Study On Early And Late Gene Expression In Mesenchymal Stem Cells Using HA/ Cuo Based Scaffold In Bone Regeneration

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ABSTRACT :

Introduction

Mesenchymal stem cells (MSCs) are multipotent cells derived from the bone marrow that migrate to injury sites, promote bone regeneration, and modulate immune responses. Gene expression, the process by which genetic information is transformed into functional proteins, is fundamental to tissue regeneration. Current regenerative strategies aim to direct cells with regenerative potential to repopulate lesions, promoting new cementum formation and connective tissue attachment.In periodontal tissue regeneration, the differentiation of mesenchymal cells into cementoblasts is critical for forming new cementum, facilitating the attachment of periodontal ligament fibers to the root and alveolar bone. The present study evaluates the expression of BMP-2 (early gene) and Type X collagen (late gene) on a novel hydroxyapatite (HA) and quercetin-coated CuO nanoparticle (CuO NPs) scaffold to understand its potential for periodontal regeneration. The aim of the study was to assess the temporal expression of BMP-2 and Type X collagen on HA/Quercetin-coated CuO NPs-based scaffolds.

Materials and Methods

The scaffold was fabricated using 1% hyaluronic acid, 0.5% carrageenan, and gelatin (6:1:3 ratio) with 10 mg quercetin-doped SrO nanoparticles. DPSCs were seeded onto UV-treated scaffolds and cultured in osteogenic media (DMEM F12, 10 mM β-glycerophosphate, 0.05 mM ascorbic acid). Temporal gene expression of BMP-2 and Type X collagen was analyzed on Days 1, 3, and 5 using qPCR, with RNA extracted via Trizol and normalized against GAPDH. All experiments were performed in triplicate.

Results and Discussion

Bioinformatic tools provide valuable insights into the complex datasets generated during gene expression analysis. A study by Stephen et al. explored transcriptional regulation by BMP in osteoblasts, identifying over 5,000 genes regulated by Dlx5 and Dlx2 using microarray technology. Similarly, our study aimed to evaluate BMP-2 expression, focusing on its role in early gene regulation and function in regenerative cells.Type X collagen, associated with late-stage chondrocyte differentiation, was also assessed to determine the scaffold's regenerative efficacy. These findings highlight the scaffold’s potential to support cellular differentiation and tissue regeneration.

Conclusion

Advancements in BMP-2 and Type X collagen-based therapies present significant potential for improving regenerative treatments. Ongoing research and translational efforts are essential to refine these approaches, enabling the development of more efficient and reliable strategies for periodontal regeneration. These advancements will contribute to overcoming current therapeutic limitations, offering better outcomes for patients in need of effective regenerative solutions.

KEYWORDS:
BMP 2, Type X collagen, Mesenchymal stem cells, gene expression

Introduction

Periodontal regeneration is a complex biological process aimed at restoring the structure and function of periodontal tissues lost due to diseases such as periodontitis. This involves the regeneration of alveolar bone, cementum, periodontal ligament, and connective tissues. Despite advances in periodontal therapy, achieving predictable and complete regeneration remains a significant clinical challenge. Traditional approaches, such as guided tissue regeneration (GTR) and bone grafting, have shown varying degrees of success but often fail

to completely restore the architecture and function of periodontal tissues.[1] Current regenerative approaches focus on biomaterials, growth factors, and bioengineered scaffolds to enhance the regenerative potential of the periodontal complex. Biomaterials such as hydroxyapatite, bioactive glass, and collagen-based scaffolds provide structural support and serve as templates for cell adhesion and tissue growth[2]. These scaffolds are often functionalized with bioactive molecules, including bone morphogenetic proteins (BMPs), vascular endothelial growth factors (VEGFs), and platelet-derived growth factors (PDGFs), to stimulate cellular recruitment, proliferation, and differentiation at the defect site.

