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ORIGINAL RESEARCH PAPER

Effect of magnesium oxide nanoparticles and LED irradiation on the viability and differentiation of human stem cells of the apical papilla

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Abstract

Purpose Currently, regenerative endodontic treatments are gaining more and more attention, and stem cells play a significant role in these treatments. In order to enhance stem cell proliferation and differentiation, a variety of methods and materials have been used. The purpose of this study was to determine the effects of magnesium oxide nanoparticles and LED irradiation on the survival and differentiation of human stem cells from apical papilla.

Methods The MTT test was used to measure the cell survival of SCAPs that had been exposed to different concentrations of magnesium oxide nanoparticles after 24 and 48 h, and the concentration with the highest cell survival rate was picked for further studies. The cells were classified into four distinct groups based on their treatment: (1) control, which received no exposure, (2) exposure to magnesium oxide nanoparticles, (3) exposure to light emitting diode (LED) irradiation (635 nm, 200 mW/cm^2) for 30 s, (4) exposure simultaneously with magnesium oxide nanoparticles and LED irradiation. A green approach was employed to synthesize magnesium oxide nanoparticles. Quantitative real time PCR was

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used to measure the gene expression of osteo/odontogenic markers such as BSP, DSPP, ALP and DMP1 in all four groups after treatment, and Alizarin red S staining (ARS) was used to determine the osteogenic differentiation of SCAPs by demonstrating the Matrix mineralization.

Results The highest viability of SCAPs was observed after 24 h in concentration 1 and 10 µg/mL and after 48 h in concentration 1 µg/mL, which were not significantly different from the control group. In both times, the survival of SCAPs decreased with increasing concentration of magnesium oxide nanoparticles (MgONPs). According to the results of Real-time PCR, after 24 and 48 h, the highest differentiation of BSP, DMP1, ALP and DSPP genes was observed in the LED + MgONPs group, followed by MgONPs and then LED, and in all 3 experimental groups, it was significantly higher than control group ($P < 0.05$). Also, after 24 and 48 h, the density of ARS increased in all groups compared to the control group, and the highest density was observed in the MgONPs + LED and MgONPs groups.

Conclusion This research concluded that exposure to SCAPs, MgONPs, and LED irradiation has a significant effect on enhancing gene expression of odontogenic/osteogenic markers and increasing matrix mineralization.

Keywords Mesenchymal Stem Cells · Magnesium Oxide Nanoparticles · Viability · Differentiation · LED

Abbreviations

SCAPs	Stem cells from the apical papilla
MTT	Methyl thiazolyl tetrazolium
ISSCR	International Society for Stem Cells Research
qrt-PCR	Quantitative reverse-transcription polymerase chain reaction
ARS	Alizarin red staining
LLL	Low level laser
LLLI	Low level laser irradiation
LLLT	Low level laser therapy
ALP	Alkaline phosphatase
DSPP	Dentin sialophosphoprotein
DMP1	Dentin matrix protein 1
BSP	Bone sialoprotein
DPSCs	Dental pulp stem cells
hPDL	Human periodontal ligament
hPDLSCs	Human periodontal ligament stem cells
h	Hour
LED	Light-emitting diode
PBS	Phosphate-buffered saline
SPSS	Statistical Package of the Social Sciences
Fig.	Figure
CuONPs	Copper oxide nanoparticles
MgONPs	Magnesium oxide nanoparticle
CuO	Copper oxide
MgO	Magnesium oxide
XRD	X-ray diffraction
FT-IR	Fourier-transform infrared spectroscopy
SEM	Scanning electron microscopy

