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Comparative Evaluation of Antioxidant and Anti-Inflammatory Properties of Bioceramic Ceremagnum Plus against E. Faecalis, Staphylococcus Aureus and Streptococcus Mutans with MTA- An Invitro Study

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KEYWORDS

Ceremagnum Plus, Magnesium-based bioceramic, Mineral trioxide aggregate, Anti-inflammatory activity, Antioxidant assay, Protein denaturation, DPPH scavenging, Vital pulp therapy, Reactive oxygen species, Bioceramic materials

ABSTRACT:

Background: Inflammation and oxidative stress play critical roles in determining the outcome of vital pulp therapy. Mineral trioxide aggregate (MTA) is widely used for pulpal repair; however, its limited capacity to modulate oxidative and inflammatory responses may compromise healing. The present study aimed to evaluate and compare the anti-inflammatory and antioxidant properties of an indigenously developed magnesium-based bioceramic material (Ceremagnum Plus, C Plus) with those of conventional MTA using in vitro assays.

Materials and Methods: The anti-inflammatory activity of both materials was assessed using the protein denaturation inhibition assay, whereas the antioxidant potential was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Ceremagnum Plus and MTA were tested at concentrations of 50, 75, 100, and 125 mg/mL, with ascorbic acid serving as the positive control. Absorbance was measured spectrophotometrically, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with the level of significance set at $p < 0.05$.

Results: Both materials demonstrated a concentration-dependent increase in anti-inflammatory and antioxidant activities. Ceremagnum Plus exhibited significantly higher protein denaturation inhibition and DPPH radical scavenging values than MTA at all concentrations ($p < 0.001$). The C Plus–$Ca(OH)_2$–MTA composite at 100 and 125 mg/mL recorded the highest inhibition values, closely approximating that of the ascorbic acid control. These findings suggest that the incorporation of magnesium within the calcium silicate matrix enhances ion release, protein stabilization, and free radical neutralization.

Conclusion: The indigenously developed Ceremagnum Plus displayed superior anti-inflammatory and antioxidant activity compared to MTA, attributed to its magnesium-enriched calcium silicate–phosphate composition and improved redox-modulating capacity. The material's ability to attenuate inflammation and oxidative stress indicates strong potential for vital pulp therapy, offering a biologically favorable environment that supports pulpal healing and reparative dentinogenesis.

1. Introduction

Inflammation and oxidative stress are central to the pathobiology of pulpal disease and to the success or failure of vital pulp therapy. Bacterial challenge and operative trauma trigger a cascade of cytokine release (e.g., TNF-α, IL-1β) and rapid generation of reactive oxygen species (ROS) within odontoblasts and resident immune cells. [1]Unchecked, this milieu sustains tissue injury, impairs angiogenesis, and delays reparative dentinogenesis. Accordingly, contemporary bioactive cements are evaluated not only for sealing ability and bioactivity, but also for their capacity to modulate inflammation and attenuate oxidative stress. [2]Mineral trioxide aggregate (MTA), a calcium-silicate–based cement introduced for endodontic use in the 1990s, has emerged as a benchmark material in this regard due to its favorable biological profile and clinical outcomes in pulp capping, pulpotomy, perforation repair, and apexification.[3]

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Chemically, MTA hydrates to form calcium-silicate-hydrate gel and portlandite [$Ca(OH)_2$], creating a sustained alkaline microenvironment (pH ~11–12) and releasing calcium ions.[4] These reactions underpin several mechanisms relevant to inflammation control: (i) buffering of acidic inflammatory exudate; (ii) precipitation of apatite that physically seals dentinal defects; and (iii) signaling through Ca^{2+}-dependent pathways that promote cell survival and differentiation.[5] In vitro studies with pulp cells and macrophage-like lines consistently show that MTA extracts reduce the expression of pro-inflammatory mediators (e.g., COX-2, iNOS) while enhancing anti-inflammatory or pro-resolving signals (e.g., TGF-β1, IL-10), alongside increased expression of odontogenic markers (DSPP, ALP, OCN) .[6] Animal implantation and pulp-capping models corroborate these findings, reporting diminished inflammatory cell infiltration, earlier organization of a collagenous matrix, and more frequent formation of a continuous, tubular reparative dentin bridge beneath MTA compared with inert or zinc-oxide–based controls.[7]