The use of nanotechnology has introduced a new dimension to periodontal regeneration by enabling the development of nanoparticles and nanocomposites with superior mechanical properties, controlled drug release capabilities, and enhanced bioactivity[3]. Nanoparticles such as copper oxide (CuO), titanium dioxide (TiO_2), and zinc oxide (ZnO) are being explored for their antimicrobial, anti-inflammatory, and osteogenic properties, making them promising candidates for periodontal applications[4]. Additionally, surface modifications and coatings on scaffolds have been shown to improve cellular attachment, promote tissue integration, and accelerate healing.Advances in tissue engineering, including 3D bioprinting and the integration of gene therapy, have further expanded the potential for periodontal regeneration. 3D bioprinting allows the precise fabrication of patient-specific scaffolds, while gene therapy techniques such as CRISPR-Cas9 provide opportunities to manipulate cellular pathways and enhance regenerative outcomes[5]. Moreover, the combination of biomaterials with stem cell-based therapies has opened new possibilities for achieving complete and predictable regeneration.

Despite these advancements, several barriers remain, including patient variability, the influence of systemic and local factors, and the long-term stability of regenerated tissues[6]. Further research is needed to optimize scaffold designs, enhance the bioactivity of regenerative materials, and translate these innovative technologies into clinically viable solutions[7].

In this study, we focus on the synthesis and characterization of copper oxide nanoparticles using extracts from *Rauvolfia serpentina*, a medicinal plant known for its phytochemical richness. These nanoparticles are integrated into a novel scaffold to evaluate their potential to promote periodontal regeneration by enhancing the expression of BMP-2 and Type X collagen, key markers in tissue repair and regeneration[8].

MATERIALS AND METHODS:

Fabrication of Scaffold

The scaffolds were fabricated using a stock solution containing 1% hyaluronic acid (HA), 0.5% carrageenan, and gelatin. These materials were blended in a 6:1:3 ratio to form a homogeneous solution. For the test group, 10 mg of quercetin-doped SrO nanoparticles was added to the solution. Following thorough mixing, 3 mL of the resulting homogeneous mixture was transferred into six-well plates. A crosslinking agent, TPP (15%), was added at a volume of 100 µL to each well. The plates were stored at -20°C for 12 hours, followed by -80°C for 24 hours. The lyophilized scaffolds were then stored under dry conditions until further use.

Cell Culture and Preparation

Human dental pulp stem cells (DPSCs) were used in this study, obtained from extracted molars with ethical approval and informed consent from the SIMATS Ethics Committee. The DPSCs were cultured in Dulbecco's Modified Eagle Medium F12 (DMEM F12), supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin. The cells were maintained at 37°C with 5% CO_2 to ensure optimal growth and proliferation. After reaching sufficient confluency, the cells were seeded onto the prepared scaffolds for analysis.

Cell Culture and Osteogenic Differentiation

Human dental pulp stem cells (DPSCs) were isolated from extracted molars, following ethical approval from the SIMATS Ethics Committee. Cells were cultured in DMEM F12 supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin at 37°C with 5% CO_2. For osteogenic differentiation, cells were seeded onto UV-treated scaffolds and cultured in differentiation media containing DMEM F12 supplemented with 10 mM β-glycerophosphate and 0.05 mM ascorbic acid, which promotes osteogenic differentiation of DPSCs.

Detection of Osteogenic Marker Gene Expression

The influence of fabricated scaffolds on early osteogenic gene expression was evaluated using quantitative real-time PCR (qPCR). RNA was extracted from cells cultured on scaffolds at Days 1, 3, and 5 using the Trizol reagent. Isolated RNA was reverse-transcribed into complementary DNA (cDNA) using oligo(dT) primers and reverse transcriptase.

qPCR was performed in a 25-µL reaction mixture containing cDNA, specific primers for BMP-2 and Type X collagen, and SYBR Green Supermix. The fluorescence of SYBR Green, a double-stranded DNA binding dye,

was used to detect specific PCR products. Relative mRNA expression levels were calculated from the threshold cycle (Ct) values and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control using the comparative Ct method (ΔΔCt). All experiments were performed in triplicate and repeated at least three times for accuracy.

Primer Sequences and Reaction Conditions

The primer sequences and annealing temperatures used for real-time PCR were as follows:

- BMP-2:
  - Forward: GGAATGACTGGATTGTGGCT (65°C)
  - Reverse: TGAGTTCTGTCGGGACACAG (65°C)
- Type X Collagen (Col X):
  - Forward: CAGATTTGAGCTATCAGACCAACA (65°C)
  - Reverse: AAATTCAAGAGAGGCTTCACATACG (65°C)

Statistical Analysis

The gene expression data were analyzed for statistical significance using ANOVA, with post hoc tests to compare different time points. Results were reported as mean ± standard deviation, with a p-value < 0.05 considered statistically significant.

