Effect of Green-Synthesized Rutin-Loaded MgO Nanoparticles-Infused Scaffold on RUNX2 and ALP Expression

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ABSTRACT:

Introduction: Bone regeneration relies on mesenchymal stem cells (MSCs) to migrate, differentiate, and promote tissue repair. Gene expression regulates this process by transforming genetic information into functional proteins. RUNX2 plays a key role in osteoblast differentiation, while ALP is an early marker of bone mineralization. This study examines the effect of a green-synthesized rutin-loaded MgO nanoparticle-infused scaffold on RUNX2 and ALP expression, assessing its potential for bone regeneration. By evaluating temporal gene expression, we aim to determine the scaffold's ability to enhance osteogenic differentiation, supporting its use in bone tissue engineering and periodontal regeneration.

Materials and Methods :The Test scaffold was fabricated using a 1% hyaluronic acid (HA), 1% carrageenan, and 1% gelatin stock solution. The polysaccharides were mixed to form a homogeneous solution, and rutin-loaded magnesium oxide (MgO) nanoparticles were added for the test group. The solution was transferred into six-well plates, followed by the addition of 100 µL of 15% tripolyphosphate (TPP) crosslinking agent. The plates were stored at -20°C for 24 hours, then at -80°C overnight, and finally lyophilized for 24 hours before being stored in dry conditions.Mesenchymal stem cells (MSCs) were seeded onto the ultraviolet (UV)-sterilized scaffolds and cultured in osteogenic media containing Dulbecco's Modified Eagle Medium (DMEM) F12, 10 mM β-glycerophosphate, and 0.05 mM ascorbic acid. Temporal gene expression of Runt-related transcription factor 2 (RUNX2) and alkaline phosphatase (ALP) was analyzed on Days 1, 3, and 5 using quantitative polymerase chain reaction (qPCR) for the Test scaffold(Rutin Loaded Magnesium Nanoparticles) and Control Scaffold (Hydroxyapatite).

Results: The test membrane demonstrated enhanced osteogenic differentiation over time, as evidenced by increased mRNA expression of ALP and RUNX2. ALP expression peaked on Day 5, indicating early bone mineralization, while RUNX2 showed significantly higher expression, suggesting improved osteoblast differentiation. Microscopic analysis revealed healthy mesenchymal stem cells (MSCs) with robust adhesion and proliferation on the scaffold. These findings highlight the test membrane's potential as a promising biomaterial for bone tissue engineering applications.

Conclusion: This study demonstrates that the green-synthesized rutin-loaded MgO nanoparticle-infused scaffold enhances osteogenic differentiation of mesenchymal stem cells (MSCs) by increasing the expression of RUNX2 and ALP. Temporal gene expression analysis showed that the scaffold supports early bone mineralization and improved osteoblast differentiation. The test membrane's positive impact on MSC adhesion and proliferation further supports its potential as an effective biomaterial for bone tissue engineering and periodontal regeneration, offering promising prospects for bone regeneration therapies.

Keywords: Magnesium Nanoparticles, Rutin, Bone regeneration, Scaffold.

INTRODUCTION:

In dentistry, bone loss can occur due to various factors, including periodontal disease, tooth loss, and trauma (1). Periodontal disease, in particular, triggers an immune response that leads to the destruction of the bone supporting the teeth (2). Tooth loss also contributes to bone resorption in the jaw, as the absence of the tooth root eliminates the stimulation necessary to maintain bone volume, resulting in gradual bone reduction. Dental trauma or certain conditions can further exacerbate bone loss, affecting the overall structure and health of the oral cavity (3). Consequently, treatment and preventive measures in dentistry focus on managing and, in some cases, regenerating lost bone to preserve dental function and aesthetics (4).

Marrow stromal cells, also known as mesenchymal stem cells (MSCs), are multipotent adult stem cells found in the bone marrow (5). These cells have the remarkable ability to differentiate into various cell types, including bone cells, cartilage, fat cells, and other connective tissues (6). MSCs play a pivotal role in tissue repair and regeneration, positioning them as essential components in regenerative medicine and tissue engineering (7). Their ability to modulate the immune system and regenerate damaged tissues has spurred significant research across diverse medical fields (8).