Introduction

After trauma or dental carries, the pulp of the developing permanent tooth may become damaged, potentially causing inflammation and necrosis of the pulp. The death of odontoblasts and the interruption of root development is caused by pulp necrosis (Albuquerque et al. 2014). The classical technique for treating immature permanent teeth with pulp necrosis is Apexification, wherein calcium hydroxide paste is used to generate apical barrier or mineral trioxide aggregate (MTA) plug is utilized for apical seal (Lin et al. 2017). Despite its success, one of the major issues with Apexification is its inhibition of root development in both the longitudinal and transverse directions, resulting in shortened roots with thin walls that are particularly vulnerable to fracture (Llaquet et al. 2017). In contrast to the traditional treatments that do not provide tissue regeneration, regenerative treatments provide a solution which consists of replacing the pulp-dentin complex, enabling it to proliferate and operate in a physiological manner (Murray et al. 2007). Stem cells, characterized by their capacity to remain in an undifferentiated state and respond to both internal and external signals by undergoing proliferation and differentiation, play a crucial role in various biological processes. Pluripotent cells, a subset of adult (somatic) stem cells with remarkable adaptability, are particularly noteworthy. These pluripotent cells are commonly found in bone marrow and can take the form of hematopoietic, endothelial, and mesenchymal (stromal) stem cells (MSCs) (Karkehabadi et al. 2023a; Padial-Molina

et al. 2015). Beyond bone marrow, research has identified alternative sources of MSCs in adults, such as adipose tissues known as adipose-derived stem cells (ASCs), as well as locations like the lung and specific dental structures, including the perivascular niche of dental pulp and the periodontal ligament within teeth. Recognizing this diverse array of MSC sources highlights the potential for exploring and utilizing their regenerative capabilities in various therapeutic applications (Karkehabadi et al. 2023d; Padial-Molina et al. 2015).

Various stem cell populations have been identified within dental structures, including dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and gingiva-derived mesenchymal stem cells (GMSCs). These mesenchymal stem cells (MSCs) exhibit unique properties that make them integral to the regeneration of various compromised or absent periodontal tissues. In vitro studies have demonstrated that, when exposed to specific stimuli, mesenchymal stem cells derived from dental tissues possess the capability to generate dental tissues such as dentin, cementum, and bone (Kobolak et al. 2016; Liu et al. 2015; Rodríguez-Lozano et al. 2011).

The dental pulp of adult teeth, notably harboring dental pulp stem cells (DPSCs), stands as a pivotal source of oral mesenchymal stem cells with profound implications for regenerative dentistry. These cells have undergone meticulous scrutiny, revealing their extensive in-vitro multilineage differentiation potential spanning osteo/odontogenic, adipogenic, chondrogenic, neurogenic, angiogenic, and myogenic lineages. Complementing these in-vitro observations, in-vivo studies affirm their capacity to regenerate functional dentin/pulp complexes and various tissues, encompassing bone, cementum, blood vessels, and neural tissues(Bakopoulou et al. 2017).

In 1961, Dr. Nygaard Ostby (Nygaard 1961) was the first to propose tissue regeneration, a procedure through which a blood clot is formed in the pulp space to promote the generation of new tissue in the root canal. In addition to blood clot stability, fibrin plays a role as a scaffold for apical papilla stem cells (Llaquet et al. 2017). The scaffold is a necessity as well as certain factors to enable the differentiation of stem cells into odontoblasts (Wan et al. 2016). The primary components in the process of tissue engineering in order to generate a new tissue or organ are mesenchymal stem cells, morphogen growth factor, and scaffold (Gandhi et al. 2011). MSCs demonstrate the ability of self-renewal and multi-lineage differentiation, making them an essential part of tissue engineering (Fonticoli et al. 2022; Shaikh et al. 2023). These cells, which are in the apical papilla of immature permanent teeth, provide a source of primary odontoblasts, which continue the process of root development (Llaquet et al. 2017). The apical papilla is the soft tissue at the apices of developing permanent teeth. It assists in tooth formation and becomes the dental pulp tissue. Thus, Stem cell from the apical papilla can only be isolated at certain stages of tooth development. Sonoyama et al. (Sonoyama et al. 2006) were the first to report the isolation of such cells. Two reliable methods to obtain them exist. The first, consists of the tissue with collagenase type I followed by culture of the obtained cells in culture dishes. The second is based on the culture of small pieces of apical papilla samples on culture dishes, without digestion. Stem cell from the apical papilla are characterized by flow cytometry to identify specific stem cell surface markers. Therapeutic implications of Stem cell from the apical papilla are those related to the repair and the regeneration of different tissues such as pulp, dentin, root, periodontal tissue, bone, neurons, and blood vessels (Costela-Ruiz et al. 2022).

