-11]. In 2001, MTA Angelus (MTA-A) was introduced as a more affordable alternative to ProRoot MTA, with a key chemical difference being the absence of calcium sulfate dihydrate—leading to a significantly shorter setting time (10 minutes for MTA-A vs. 165 minutes for ProRoot MTA) [9,12-13]. To further enhance MTA's clinical performance, various additives such as methylcellulose, calcium chloride, zinc oxide, eugenol, and chitosan have been explored [8,14]. Among these, the incorporation of bacterial cellulose nanocrystals (BCNCs) has shown promising improvements in the physiochemical properties of MTA.

A variety of materials, including plants and certain bacterial species, can supply cellulose [8,14]. Among these, bacterial cellulose is an eco-friendly, naturally occurring hydrogel which has drawn considerable interest in the field of dentistry. It is an unbranched polymer composed of (1→4) β-glycosidic linked glucose units and is synthesized as nanofibrils by aerobic bacteria, most notably species from the Komagataeibacter genus, with

Komagataeibacter xylinus being the primary producer using glucose as a carbon source [15-16]. Bacterial cellulose possesses several advantageous features, such as interconnected porosity, excellent water retention, high biocompatibility, and moldability, along with mechanical properties that closely resemble those of some hard and soft tissues [8,15-16]. It is also non-toxic, hydrophilic, non-allergenic, and biodegradable when appropriately modified, making it highly suitable for medical and dental applications [15-16]. Furthermore, the U.S. Food and Drug Administration (FDA) has recognised it as "Generally Recognised as Safe" (GRAS) for use in food [17-18]. In India, it has been developed by the Council of Scientific and Industrial Research – Indian Institute of Integrative Medicine and is categorized under code 42 by the Drugs Controller General of India (DCGI) for use in dental materials[18]. These attributes make bacterial cellulose a promising biomaterial for various dental and oral health applications.

Since the mid-1930s, X-ray diffraction (XRD) has been employed for quantitative phase analysis [19]. It remains a highly effective technique for identifying and analyzing the crystalline phase composition of materials, offering crucial insights into their structural characteristics and potential clinical applicability [9,19]. Therefore this study aimed to evaluate and compare the crystalline structures of conventional MTA and Bacterial Cellulose-reinforced MTA (BC-MTA) using XRD analysis.

Type of study

This in vitro study was conducted under a controlled experimental environment, allowing for accurate data collection and a reliable comparison of the crystalline structures of MTA and BC-MTA. The materials were divided into two groups: Group 1 comprised conventional MTA (*MTA Plus*™), while Group 2 included BC-MTA (Vedayukt India Private Limited).

Preparation of BC-MTA

In this study, bacterial cellulose was utilized as a reinforcing agent, recognized as GRAS under Title 21 CFR 182.1 of the Federal Food, Drug, and Cosmetic Act (Section 321(s)). This designation reflects a thorough scientific evaluation and validation by independent expert panels [8]. All sample preparations were carried out meticulously within a strictly controlled laboratory environment to ensure both reproducibility and experimental accuracy.

For the formulation of the BC-modified MTA, a carefully measured composition was employed, consisting of 33.34% by weight Bacterial Cellulose Nanocrystals (BCNCs) and 66.66% by weight conventional MTA [20]. The components were thoroughly blended to achieve a homogenous mixture, ensuring uniform dispersion of BCNCs throughout the matrix. To preserve the physicochemical integrity of the material and minimize the risk of photo degradation, the prepared mixture was stored in amber-colored bottles under cool and dark storage conditions [8].

Sample preparation

A cylindrical plexiglass mold measuring 6 mm in diameter and 4 mm in height was custom-fabricated in compliance with ASTM E384 standards using a Computer Numerical Control (CNC) laser cutting system. The mold fabrication was conducted under optimized conditions—utilizing 4200 watts of power, a cutting speed of 4400 mm/min, and a pressure of 0.8 MPa—at the Saveetha Institute of Dental Sciences and Research, Chennai, India.

The BC-reinforced MTA was mixed using a water-to-powder ratio of 3:1, whereas the conventional MTA was prepared following the manufacturer's recommended protocol, using a standard 3:1 powder-to-water ratio [8, 20]. After mixing, all samples were placed in a fully

saturated environment and incubated at 37°C for 24 hours to facilitate complete setting and material stabilization [8].

XRD analysis

For XRD analysis, the set material samples from both groups were first crushed into a fine powder using an agate mortar and pestle to ensure uniformity. The powdered specimens were then appropriately mounted for examination. XRD measurements were performed by randomly selecting five points from each sample to ensure representative analysis [21]. The analysis utilized a Philips diffractometer system, including Model 1130/96 (generator) and PW1050/24 (goniometer), operating with CuKα radiation across angular intervals ranging from 4° to 70°and at operating conditions of 40 mA and 45 kV. Phase identification was conducted using DIFFRAC.EVA software (Bruker, Billerica, USA), supported by data from the International Centre for Diffraction Data (ICDD) database (Newtown Square, USA) [21-22]. Figure 1 depicts the schematic illustration of the methodological framework, outlining the process from sample preparation to XRD analysis

Results

The XRD results of the samples are shown in Figures 1-2.