"Green synthesis" methods, which utilize environmentally friendly techniques, have led to the development of rutin-doped magnesium oxide (MgO) nanoparticles embedded within scaffolds. These materials show promise in influencing cell behavior, particularly in the context of bone cell differentiation and development (9). Understanding the effects of these materials on biological systems, such as MSCs, offers valuable insights into their potential to promote bone tissue regeneration and repair (10). This research aims to explore how these biomaterials affect MSC behavior, offering exciting prospects for regenerative medicine applications (11).

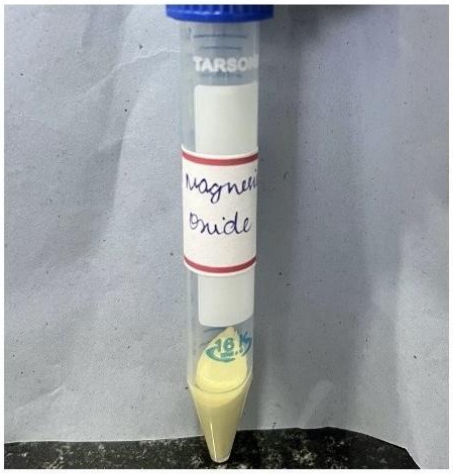
The investigation of a green-synthesized rutin-doped MgO nanoparticle-impregnated scaffold on the expression of RUNX2 (a transcription factor essential for bone formation) and ALP (alkaline phosphatase, crucial for bone mineralization) in MSCs holds significant promise for advancing bone tissue engineering. Understanding how these components influence gene expression and protein synthesis related to bone development in MSCs is crucial for their potential application in regenerative medicine. This study aims to examine the effects of these bioengineered materials on osteogenic differentiation, which could have a profound impact on future therapeutic strategies for bone-related disorders.

MATERIALS AND METHODS:

The test scaffold was prepared by dissolving 1% hyaluronic acid (HA), 1% carrageenan, and 1% gelatin in distilled water to form a homogeneous solution. Rutin-loaded magnesium oxide (MgO)

nanoparticles were incorporated into the solution for the test group, while hydroxyapatite was used for the control scaffold. The solution was transferred into six-well plates, and 100 µL of 15% tripolyphosphate (TPP) was added as a crosslinking agent. The plates were stored at -20°C for 24 hours, frozen at -80°C overnight, and lyophilized for 24 hours as shown in Figure 1a,b,c. The dried scaffolds were stored under dry conditions until further use.Mesenchymal stem cells (MSCs) were seeded onto ultraviolet (UV)-sterilized scaffolds and cultured in osteogenic media composed of Dulbecco's Modified Eagle Medium (DMEM) F12, supplemented with 10 mM β-glycerophosphate and 0.05 mM ascorbic acid. Temporal gene expression of Runt-related transcription factor 2 (RUNX2) and alkaline phosphatase (ALP) was analyzed on Days 1, 3, and 5 using quantitative polymerase chain reaction (qPCR) to assess osteogenic differentiation. Gene expression results for the test scaffold (rutin-loaded MgO nanoparticles) were compared with those of the control scaffold containing hydroxyapatite.

a)



b)

c)

