

BIOSYNTHESIS AND CHARACTERIZATION OF MAGNESIUM OXIDE DOPED WITH POLYINDOLE AND ITS WOUND HEALING PROPERTIES

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ABSTRACT

Magnesium oxide is recognized for its antimicrobial characteristics, stability, and biocompatibility, rendering it a significant material for biomedical applications. MgO was amalgamated with polyindole, a conductive polymer, to create a composite with augmented biological activity. The composite's structural and morphological characteristics were analyzed using FT-IR, XRD, SEM, and EDAX to verify its composition and crystallinity. The Magnesium oxide doped polyindole composite demonstrated significant antibacterial efficacy, essential for infection prevention in wound healing. Furthermore, it stimulated fibroblast proliferation and expedited wound healing, indicating its promise in tissue regeneration. The integration of polyindole enhanced the material's conductivity and its interaction with biological systems, hence enhancing its efficacy. The findings indicate that the Magnesium oxide doped Polyindole composite is a viable material for wound healing and regenerative medicine, providing both antimicrobial protection and improved biocompatibility.

INTRODUCTION

The advancement of environmentally friendly methods for the synthesis of nanoparticles is becoming a significant area within nanotechnology[1,2]. Recently, various groups have successfully synthesized nanoparticles using extracts from unicellular organisms such as bacteria and fungi, as well as from different parts of plants[3-11]. The ongoing application of nanomaterials produced through green synthesis has risen due to their cost efficiency, environmentally friendly characteristics, and speed of production [12]. Metal oxides such as Magnesium Oxide(MgO), Zinc Oxide(ZnO) are extensively utilized in nanomaterial fabrication due to their stability under high temperatures and safety[13].

The synthesis involving chemicals requires intense radiation and various harmful substances as reducing and stabilizing agents, which pose risks to both humans and animals. This eco-friendly synthesis method involves the synthesis of nanoparticles through a single-step, pollution-free process that requires minimal

energy to initiate the reaction, resulting in a shorter preparation time compared to alternative methods. The primary benefit of green synthesis lies in its cost efficiency, utilizing biological species or plants as reducing agents that are abundantly available[14,15].

Amaranthus viridis is a perennial foliage vegetable that is short-lived and is a member of the Amaranthaceae family[16-18]. Amaranthus species are widely cultivated and consumed in Mexico, Central America, the Philippines, China, India, Indonesia, Malaysia, and Southern and Eastern Africa[19-22]. Synthesis of nanocomposites using conjugated polymers with metals had drawn much the attention nowadays due to their thermal and electrical properties[23]. Indole is a well-known heterocyclic compound utilized in pharmacology as a bioactive intermediate, as well as in agriculture and material sciences. It also demonstrates a wide range of biological activities, including antimicrobial, antifungal, and antioxidant effects. Recent reports indicate that bis (5-methoxy 3-Indoxyl) has been utilized in DNA-based biosensors. Polyindole(PIn) is an electroactive

polymer characterized by high thermal stability and notable redox properties. Pln is suitable for multiple applications, including organic electronics [24], electro-catalysis [25], batteries, anticorrosion coatings [26], and sensors [27].

2. MATERIALS AND METHODS

2.1 Materials

Amaranthus vidiris, Sodium Lauryl Sulfate, Indole, Ethanol, Distilled water, Buffer($\text{CH}_3\text{COONa} - \text{CH}_3\text{COOH}$), Iron(III)chloridehexahydrate($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Magnesium Nitrate, Sodium hydroxide

2.2 Preparation of leaf extract

Amaranthus vidiris leaves were carefully cleaned with running tap water, followed by distilled water. The washed leaves were boiled for around 15 minutes and allowed to cool. Following that, the extract is filtered using Whatmann No. 1 filter paper and stored for future use.

2.3 Biosynthesis of MgO nanoparticles

20ml of leaf extract was combined with 0.2M of Magnesium Nitrate, which was added dropwise, followed by 1M of NaOH with steady stirring for around 3 hours at 80°C . With the addition of Magnesium Nitrate, the colour turns to white within a few minutes, indicating the presence of MgO. The solution was then centrifuged, the precipitate was rinsed with distilled water and ethanol to eliminate contaminants, and dried in a hot air oven for a few hours.