Beyond cytokine modulation, emerging evidence indicates that calcium-silicate cements exhibit measurable antioxidant behavior.[8] MTA has demonstrated scavenging activity in chemical assays (e.g., DPPH, ABTS, FRAP) and mitigation of intracellular ROS in peroxide-challenged pulp cells, effects often attributed to hydroxyl ion release, surface redox interactions on calcium-silicate hydrates, and adsorption/neutralization of oxidizing radicals at the material interface. [9]By lowering ROS burden, MTA can support mitochondrial function, preserve cytoskeletal integrity, and favor a pro-healing phenotype in pulp fibroblasts and stem cells. This redox modulation may also intersect with inflammation resolution, given the crosstalk between ROS signaling and NF-κB/MAPK pathways.[10]

Clinically relevant models extend these observations: when compared with conventional calcium hydroxide dressings, MTA-treated pulps show reduced necrotic zones, fewer multinucleated giant cells, and more advanced mineralized barrier formation at early time points, suggesting a combined anti-inflammatory and pro-repair effect rather than mere tissue tolerance. [11]Notably, the biological response appears time-dependent and conditioned by the material's sustained ion release and stable high pH, both of which are sensitive to powder–liquid ratio, moisture availability, and the presence of radiopacifiers or accelerators in proprietary formulations.[12]

Nevertheless, important gaps remain. Reported antioxidant magnitudes vary across test systems, and few studies standardize the surface area–to–volume ratio or aging of set cements before testing, parameters that strongly influence ion elution and reactivity. Likewise, while short-term suppression of inflammatory markers is well documented in vitro, longitudinal human data quantifying persistence of these effects in vivo are limited. [13]Comparative studies versus newer calcium-silicate materials (e.g., fast-setting, zirconium-based formulations) also yield mixed results, likely reflecting differences in particle morphology, hydration kinetics, and trace phase chemistry.

Given the central role of oxidative stress and inflammation in pulpal healing, and the biological plausibility that MTA's chemistry can favorably modulate both, a focused evaluation of MTA's anti-inflammatory and antioxidant properties using standardized, complementary assays is warranted. In this context, protein-denaturation inhibition can model anti-inflammatory potential by reflecting the material's ability to stabilize proteins against heat-induced conformational damage, while free-radical scavenging assays (e.g., DPPH with ascorbic acid as a reference) provide a quantitative index of antioxidant capacity.[14] The present study synthesizes available evidence and augments it with experimental testing to clarify the extent to which MTA exerts clinically meaningful anti-inflammatory and antioxidant effects, thereby informing material selection and protocols for vital pulp therapy.

2. Methodology

The experimental magnesium-based bioceramic material (Ceremagnum Plus) was synthesized by combining potassium dihydrogen phosphate (KH_2PO_4) and magnesium oxide (MgO) in a 1:1 molar ratio. The mixture was homogenized and sintered at 900 °C for 3 hours to form potassium magnesium phosphate ($KMgPO_4$). After cooling, the sintered mass was finely ground to obtain a uniform powder. To this, 35 wt% calcium silicate ($CaSiO_3$) and 2 wt% zirconyl nitrate [$ZrO(NO_3)_2$] were added in distilled water and mixed thoroughly, followed by incorporation of 35 wt% of the synthesized $KMgPO_4$ to generate the primary bioceramic

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matrix. For the test formulation, 975 mg of $KMgPO_4$, 900 mg of $CaSiO_3$, 32.4 mg of zirconium oxide (ZrO_2), and 65.1 mg of cerium oxide (CeO_2) were used as radiopacifiers, along with 39.45 mg of sodium fluoride (NaF) to enhance bioactivity. All powders were transferred to sterile Eppendorf tubes and co-ground for 10 minutes to ensure homogeneity, after which the radiopacifiers were incorporated. 100 µL of distilled water was added using a micropipette and triturated manually to obtain a smooth, uniform paste. Commercial MTA Angelus (700 mg) served as the control material.[15]