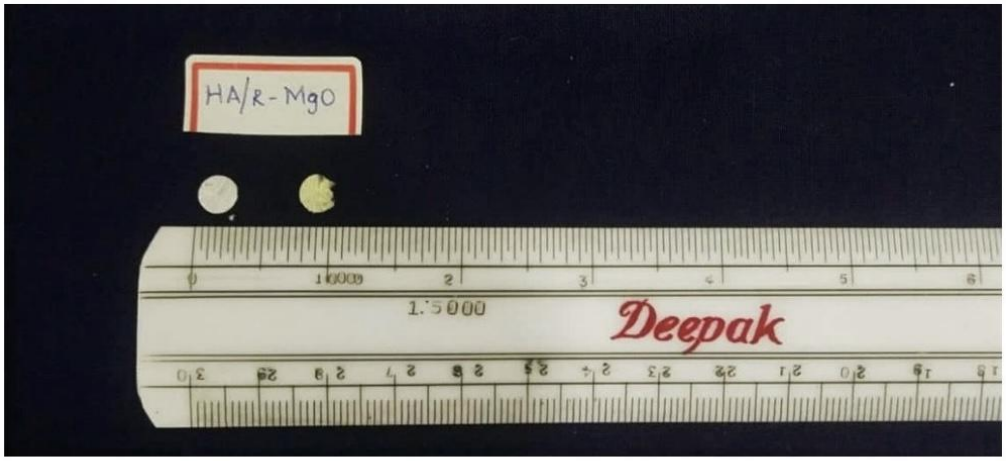


FIGURE 1: a) Magnesium nanoparticles b) Centrifugation of Rutin solution and magnesium nanoparticles c) Scaffold formation

RESULTS AND DISCUSSION:

The bar graph in Figure 2 illustrates the mRNA fold expression of alkaline phosphatase (ALP) over Days 1, 3, and 5 for both the Control (blue) and Test Membrane (orange) groups. On Day 1, ALP expression is relatively low, with no significant difference between the two groups. By Day 3, ALP expression increases in both groups, with the test membrane showing a slightly higher fold expression compared to the control. On Day 5, ALP expression reaches its peak, with the test membrane exhibiting a greater increase than the control. This trend suggests that the test membrane enhances osteogenic differentiation over time, as ALP is an early marker of bone formation. The higher expression observed on Day 5 in the test membrane group indicates its potential to promote bone mineralization, making it a promising candidate for bone regeneration applications.