2.4 Synthesis of MgO doped Polyindole

1g of MgO nanoparticles was mixed with 5g of Sodium Lauryl Sulphate in thirty millilitres of distilled water and exposed to ultrasonication for fifteen minutes. 0.1M of indole was included into the previously prepared solution and subjected to ultrasonication for about 30 minutes. Add 2.7 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and stir for about 7 hours, thereafter allowing it to equilibrate at the room temperature for 24 hours. It was further filtered with ethanol and distilled water, then dried at normal temperature.

2.5 Invitro wound healing assay

The efficacy of MgO doped Pln (50 $\mu\text{g}/\text{ml}$) in promoting wound healing was assessed through a scratch wound healing assay utilising human dermal fibroblast (HDF) cells. Cells were cultivated in DMEM supplemented with 10% foetal bovine serum (FBS), 1% antibiotic-antimycotic solution, and high glucose. The samples were maintained in a humidified incubator at 37°C with 5% CO_2 . The study employed cells at passage 15, with subculturing performed bi-daily.

A monolayer exhibiting 80-100% confluence was established by seeding HDF cells in 12-well plates at a density of 0.25 million cells per well, followed by a 24-hour incubation period. Upon reaching confluence, a cross-shaped mark was created in each well utilising a sterile 200 μL pipette tip. The pipette tip was maintained in a perpendicular position to the well bottom to ensure uniformity in scratch width. Following the careful removal of detached cells through two rinses with DMEM, the remaining adherent cells were treated with FPP at a concentration of 50 $\mu\text{g}/\text{ml}$. The control wells included untreated cells and a standard control group administered 50 $\mu\text{g}/\text{ml}$ of povidone iodine. The plates were incubated for 48 hours at 37°C with 5% CO_2 to promote wound healing.

Scratch wound images were captured at 0, 24, and 48 hours using an inverted biological microscope (Olympus CKX415F, Japan), with consistent magnification settings maintained throughout the process. ImageJ software quantified the degree of wound closure, employing the following formula to calculate the percentage of wound healing:

$$\text{Wound Healing Score (\%)} = (\text{Initial Area} - \text{Final Area}) / \text{Initial Area} \times 100$$

Three replicates of the experiment were performed, and the results were expressed as mean \pm standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 FT - IR Spectroscopy