Figure 1: Anti oxidant Assay

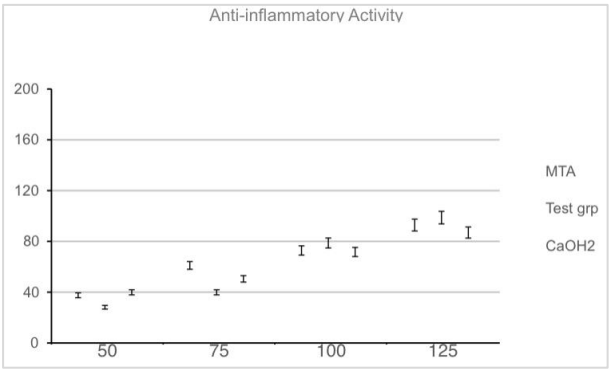
Figure 2: Anti-inflammatory Assay

The anti-inflammatory activity was evaluated using the protein denaturation inhibition assay, in which 0.5 mL of test solution was mixed with 0.45 mL of egg albumin and 0.05 mL of phosphate-buffered saline (pH 6.4), incubated at 37 °C for 20 minutes, and then heated at 70 °C for 5 minutes. The absorbance was recorded at 660 nm, and the percentage inhibition was calculated relative to the control. The antioxidant potential was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, where 1 mL of DPPH solution (0.1 mM in methanol) was mixed with 1 mL of each sample at concentrations of 50, 75, 100, and 125 mg/mL, incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm. [16]Ascorbic acid was used as the positive control in both assays. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with a significance threshold of $p < 0.05$.

3. Results

Anti-Inflammatory Activity:

The anti-inflammatory potential of the test materials was evaluated using the protein denaturation inhibition assay, with the percentage inhibition taken as a measure of activity. The test samples included varying concentrations of MTA, Ceremagnum Plus (C Plus)–$Ca(OH)_2$ combinations, and control groups.



Graph 1

As shown in Graph 1, a concentration-dependent increase in anti-inflammatory activity was observed across all test groups. Among the tested materials, Ceremagnum Plus (C Plus) formulations exhibited a significantly higher inhibition of protein denaturation when compared to MTA alone.

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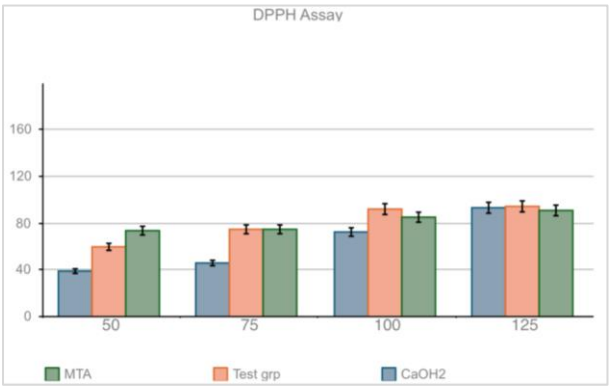
At 50 mg/mL, MTA displayed the lowest percentage inhibition, indicating limited anti-inflammatory effect at this concentration. In contrast, the C Plus–$Ca(OH)_2$ combinations demonstrated markedly enhanced activity, with the C Plus–$Ca(OH)_2$–MTA (100 mg/mL) and C Plus–$Ca(OH)_2$ (125 mg/mL) groups achieving the highest inhibition percentages. The increase in anti-inflammatory activity with increasing concentration suggests that the presence of magnesium and calcium components within Ceremagnum Plus contributes to greater stabilization of protein structures against heat-induced denaturation.

The results therefore establish that Ceremagnum Plus exhibited superior anti-inflammatory activity compared to MTA, indicating a better potential to modulate inflammatory responses associated with pulpal irritation or tissue injury.

Antioxidant Activity:

The antioxidant activity of the materials was assessed using the DPPH free radical scavenging assay, and the percentage inhibition was calculated relative to the ascorbic acid standard.



Graph 2

As represented in Graph 2, Ceremagnum Plus formulations exhibited a pronounced scavenging effect, with higher inhibition percentages compared to MTA across all tested concentrations (50, 75, 100, and 125 mg/mL). The radical scavenging capacity increased proportionally with the concentration of the sample.

At 50 mg/mL, MTA showed minimal scavenging activity, while Ceremagnum Plus–$Ca(OH)_2$ (100 mg/mL) and Ceremagnum Plus–$Ca(OH)_2$–MTA (125 mg/mL) demonstrated the highest DPPH inhibition percentages, approaching that of the Vitamin C positive control.

This trend suggests that the incorporation of magnesium bioactive components in Ceremagnum Plus enhances the ability to neutralize free radicals, thereby potentially protecting pulpal cells from oxidative damage.[14] The higher DPPH scavenging effect indicates that Ceremagnum Plus can effectively mitigate reactive oxygen species (ROS), which play a key role in pulpal inflammation and delayed healing.