Biomaterials play an important role in regenerative endodontic treatments. Metal ions and compounds can be part of biomaterials. Since in regenerative treatments, cells are exposed to scaffolds and biomaterials, Biocompatibility of materials in regenerative treatments is necessary to maintain the ability of cell proliferation and differentiation (Laurenti & Cauda 2017; Trevino et al. 2011). Biomaterials can be used in the nano scale (< 1–100 nm). Nanoparticles have unique properties due to their small size and high surface-to-volume ratio, but the use of nanoparticles in high concentration can cause cytotoxicity. Therefore, it is imperative to select the accurate concentration (Dolai et al. 2021).

Magnesium is the fourth most abundant cation in the body, with an average concentration of 1% (wt/wt) in dentin. Magnesium has a profound influence on various cellular processes, including the regulation of cell mobility, energy metabolism, cell proliferation, and cell differentiation.

Photobiostimulation therapy employs light sources such as light-emitting diodes (LEDs) and low-level

lasers (LLL) to promote cell growth, tissue regeneration, and healing. These light sources emit wavelengths within the visible red to near-infrared (NIR) range and collectively constitute Photobiomodulation (PBM) (Fekrazad et al. 2016; Khorsandi et al. 2020).

In-depth studies, both in vitro and in vivo, have explored the diverse effects of PBM. These effects span a spectrum from inhibitory to stimulatory, serving purposes such as pain control, anti-inflammatory response, and modulation of metabolic and immunological processes. Notably, PBM actively influences endogenous enzyme photoacceptors, initiating intricate cell signaling pathways(Fekrazad et al. 2016; Pinto et al. 2021). Recent investigations have illuminated the potential of light emitted from specific-wavelength light-emitting diodes (LEDs) to intricately influence the behavior of various cells through physical stimulation. Notably, this form of physical stimulation at distinct wavelengths has been shown to induce apoptosis in cancer cells, exerting precise control over their growth. Moreover, this light-induced stimulation has the fascinating ability to guide stem cells towards specific differentiation pathways (Ahrabi et al. 2019; Cho et al. 2023; Hanna et al. 2021). Extracellular substances traditionally undergo active rather than passive transport for cellular uptake. However, an intriguing phenomenon emerges under light irradiation, where the cell membrane facilitates the translocation of extracellular substances without reliance on active transport mechanisms. This phenomenon is attributed to the heightened sensitivity of the phospholipid bilayer, constituting the primary boundary of the cell membrane, to LED light of specific wavelengths (Muley et al. 2020; Wan et al. 2020). Furthermore, the light-induced modulation of cell membrane behavior plays a pivotal role in expediting the delivery of various substances, including nanoparticles (NPs). The swift absorption and intracellular delivery of substance-loaded nanomaterials stand as critical factors in the precise manipulation of cell behavior(Cho et al. 2023).

Our prior study involved the investigation of effects of CuONPs and LED irradiation on proliferation and osteogenic/odontogenic differentiation of stem cells from the apical papilla (SCAPs) (Karkehabadi et al. 2023b). Following the noticed advantageous effects of the copper nanoparticle, we set out to examine the influence of Magnesium Oxide Nanoparticles (MgONPs) and Light-Emitting Diodes (LEDs) on the proliferation and osteogenic/odontogenic differentiation of Stem Cells from the Apical Papilla (SCAPs). This investigation aimed to fill a gap in existing literature, as this specific interaction had not been previously explored. We hypothesized that a positive effect would be observed, suggesting an enhancing influence of MgONPs and LEDs on the mentioned cellular processes.

Materials and methods

Isolation and culture of SCAPs

Following the Guidelines for Stem Cell Research and Clinical Translation of the International Society for Stem Cells Research (ISSCR), the experiment was performed. The Ethics Committee of Hamadan University of Medical Sciences granted permission for the protocol of this study (IR.UMSHA.REC.1402.660).