RESULT:

For **early gene expression**, there is a significant upregulation from **day 1 to day 5**, suggesting that the gene is actively transcribed soon after the initial stimulus or condition. However, an interesting deviation occurs on **day 3**, where a temporary drop in expression is observed before it resumes its increase. This fluctuation could be due to regulatory mechanisms such as feedback inhibition, transient repression, or cellular adaptation processes that momentarily slow down transcriptional activity before recovery and continued expression.

In contrast, **late gene expression** follows a different trajectory. Unlike early genes, late genes do not exhibit an immediate increase in transcription. Instead, their expression gradually builds up over time in a steady manner. This suggests that late genes may require prior activation of upstream regulatory elements, the accumulation of necessary transcription factors, or the completion of early gene-related processes before their activation. By **day 5**, the late genes reach their peak expression, indicating that their role may be crucial at a later stage of the cellular or molecular response.

This differential gene expression pattern highlights the **temporal regulation of genetic activity**, where early genes respond rapidly to stimuli, potentially initiating key processes, while late genes contribute to sustaining or completing the biological response at a later phase.

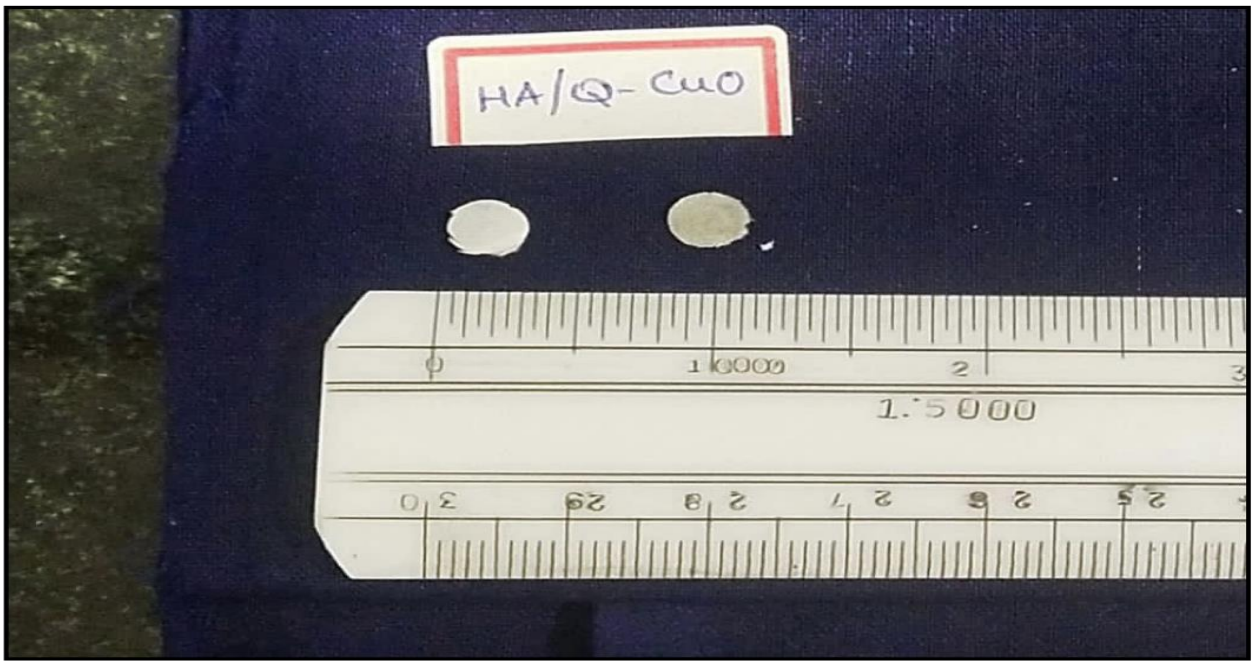

Figure 4: Scaffold measurement

Group - QUERCETIN CONTROL	Day 1 (ng/µL)	Day 3 (ng/µL)	Day 5 (ng/µL)
CuO	15.1	6.5	0.7