4. Discussion

The success of vital pulp therapy depends largely on the ability of the restorative biomaterial to suppress inflammation, mitigate oxidative stress, and create a biologically favorable environment that supports pulpal healing and reparative dentinogenesis. In the present investigation, the indigenously developed magnesium-based bioceramic material, Ceremagnum Plus (C Plus), demonstrated markedly superior anti-inflammatory and antioxidant activities when compared with conventional mineral trioxide aggregate (MTA). These findings were confirmed through protein denaturation inhibition and DPPH radical scavenging assays, which collectively validated the material's potential to modulate inflammatory and oxidative responses at the tissue interface.[16]

Inflammation is a key determinant of pulpal healing outcomes, serving both protective and destructive roles depending on its intensity and duration.[17] In this context, the protein denaturation inhibition assay serves as a reliable in vitro indicator of a material's capacity to prevent inflammatory protein alteration. The present results revealed that Ceremagnum Plus exhibited a significantly higher percentage of protein denaturation inhibition than MTA at all tested concentrations ($p < 0.05$), indicating greater protein stabilization and suggesting an enhanced ability to minimize inflammatory reactions in biological systems. The superior anti-inflammatory activity of Ceremagnum Plus can be attributed to its composition rich in magnesium silicate and calcium hydroxide, both of which promote an alkaline microenvironment that facilitates the sustained release of calcium ions. [18]This ionic environment encourages hydroxyapatite formation and has been shown to stimulate the release of anti-inflammatory cytokines such as TGF-β1 and IL-10,

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while simultaneously downregulating pro-inflammatory mediators including TNF-α, IL-1β, and COX-2, as documented in previous studies on MTA and related calcium silicate materials. [19]Moreover, the presence of magnesium ions (Mg^{2+}) plays a critical regulatory role in inflammation through suppression of the NF-κB signaling pathway, which is central to the transcriptional activation of inflammatory cytokines. This suggests that magnesium incorporation in Ceremagnum Plus contributes significantly to its enhanced anti-inflammatory performance by modulating intracellular signaling pathways that govern inflammation resolution.[20]

In addition to inflammation control, oxidative stress represents another critical factor influencing pulpal cell survival and regenerative potential. Excessive production of reactive oxygen species (ROS) can induce mitochondrial dysfunction, DNA damage, and cellular apoptosis, ultimately compromising healing. [21]The present study demonstrated that Ceremagnum Plus possessed a markedly higher DPPH radical scavenging activity compared with MTA ($p < 0.001$), indicating robust antioxidant potential.[22] The scavenging effect increased proportionally with concentration, and at 125 mg/mL, the inhibition levels approached those of the ascorbic acid standard. The antioxidant behavior of calcium silicate-based materials has been associated with hydroxyl ion release, which helps neutralize radicals and maintain redox balance. [23]The inclusion of magnesium oxide and phosphate phases in Ceremagnum Plus likely augments this effect, as magnesium ions are known to engage in redox buffering reactions that stabilize reactive intermediates. Previous studies have also shown that magnesium-enriched bioactive cements can enhance antioxidant enzyme activity, upregulating superoxide dismutase (SOD) and glutathione peroxidase (GPx), thereby reinforcing the cellular defense against oxidative injury .[24] The combined release of Ca^{2+} and Mg^{2+} ions at a sustained high pH may thus provide additional protection against oxidative stress–induced DNA and membrane damage, preserving mitochondrial integrity and supporting odontoblastic differentiation.

These findings align with and extend earlier reports comparing MTA with newer bioceramics. Since its introduction, MTA has been recognized as the benchmark material for vital pulp therapy due to its sealing ability, biocompatibility, and capacity to induce mineralized tissue formation. However, MTA's drawbacks—including prolonged setting time, handling difficulties, and inconsistent ion release—have driven the development of modified formulations. Previous literature has suggested that although MTA possesses intrinsic bioactivity, its anti-inflammatory and antioxidant properties remain moderate relative to emerging materials.[25] The present results corroborate these findings, demonstrating that magnesium enrichment significantly enhances biological efficacy. The improved performance of Ceremagnum Plus indicates that the synergistic effects of magnesium and calcium silicate phases not only enhance ROS scavenging capacity but also provide better protein stabilization and cytokine modulation compared with conventional MTA.[26]

From a biological and clinical standpoint, the dual anti-inflammatory and antioxidant potential of Ceremagnum Plus holds significant implications for vital pulp therapy. During procedures such as pulp capping or pulpotomy, the material comes into direct contact with vital tissue that is metabolically active and highly responsive to oxidative and inflammatory stimuli. The ability of Ceremagnum Plus to reduce protein denaturation and neutralize free radicals can help establish a microenvironment conducive to tissue regeneration, thereby accelerating odontoblastic differentiation and dentin bridge formation. Furthermore, the reduction of local inflammation may enhance cell adhesion and migration, while the antioxidant function protects essential growth factors and signaling molecules from oxidative degradation, promoting a more controlled and sustained healing response.