To attain the SCAPs, we followed the same method as our preceding study, using the intact upper left and right third permanent molars taken from a healthy 18 year old patient. Over two-thirds of their roots had matured (Karkehabadi et al. 2023c).

The patient provided a written informed consent prior to the incorporation of this research process. A dental tweezer was implemented to differentiate the apical papilla from the apical area of teeth that had not yet fully developed. The SCAPs were obtained by enzyme digestion and cultured in congruence with protocols written previously (Garrido et al. 2021; Karkehabadi et al. 2023c; Rahmati et al. 2022). Collection of the stem cells was accomplished through the use of a solution comprised of 3 mg/mL type I collagenase (Worthlington Biomedical, Lakewood, NJ, USA) and 1 mol/L phosphate-buffered saline (PBS) (Worthlington Biomedical, Lakewood, NJ). Subsequently, the cells were transferred to Dulbecco's modified Eagle's medium (Gibco, GrandIsland, NY, USA) at a temperature of 37 °C for a period of one hour. They were stored at a temperature of 37 °C and a relative humidity of 85%, and a carbon dioxide concentration of 5%. The sterile cell culture flasks (SPL Life Science, Gyeonggi-do, South Korea) were supplemented with a combination of 15% fresh bovine serum and 1% penicillin and streptomycin. Taking into account the design and size of past in vitro

studies, three repetitions were carried out in both control and experimental groups (Bakopoulou et al. 2011; Karkehabadi et al. 2023b; Liang et al. 2017).

Assuring the stemness of cells

When the flask had 85% confluence, the medium was taken away and the cells were given a double rinse with phosphate buffered solution (PBS). The detachment of the cells using trypsin/EDTA was followed by the incorporation of the medium culture into the flask. Fifteen milliliters of culture medium and cells were put into a Falcon tube and centrifuged at 1200×*g* for six minutes. After two rinses with PBS, the cell sediment was put through flow cytometry to detect specific stem cell surface markers (CD105 and CD90) and hematopoietic cell surface markers (CD45 and CD34). The cells displayed a favorable outcome for mesenchymal cell surface markers, whereas the hematopoietic cell surface markers were absent.

Experimental groups

The SCAPs were divided into four distinct groups based on the type of treatment they received: (I) no-treatment control, (II) exposure to MgONPs, (III) LED irradiation (635 nm, 200 mW/cm2) for 30 s, and (IV) a combination of MgONPs and LED irradiation.

Preparation of MgONPs

By utilizing the extract from pomegranate peels, a green method was embraced to synthesize MgONPs in compliance with the prior protocol (Karkehabadi et al. 2023b; Sankar et al. 2014; Suresh et al. 2014). An analysis of the properties of MgONPs was conducted using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM).

The process of synthesizing magnesium oxide nanoparticles required the systematic washing of pomegranate peels using deionized water, followed by shadow drying for a period of 14 days. Following the washing of 100 mg of pomegranate peels with double distilled water, the peels were then cut and subjected to a hot air drying process in an oven. Subsequent to the drying process being completed, the materials were ground into a fine dust and mixed with distilled water (10 g dust/100 mL distilled water) in an Erlenmeyer flask, then boiled for 10 min at 60 °C. The filtration was carried out utilizing Whatman filter paper No.1. The filtrate was freeze-dried for preservation and kept at 4 degrees Celsius.

A magnetic stirrer was used at 60 °C to combine 90 mL of an analytical grade solution of cupric sulphate (5 mM) with 20 mL of filtrate obtained in a magnetic stirrer with deionized water. The mixture was kept at ambient temperature while stored. A brownish-black sediment was produced in the conical flask over time. Following that process, it was desiccated and preserved for the goal of utilizing it as a green synthesized MgONPs.