Table 2: scaffold measurement growth in micrometer

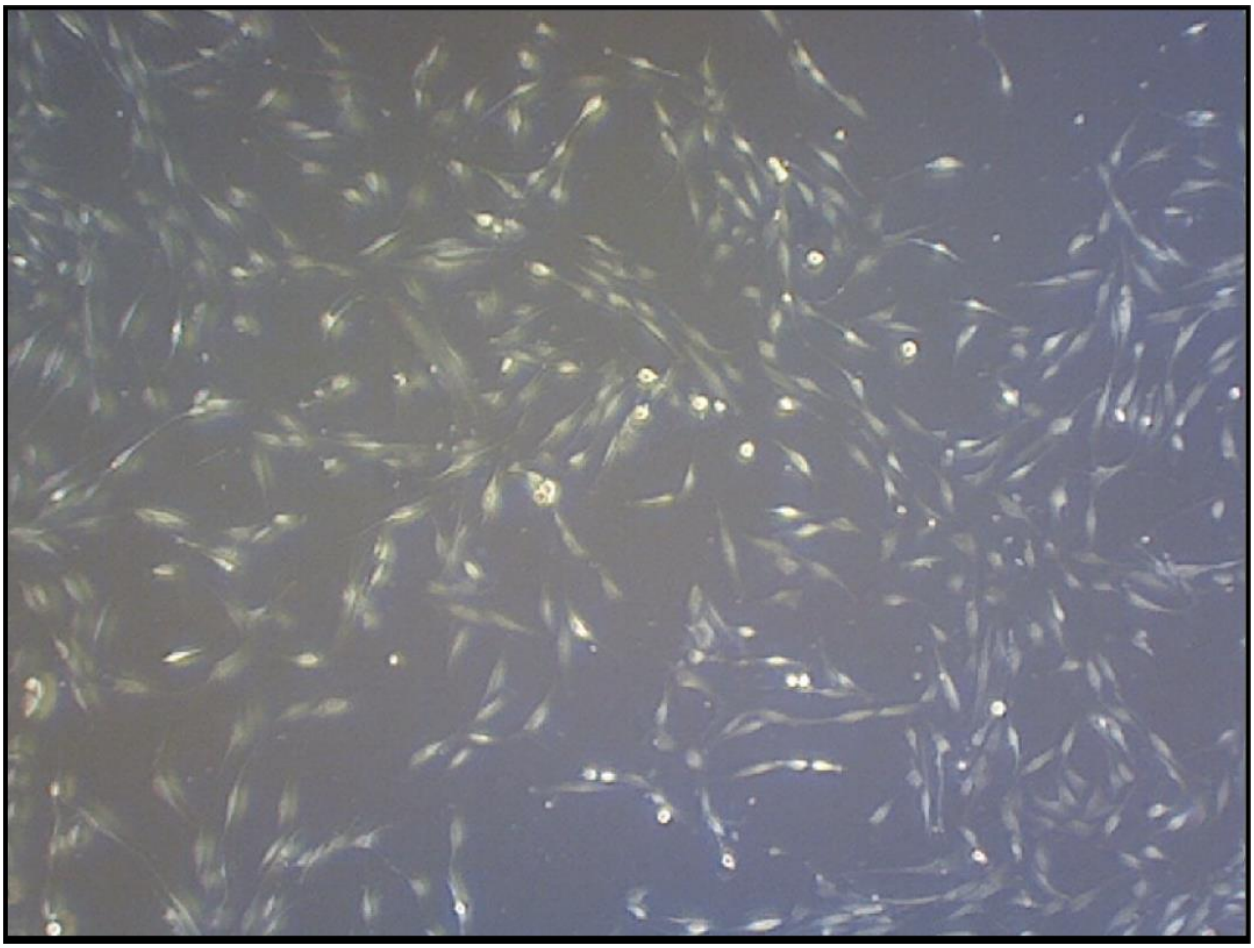

Figure 5: Microscopic picture of mesenchymal stem cells

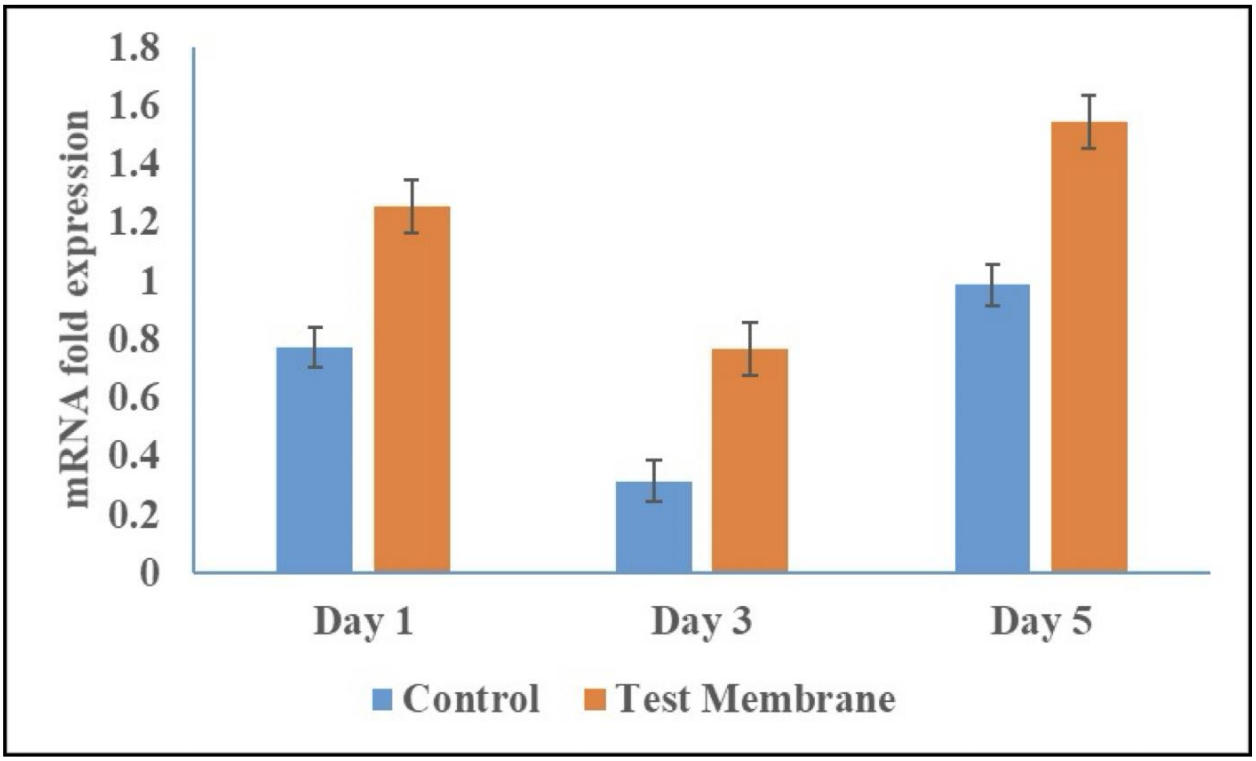

Figure 6: The graph shows the gene expression of early gene - BMP 2

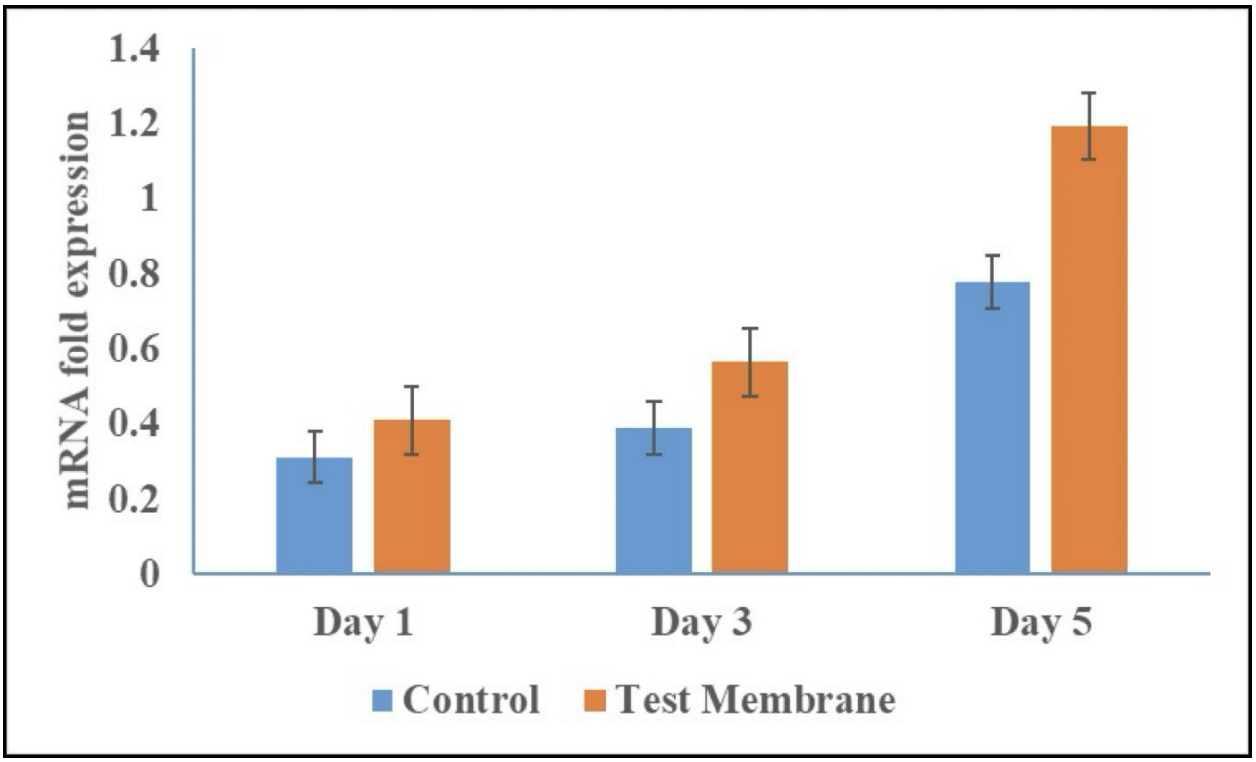

Figure 7: The graph shows the gene expression of late gene - Collagen type X

DISCUSSION:

The results from this study shows that the gene expression of early gene is well significant in day 1 where as the late gene from the same scaffold is not very expressive in the first day, in Day 3 the early gene expression was comparatively lower than the expression in day 1 unlike for late gene where day 3 expression was on a gradual rise, Day 5 both the early and late genes were well expressive and thus promoting nine forming activity[9].

In another study on scaffold in microbial resistant era, These scaffolds not only showed good antibacterial and cytocompatibility results in vitro, but also performed well in an in vivo rabbit model, demonstrating their potential for bone regeneration