The biological mechanisms underlying these effects can be attributed to a combination of physicochemical and ionic processes. Hydration of the magnesium–calcium silicate matrix results in the release of $Ca(OH)_2$ and $Mg(OH)_2$, elevating the local pH and buffering inflammatory acids. [27]Simultaneously, released calcium and magnesium ions influence intracellular signaling cascades, including MAPK and NF-κB pathways, which regulate cytokine production and cellular differentiation. The capacity of these ions to quench reactive oxygen species stabilizes redox homeostasis and protects cellular components from oxidative damage. [28]Additionally, the bioactive surface of the material promotes the deposition of a calcium phosphate layer, which further supports

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biomineralization and contributes to the overall resolution of inflammation and oxidative stress.[29] These interrelated mechanisms explain the superior in vitro outcomes observed with Ceremagnum Plus.[30]

Despite the promising results, certain limitations must be acknowledged. The present investigation utilized in vitro assays, which, although informative, cannot fully replicate the dynamic inflammatory and oxidative conditions within the clinical pulp environment. Future research employing animal pulp exposure models and in vivo oxidative stress biomarkers is essential to validate the long-term biological relevance of these findings. Moreover, further studies exploring the temporal stability, ion release kinetics, and sustainability of the observed effects following clinical application would be valuable for translating this material into practical therapeutic use.

In summary, the results of this study demonstrate that the magnesium-based bioceramic Ceremagnum Plus exhibits significantly higher anti-inflammatory and antioxidant activities than conventional MTA, with effects that are both concentration-dependent and statistically significant ($p < 0.001$). The improved biological response can be attributed to its magnesium-enriched composition, enhanced ionic release, and superior redox modulation. Collectively, these properties position Ceremagnum Plus as a promising next-generation material for vital pulp therapy, capable of providing a biologically favorable environment that supports pulpal healing, minimizes inflammation, and promotes dentin regeneration.

5. Conclusion

Within the limitations of this in vitro study, the findings demonstrate that the indigenously developed magnesium-based bioceramic, Ceremagnum Plus (C Plus), exhibits significantly superior anti-inflammatory and antioxidant properties compared to conventional mineral trioxide aggregate (MTA).

The anti-inflammatory assay revealed that Ceremagnum Plus effectively inhibited protein denaturation in a dose-dependent manner, with inhibition values significantly higher than MTA ($p < 0.05$). Similarly, in the antioxidant (DPPH radical scavenging) assay, Ceremagnum Plus demonstrated enhanced free radical scavenging activity approaching that of the Vitamin C control, confirming its potent redox-modulating potential.

These results suggest that the incorporation of magnesium oxide and calcium silicate phases within Ceremagnum Plus contributes to improved ion release, enhanced protein stabilization, and increased reactive oxygen species (ROS) neutralization. Such properties collectively create a biologically favorable microenvironment that supports reduction of pulpal inflammation, enhancement of cellular defense mechanisms, and promotion of reparative dentinogenesis.

Clinically, these dual biological effects hold significant potential for vital pulp therapy, particularly in cases where inflammation and oxidative stress compromise healing. By offering both anti-inflammatory modulation and antioxidant protection, Ceremagnum Plus may outperform conventional calcium silicate materials like MTA in sustaining pulpal vitality and accelerating dentin bridge formation.

Future investigations should focus on:

1. Long-term in vivo validation of these findings in pulp exposure and regeneration models.
2. Comparative evaluation with other modern bioceramics to establish its clinical superiority.
3. Mechanistic studies on the molecular pathways underlying its biological effects, especially in oxidative stress regulation and cytokine modulation.

In conclusion, Ceremagnum Plus represents a promising next-generation bioactive material that combines anti-inflammatory efficacy and antioxidant potential, thereby offering a more physiologically compatible approach for vital pulp preservation and regenerative endodontic procedures.

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Conflicts of Interest:

There are no conflicts of interest.

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