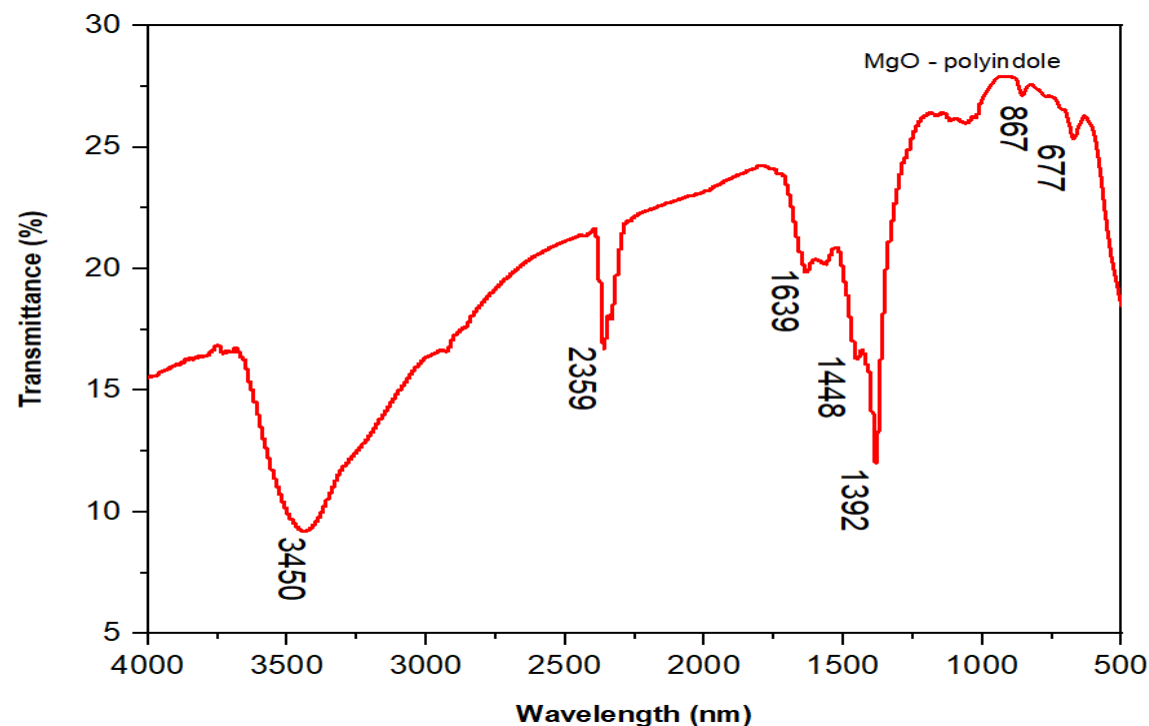


Fig 1. FT-IR spectroscopy of MgO doped Pln

The characteristic vibrational bands obtained from the FT-IR spectrum of MgO-Pln support these results and confirm the occurrence of the Pln and MgO interactions. The strong IR band at 3450 cm^{-1} is due to the N-H stretching vibration. Furthermore, bands at 1448 and 1639 cm^{-1} are due to the stretching mode characteristic of the benzene ring in Pln [28].

Lastly, the broad peak at 665 cm^{-1} is due to Mg-O stretching, demonstrating the fact that MgO is embedded within the polymer matrix. The observed shifts and intensity therefore indicate strong binding interaction between MgO and Pln which could possibly tune the electronic and structural property.

3.2 Scanning Electron Microscope (SEM)

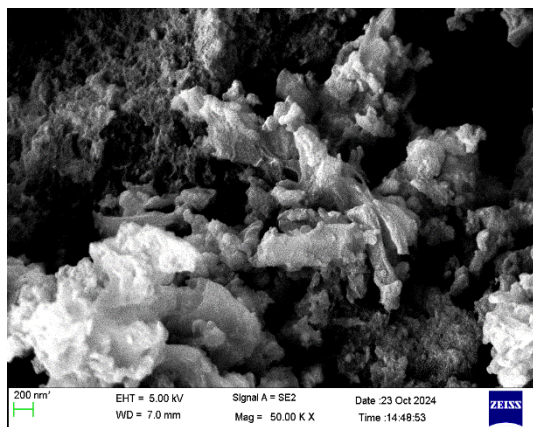
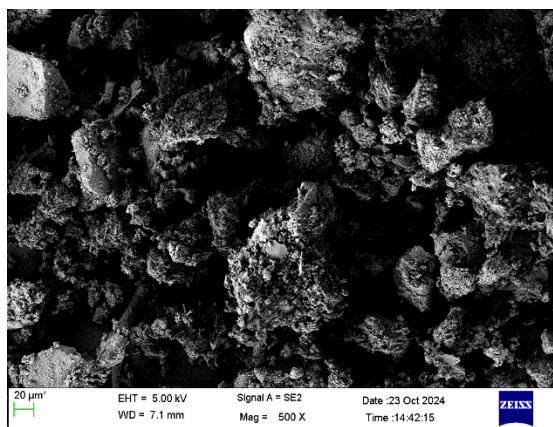


Fig 2. SEM image of MgO doped PIn

The SEM image of MgO doped PIn is shown in Fig.2. The SEM image reveals the surface morphology and aggregation of small particles in the form of clumsy globules with

smooth/rough surface.