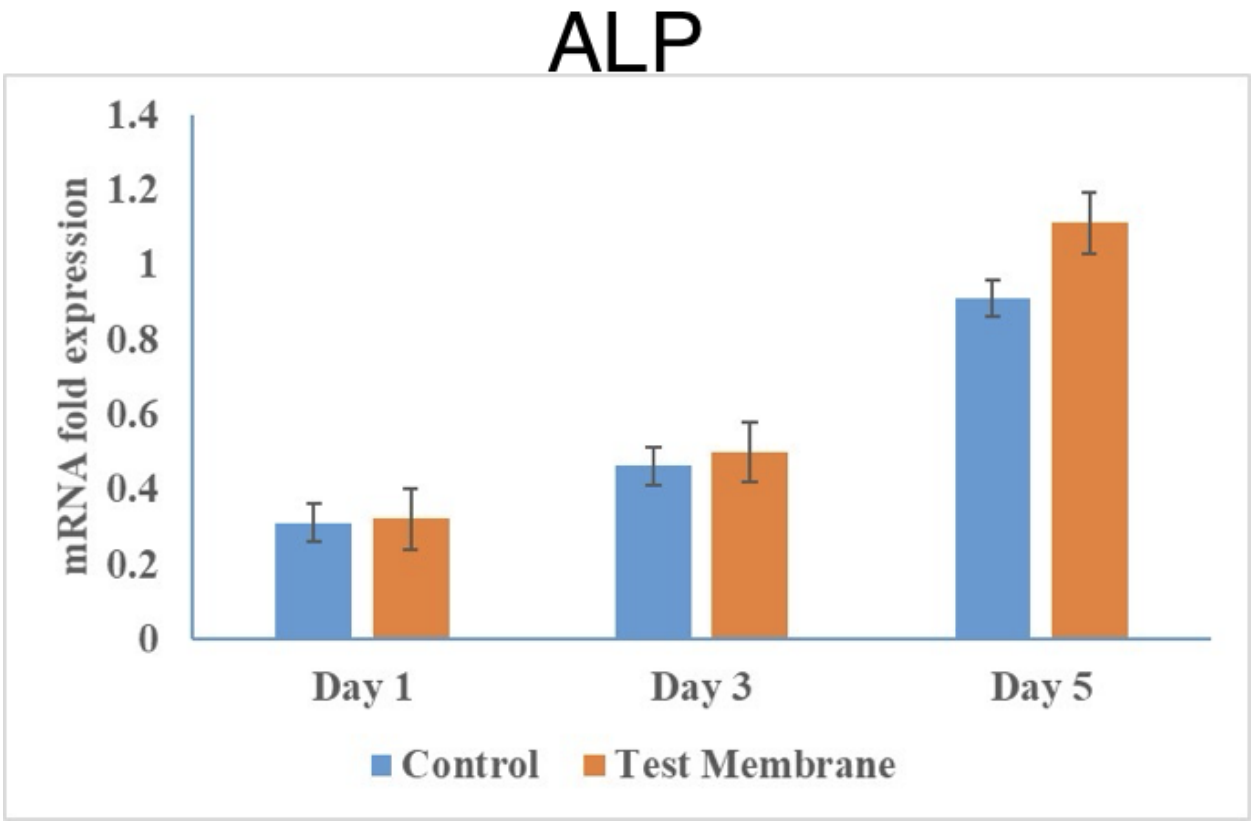


FIGURE 2: Graph showing the ALP activity of Test(Rutin Loaded Magnesium nanoparticles) and Control Membrane (Hydroxyapatite)

The bar graph in Figure 3 depicts the mRNA fold expression of RUNX2 over Days 1, 3, and 5 for both the Control (blue) and Test Membrane (orange) groups. On Day 1, RUNX2 expression is relatively low but slightly higher in the test membrane group compared to the control. By Day 3, expression increases in both groups, with the test membrane showing a greater fold expression, suggesting enhanced osteogenic differentiation. On Day 5, RUNX2 expression peaks, with a significantly higher expression in the test membrane group than in the control. RUNX2 is a key transcription factor regulating early osteoblast differentiation, and its elevated expression in the test membrane group suggests enhanced osteogenic potential and bone regeneration capability. The observed trend indicates that the test membrane effectively promotes osteoblast differentiation over time, making it a promising biomaterial for bone tissue engineering applications.