Figure 1 presents XRD analysis of conventional MTA that revealed characteristic diffraction peaks corresponding to several key elements. Strongest peaks were observed at 7.89° 2θ for carbon (C) and 28.29° 2θ for oxygen (O)**.** Additional distinct peaks were seen at 18.99° (Na), 32.47° (Si), 35.87° (Cl), 47.07° (Ca), 57.64° (Cu), and 78.10° (K), indicating the presence of sodium, silicon, chloride, calcium, copper, and potassium, respectively. The presence of calcium and silicate-related components supports the bioactive nature of MTA and its ability to contribute to hard tissue formation.

On the other hand **f**igure 2 presents XRD analysis of BC-MTA, highlighting the elemental composition and crystalline structure of each material.It exhibited multiple sharp peaks at higher 2θ values. The most prominent peaks were observed at 45.58° (C), 49.31° (Na), 53.20° (K), 54.88° (Cl), 56.88° (Ca), and 76.38° (Cu). Notably, a significant diffraction peak was recorded at 57.68°**,** corresponding to tungsten (W)**,** which was absent in the conventional MTA pattern. The oxygen (O) peak was detected at 59.77°**.**

The XRD analysis revealed that both materials—conventional MTA and BC-MTA—shared the same primary constituents: carbon (C), oxygen (O), chloride (Cl), and calcium (Ca). However, bismuth oxide was detected exclusively in conventional MTA and was absent in BC-MTA. The presence of tungsten in BC-MTA indicates a compositional alteration, likely resulting from the incorporation of bacterial cellulose. Additionally, the broader peak distribution and reduced intensity observed in BC-MTA suggest lower crystallinity, which may be due to the cellulose matrix disrupting the regular formation of the crystal lattice.

Discussion

Determining the primary compounds present in a material is crucial for understanding its physical, chemical, and mechanical behavior. XRD is a powerful analytical technique used to identify these constituents [23]. The method works by analyzing the unique diffraction pattern produced by each crystalline phase, characterized by distinct Bragg's peaks—each with a specific intensity (y-axis) and corresponding diffraction angle. Phase identification is carried out by comparing the specimen's diffraction data with an extensive database of standard patterns provided by the International Centre for Diffraction Data (ICDD) [9,19]. In our study, XRD analysis

was employed to determine the chemical composition of two materials: conventional MTA and BC-MTA.

MTA is composed primarily of Portland cement, whose key constituents have been thoroughly investigated. It predominantly consists of crystalline phases, with calcium silicate hydrate being the sole amorphous component [9]. Our results demonstrated that both conventional MTA and BC-MTA share key elemental components, namely carbon (C), oxygen (O), calcium (Ca), and chloride (Cl), consistent with the known composition of MTA, which primarily consists of tricalcium silicate, dicalcium silicate, and calcium aluminate phases [24-25]. The presence of these constituents supports the retention of the core chemical framework even after reinforcement with bacterial cellulose. Calcium, in particular, plays a central role in the bioactivity and hard tissue induction potential of MTA due to its involvement in calcium hydroxide release and hydroxyapatite formation upon setting [26].

However, a notable distinction was observed in the radiopacifying agents. Bismuth oxide, which is traditionally added to MTA to enhance its radiopacity, was detected in conventional MTA but was absent in the BC-MTA formulation. Instead, tungsten was present in BC-MTA. This substitution may have been introduced to mitigate some of the known drawbacks associated with bismuth oxide, such as tooth discoloration and interference with hydration and setting reactions [27-28]. Tungsten, particularly in the form of calcium tungstate or elemental tungsten, has been investigated as a potential alternative radiopacifier due to its favorable biocompatibility and radiographic visibility [29].

Moreover, the XRD patterns indicated a broader peak distribution and lower peak intensity in BC-MTA compared to conventional MTA, suggesting reduced crystallinity. This phenomenon may be attributed to the integration of bacterial cellulose into the MTA matrix. Bacterial cellulose is a highly pure, nanofibrous polysaccharide with an extensive hydrogen-bonded network, which could interfere with the regular lattice formation of the crystalline components during the setting of the cement [30]. Reduced crystallinity has been linked to altered hydration kinetics and potentially improved ion release due to a more disordered matrix, which may enhance bioactivity [30].