3.3 Energy Dispersive X-ray Analysis (EDAX)

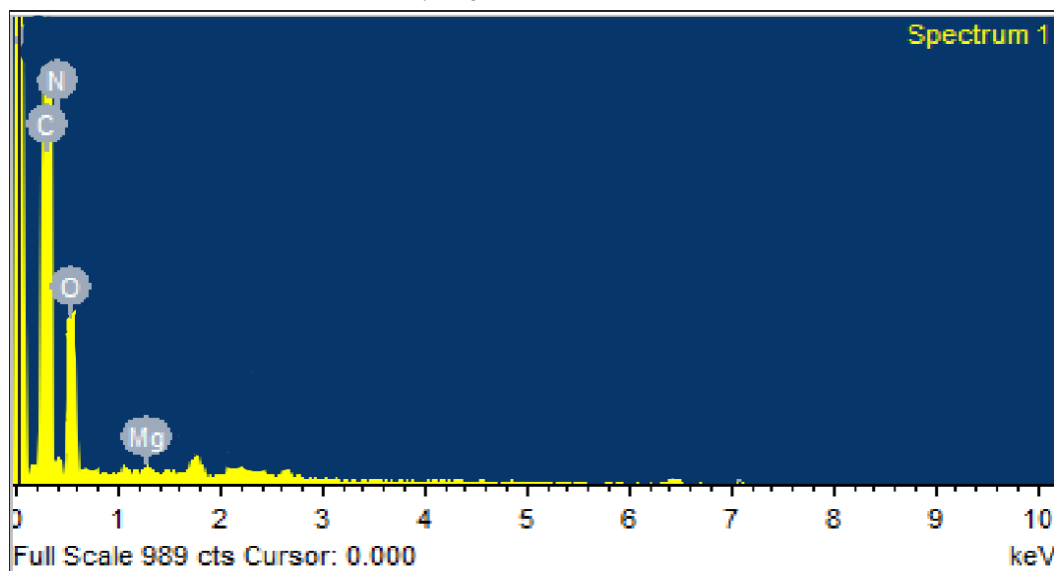


Fig 3. EDAX of MgO doped PIn

Using EDAX Analysis, the MgO doped PIn has a composition that is entirely composed of elements. Based on the information shown in figure 3, the spectrum provides evidence that the sample contains magnesium (Mg), oxygen (O), nitrogen (N), and carbon

(C). The peak energies of Mg are 1.2 KeV, O is 0.6 KeV, while C and N are both 0.3 KeV.

3.4 X-Ray Diffraction Analysis(XRD)

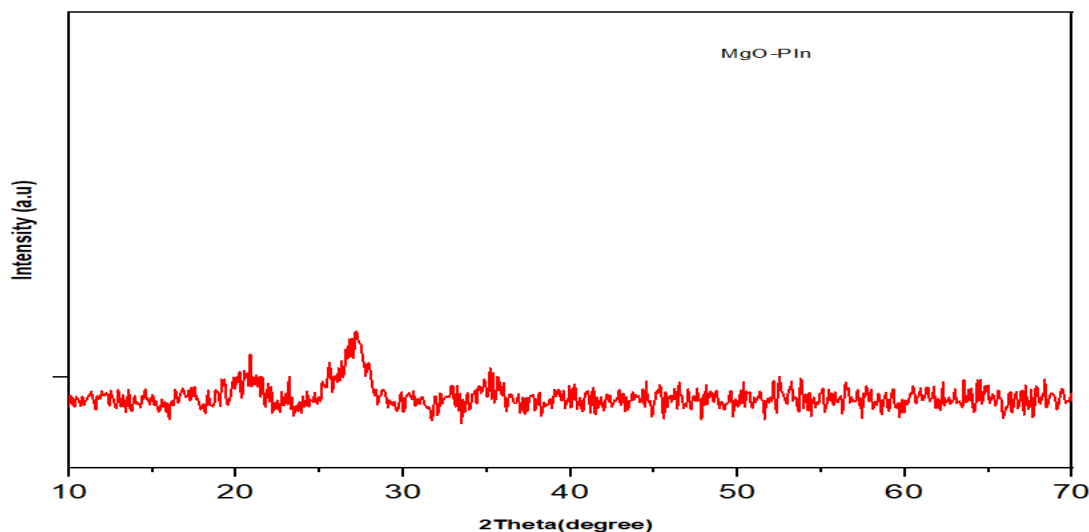


Fig 4. XRD of MgO doped PIn

In Fig 4, the observed peaks at 20.84° confirms the presence of polyindole. The diffractogram reveals that the material that was synthesized is amorphous, as evidenced by the absence of well-defined peaks and the presence of a wide peak that arises in the area of $18-28^\circ$ and the peak at 27.30° and 35.26° confirms the presence of MgO nanoparticles [29,30].

4. APPLICATION

4.1 Wound healing on MgO doped PIn

A scratch wound healing experiment was conducted on human dermal fibroblast (HDF) cells for a duration of 48 hours. The experiment involved the use of MgO doped PIn at a concentration of $50 \mu\text{g/ml}$. According to the formula, the percentage of wound closure was calculated by dividing the initial area by the final area and then multiplying the result by 100.