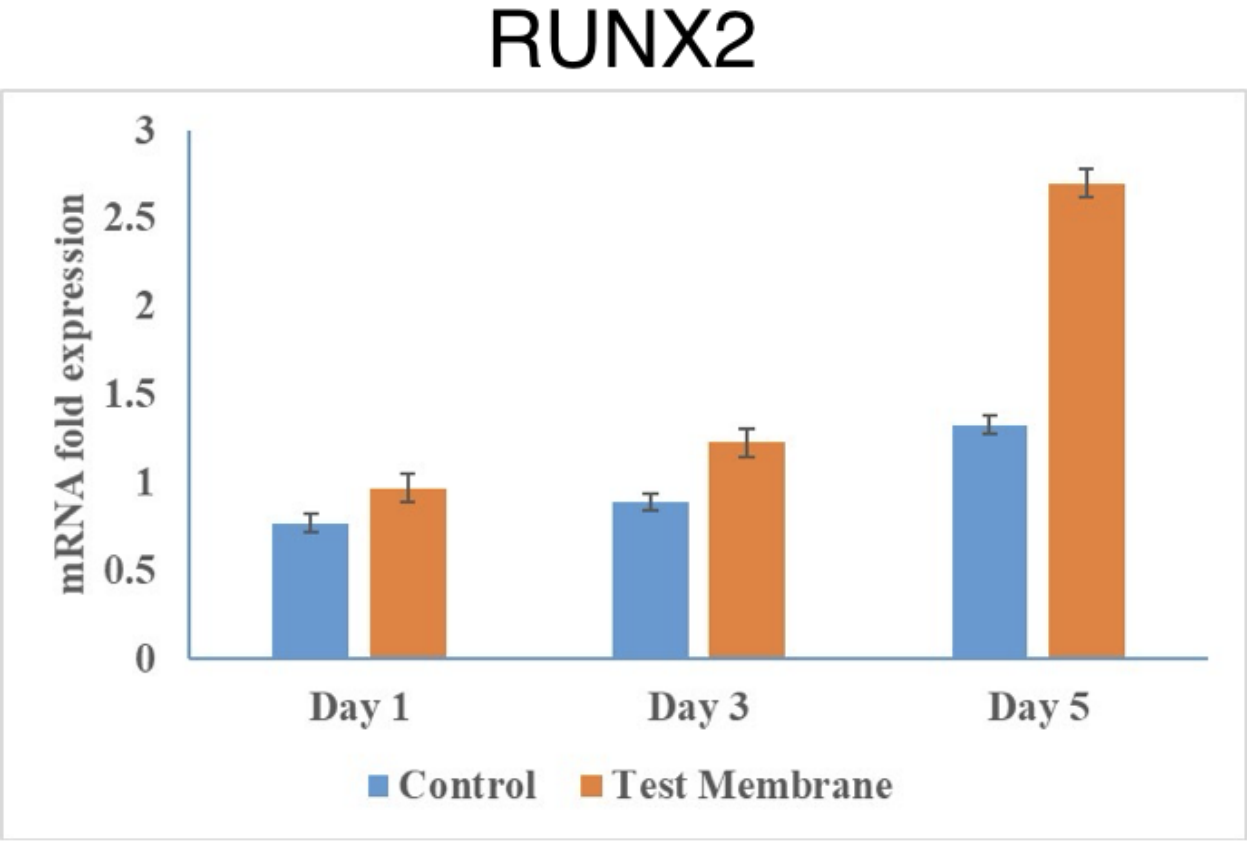


FIGURE 3- Graph showing the RUNX2 activity of Test(Rutin Loaded Magnesium nanoparticles) and Control Membrane (Hydroxyapatite)