due to their compatibility, antimicrobial capacity and mechanical properties[10] . The production of porous metal alloys with powerful antimicrobial properties by AM for potential biomedical applications has recently been reported[11]. Like in our current study where This method aligns with sustainable and eco-friendly principles, mitigating the ecological impact associated with traditional chemical synthesis[12].
In a similar study This work first summarizes the skin wound healing process and relates evaluation parameters and then reviews the advanced functions of hydrogel dressings such as antimicrobial property, adhesion and hemostasis, anti-inflammatory and anti-oxidation, substance delivery, self-healing, stimulus response, conductivity, and the recently emerged wound monitoring feature, and the strategies adopted to achieve these functions are all classified and discussed[13]. Furthermore, applications of hydrogel wound dressing for the treatment of different types of wounds such as incisional wound and the excisional wound are summarize , whereas in out current study we use quercetin doped CuO nanoparticles with Gelation for its wound healing properties[14]

RECOMMENDATION / SCOPE OF FUTURE RESEARCH:
This study will help in advancement of regeneration therapies. With improved regulations of desired genes we can make use if it's functional in cell differentiation and improve self regeneration of human tissues.

CONCLUSION:
In conclusion, further advancements in the science and translation of BMP 2 and collagen X - based therapies are required, and this growing body of knowledge will be consistently more successful than current treatments. The green synthesis and characterization of CuO nanoparticles from Rauvolfia serpentina hold promise for the development of sustainable, environmentally benign nanomaterials with multifaceted applications, making this research an exciting frontier in materials science and nanotechnology.

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CONFLICT OF INTEREST:
The authors declare that there were no conflicts of interest in the present study.

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