Following a period of 48 hours, the control group that had not been treated saw a wound healing rate of 61%, indicating the migration and proliferation of fibroblast cells. Within a span of 48 hours, the cells that were subjected to treatment with $50 \mu\text{g/ml}$ of MgO doped PIn shown a significant enhancement in wound closure, reaching a level of 95-100%. This high pro-

migratory and proliferative impact was seen. The normal control, which consisted of Povidone Iodine at a concentration of $50 \mu\text{g/ml}$, exhibited a higher rate of wound healing, although it had a poorer closure rate compared to the MgO doped PIn. In order to demonstrate that MgO doped PIn facilitates fibroblast migration and proliferation, the progressive improvement in wound closure is demonstrated. It is possible that MgO doped PIn might facilitate early wound healing by promoting cellular mobility and extracellular matrix remodelling. This is because of the rapid closure rate that it exhibits within the first twenty-four hours. The fact that it has almost completely recovered after forty-eight hours is evidence that it increases fibroblast activity. The production of extracellular matrix proteins and growth factors by fibroblasts is essential for the regeneration of tissue, which in turn facilitates angiogenesis and the healing of the skin. Because FPP has the potential to stimulate the development of new blood vessels, it is an effective treatment for skin healing. The enhanced wound healing of cells treated with MgO-doped PIn lends credence to this assertion. The results of this study suggest that MgO-doped PIn and HDF cells may be able to facilitate faster wound healing.