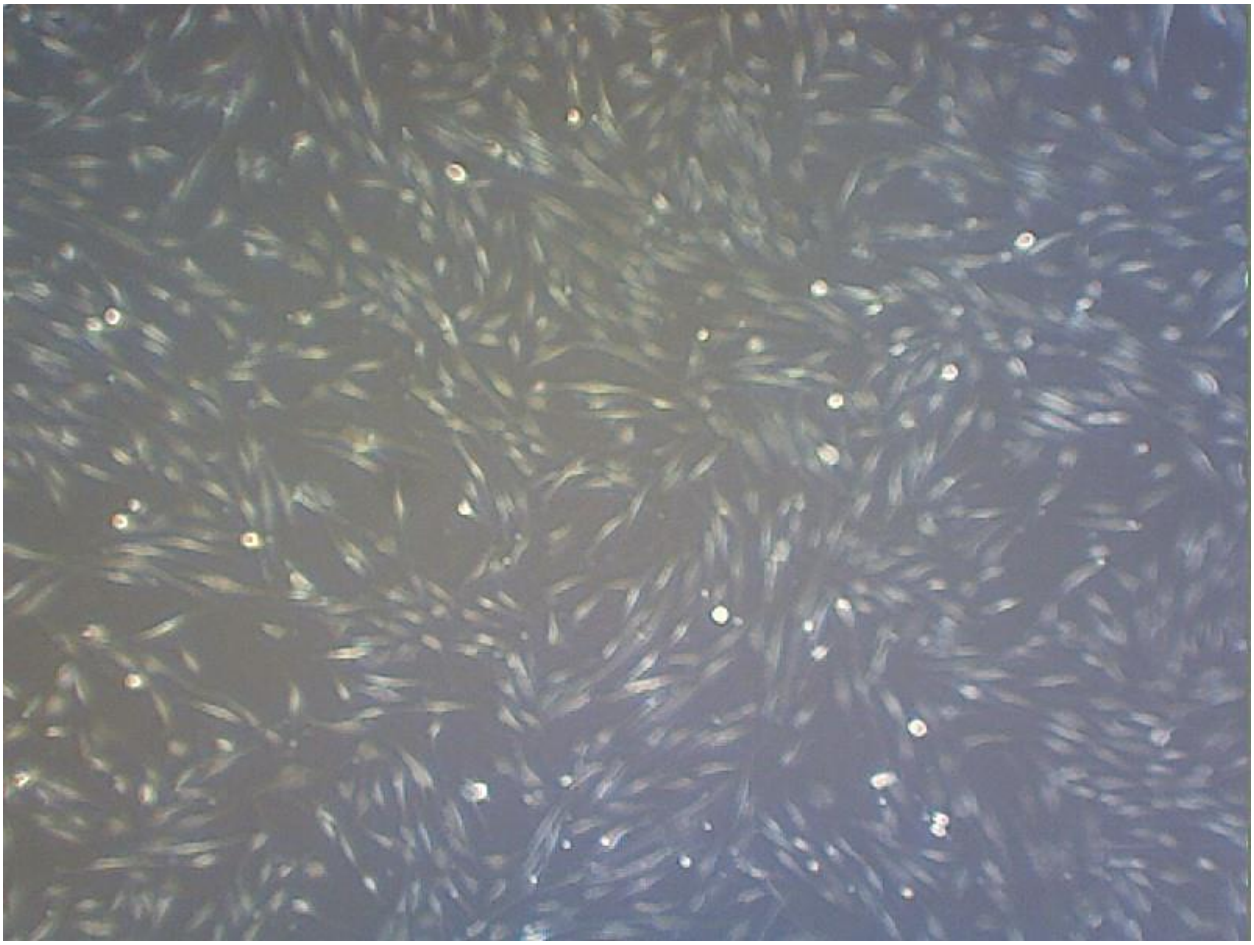


FIGURE 4: Microscopic image of mesenchymal stem cells (MSCs) cultured on the test scaffold at Day 5.

Microscopic analysis at Day 5 as shown in Figure 4 revealed healthy mesenchymal stem cells (MSCs) cultured on the test scaffold, displaying a characteristic spindle-shaped morphology indicative of active cell growth and differentiation. The cells were evenly distributed across the scaffold surface, demonstrating robust adhesion and proliferation. This observation corresponds to the qPCR results, which showed a peak in ALP expression at this time point, suggesting enhanced osteogenic differentiation and early bone mineralization. The cellular morphology and confluency observed further support the scaffold's potential to promote cellular functions essential for bone tissue engineering.

Magnesium oxide (MgO) nanoparticles have attracted significant attention for their biocompatibility, antibacterial properties, and ability to support bone regeneration (13). This study incorporated rutin into MgO nanoparticles to further enhance their bioactivity, especially

for bone tissue engineering (14). The results indicate that rutin could positively influence the osteoinductive properties of MgO nanoparticles. Rutin has demonstrated antioxidant, anti-inflammatory, and potential osteogenic properties, all of which could enhance bone regeneration (15). By loading rutin onto MgO nanoparticles, the scaffold not only provided structural support but also promoted osteoblast differentiation, accelerating the healing process (16). The scaffold's ability to deliver bioactive signals may further stimulate bone regeneration (17), with the controlled release of rutin leading to more sustained and localized effects on bone repair, optimizing healing compared to systems with faster or insufficient release rates (18). The interaction between the scaffold, MgO nanoparticles, and rutin may activate signaling pathways that promote osteogenic differentiation (19). The antioxidant properties of rutin may reduce oxidative stress, which can hinder osteoblast differentiation, while MgO nanoparticles enhance cell adhesion and provide mechanical cues crucial for differentiation (20). These findings align with previous studies suggesting that MgO nanoparticles, combined with bioactive compounds like rutin, create a promising environment for bone tissue engineering.While the results of this study highlight the potential of the rutin-loaded MgO nanoparticle-infused scaffold in promoting osteogenic differentiation, several limitations must be acknowledged. The limitations of the study was that it is in vitro analysis , and the findings may not fully replicate the complex in vivo environment where additional factors, such as immune response and vascularization, could influence scaffold performance. Second, the long-term effects of the scaffold on bone regeneration and its biocompatibility were not assessed beyond Day 5, limiting our understanding of its effectiveness over extended periods. Additionally, the study did not evaluate the mechanical properties or degradation rates of the scaffold, which are critical for its application in clinical settings. Further research, including in vivo studies and long-term assessments, is needed to fully establish the scaffold's potential for bone regeneration therapies.

CONCLUSION

In conclusion, the green-synthesized rutin-loaded MgO nanoparticle-infused scaffold demonstrated significant potential in enhancing bone regeneration. The study showed that the scaffold promoted osteogenic differentiation, as evidenced by the increased expression of RUNX2 and ALP in mesenchymal stem cells (MSCs). ALP expression peaked on Day 5, indicating early bone mineralization, while RUNX2 expression was notably higher, suggesting improved osteoblast differentiation. Microscopic analysis confirmed the healthy adhesion and proliferation of MSCs on the scaffold. These results support the use of the test scaffold as a promising biomaterial for bone tissue engineering and periodontal regeneration, offering an innovative approach to enhancing bone repair and regeneration.

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