Previous studies have highlighted that material crystallinity can directly influence mechanical strength, solubility, and bio-interactive behavior. While high crystallinity often correlates with enhanced strength, lower crystallinity may facilitate faster calcium ion release and better biological responses, especially in pulpal and periapical tissues [31]. Therefore, the observed structural modifications in BC-MTA could have both advantageous and disadvantageous implications depending on the clinical application.

The presence of tungsten in BC-MTA also opens up new avenues for exploration. Some reports suggest that tungsten compounds may contribute to antimicrobial activity or altered setting kinetics, though more research is needed to confirm such effects. The observed compositional differences imply that the incorporation of bacterial cellulose does more than merely reinforce the matrix—it may also influence the chemical formulation and final physicochemical behavior of the material.

Overall the XRD findings of this study reveal that although both conventional MTA and BC-MTA share similar core constituents, the replacement of bismuth oxide with tungsten and the reduced crystallinity in BC-MTA reflect significant compositional and structural modifications. These alterations may affect the material's biological and mechanical performance. Future investigations should explore the long-term clinical

outcomes and in vitro/in vivo bioactivity of BC-MTA, particularly focusing on how these compositional changes influence its sealing ability, cytocompatibility, and regenerative potential.

Figure 1: Schematic illustration methodological framework: from sample preparation to XRD analysis

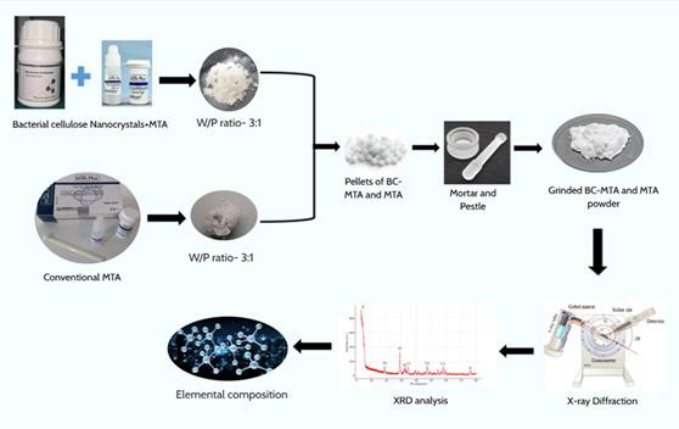


Figure 2: XRD analysis of conventional MTA

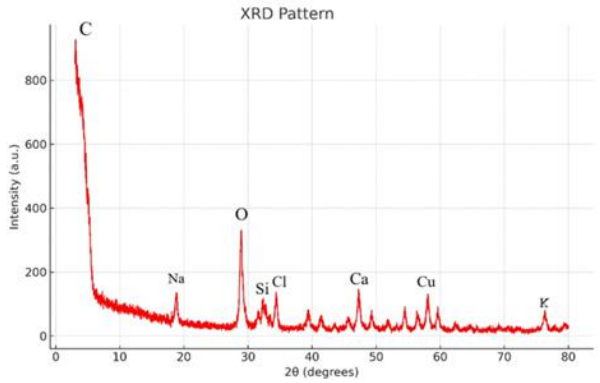
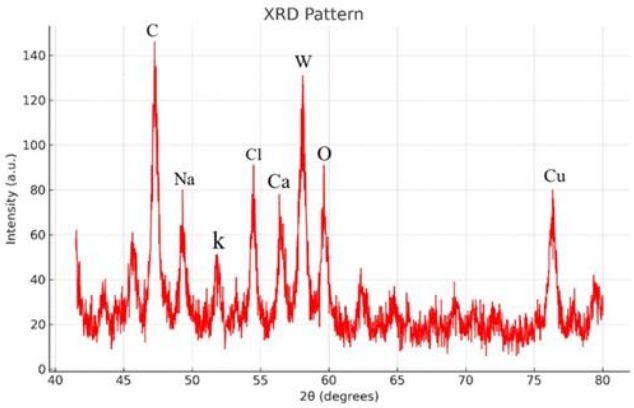


Figure 3: XRD analysis of BC-MTA



Conclusion

XRD analysis revealed that both conventional MTA and BC-MTA share similar major constituents, but differ in radiopacifying agents—bismuth oxide in MTA and tungsten in BC-MTA. The reduced crystallinity observed in BC-MTA is likely due to bacterial cellulose integration, which may affect its setting and biological properties. These findings suggest that cellulose reinforcement not only modifies the composition but may also influence the clinical behavior of MTA-based materials.

Abbreviations BC- Bacterial cellulose, BCNC- bacterial cellulose nanocrystals, MTA- Mineral Trioxide Aggregate, BC-MTA- bacterial cellulose-reinforced MTA, XRD- X-ray diffraction analysis, C- carbon, O- oxygen, Ca- calcium, cl-chloride, MTA-A-MTA Angelus, GRAS- Generally Recognised as Safe, DCGI- Drugs Controller General of India, ICDD-International Centre for Diffraction Data

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