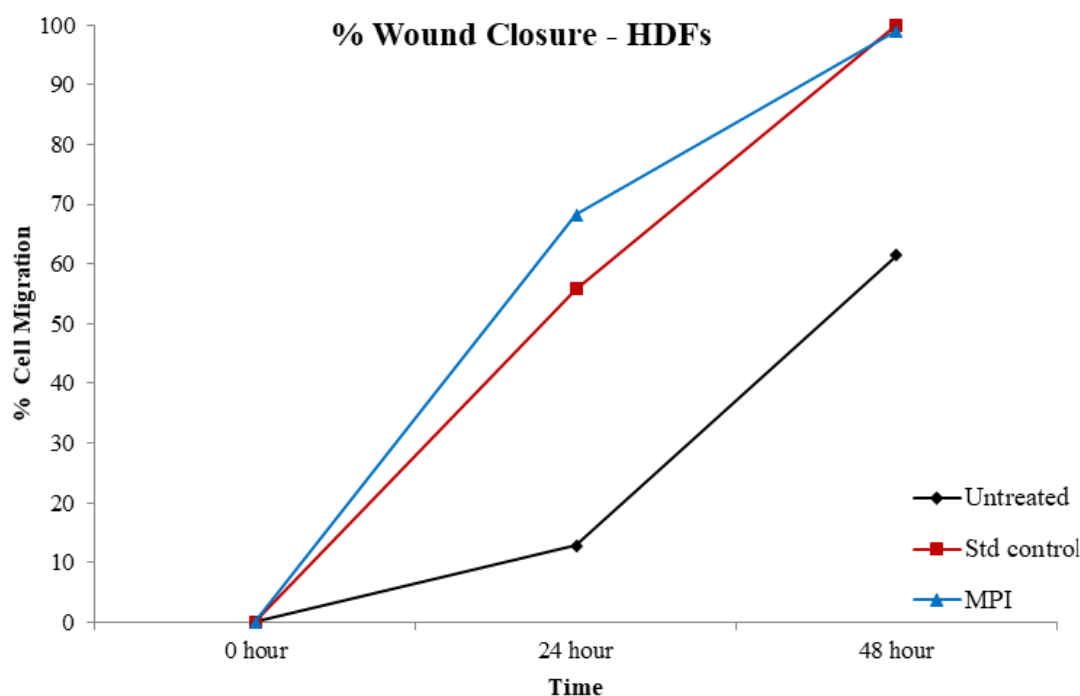


Fig 5. Wound healing graph of MgO doped PIn

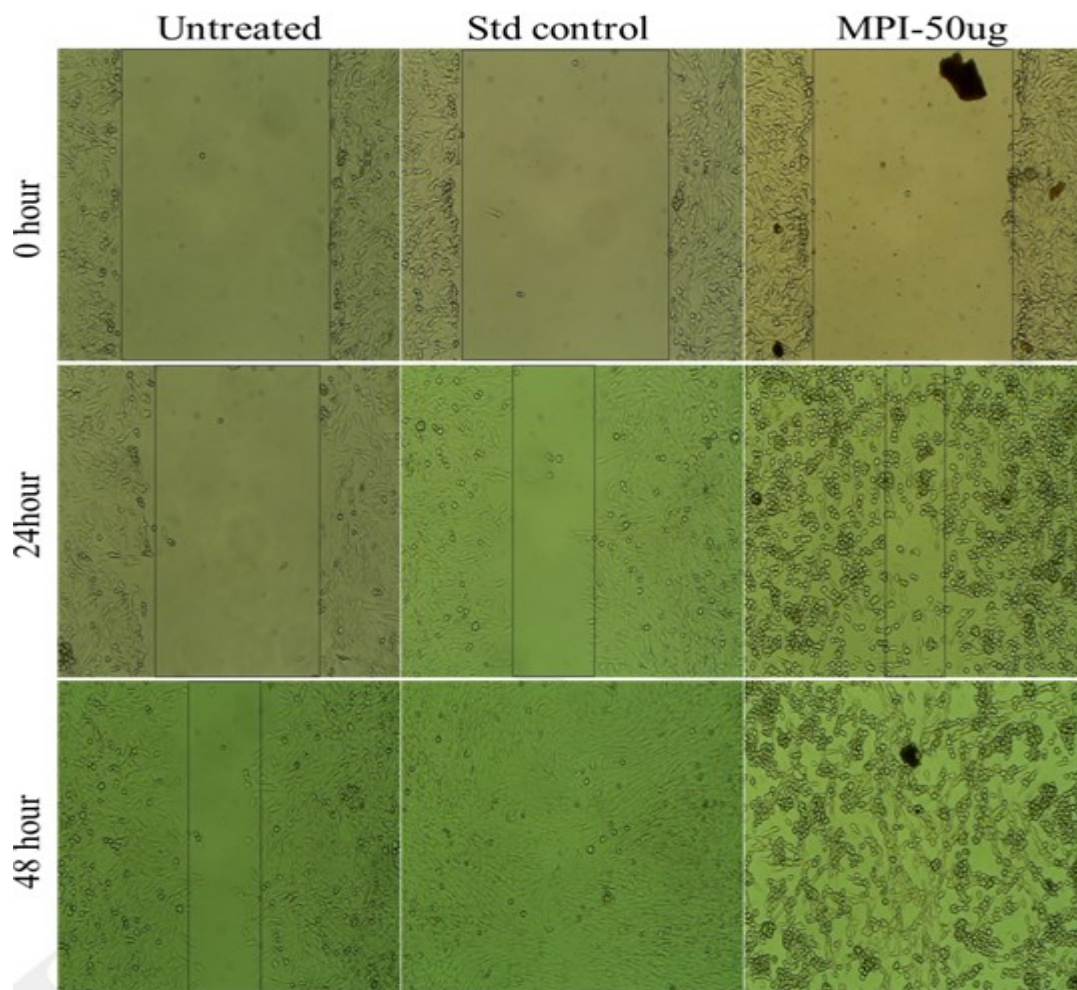


Fig 6. Wound healing image of MgO doped Pln

CONCLUSION

The research effectively synthesized and characterized MgO doped Pln through an environmentally sustainable biosynthesis method. Structural and morphological analyses conducted through FT-IR, XRD, SEM, and EDAX validated the successful formation of the composite. The antibacterial properties of MgO doped Pln underscore its potential for infection prevention, positioning it as a promising material for biomedical applications.

The in vitro wound healing assay indicated that MgO doped Pln markedly improved fibroblast proliferation and wound closure, achieving 95-100% healing within 48 hours, surpassing the control group. The enhanced wound healing capacity indicates that MgO doped Pln facilitates tissue regeneration through the promotion of cell migration.

The study concludes that MgO-Pln is a promising biomaterial for wound healing applications, providing antimicrobial protection and improved biocompatibility. The conductive properties enhance its potential applications in tissue engineering and regenerative medicine. Future research should investigate in vivo studies to confirm its efficacy and safety for clinical applications.

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7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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