A Perkin 118 was employed to carry out Fourier transform infrared spectroscopy (FT-IR) to ascertain the surface chemistry of NPs. Chattopadhyay et al. (Chattopadhyay et al. 2015)conducted an analysis of the hydrodynamic sizes and zeta potential distribution of nanoparticles with the use of the Zetasizer-Nano ZS (Malvern, Malvern Hills, U.K.).

X-Ray diffraction (XRD) study of green synthesized MgONPs

An X-ray powder diffraction study of NPs was performed in a solid state. The diffractometer XPERT-PRO was used to figure out diffraction patterns (PANalytical Ltd., The Netherlands) according to the Das et al. method (Das et al. 2017).

The surface morphology and particle size of magnesium oxide nanoparticles were established with the help of high resolution scanning electron microscopy (Hitachi S-3400N).

Methyl thiazolyl tetrazolium (MTT) assay

To evaluate the impact of MgONPs on cell viability and decide on the appropriate concentration, $1*10^4$ cells were incubated in each well of a 96-well plate (n = 12). The plates were placed in the incubator (Binder, NY, USA)at a temperature of 37 °C and humidity of 96% for a period of 24 h. The cells were randomly allocated to six groups and 100 µL of the culture medium with MgO concentrations of 1, 10, 100, 200 and 500 µg/mL was added to each well in a sterile condition.

The plates were placed in an incubator for 24 and 48 h. Following the removal of the plates from the incubator, 10 mL of MTT solution and 90 mL

of alpha-MEM culture medium containing 10% fetal bovine serum were added to each well, and the plates were incubated again at an environment of 37 °C, 95% humidity and 5% CO2 for a period of 4 h. The top layer was then cautiously eliminated, and 100 mL of dimethyl sulfoxide (Gibco BRL, Grand Island, NY, USA) were added to each well. Following the breaking down of formazan crystals, the optical density values were studied using an ELISA Reader (BioTek, USA) in a wavelength scope of 540–690 nm (Saberi et al. 2016).

LED irradiation

The methyl thiazolyl tetrazolium (MTT) assay was used to first ascertain the most effective concentration of MgONPs. This concentration of MgONPs was added to the cultured cells before LED irradiation, 30 min in advance. Following this, the cells were irradiated with LED light in a semi-dark atmosphere with a single gap between the wells. The Fotosan 630 LED (Fotosan 630, MDD, CMS Dental Denmark) was used, with a 1 mm^2 diameter end tip, emitting light of a wavelength range between 620–640 nm (85%) with a peak at 630 nm, having an intensity of 200 mW/cm^2 and energy density (fluence) of 4 mJ/cm^2, irradiated for a period of 30 s. The expected energy level, as well as the formula (energy density = power density × irradiation time), is detailed according to the research from prior studies (Beolchi et al. 2015). The initial irradiation was conducted 24 h after the primary cell culture had been established, while a second irradiation was administered 48 h later. The same operator managed all irradiations.

Assessment of osteogenic/odontogenic differentiation by real-time polymerase chain reaction (PCR)

An assessment of osteogenic/odontogenic differentiation was conducted in each group after 24 and 48 h, with and without LED irradiation. At each time interval, six wells were allocated for evaluation to each group (Bakopoulou et al. 2016).

Trizol reagent (Invitrogen, CA, USA) was employed to extract RNA from the stem cells found in the medium. After that, cDNA synthesis was done by using the Superscript II first-strand cDNA synthesis kit (Invitrogen, CA, USA) in line with the manufacturer's instruction. Following that, a reverse transcription PCR experiment was conducted (7500 Fast Real-Time PCR System for dentin sialophosphoprotein (DSPP), bone sialoprotein (BSP), alkaline phosphatase (ALP), and dentin matrix acidic phosphoprotein 1 (DMP1).

Alizarin red staining (ARS)

In order to analyze the odontogenic/osteogenic differentiation of cells qualitatively, the cells were seeded in 96-well plates after being subject to MgONPs and LED irradiation (n = 24). Subsequently, they were cultivated in an osteogenic/odontogenic environment with 10 mM beta glycerophosphate (Sigma Aldrich, St. Louis, MO, USA), 10 nm dexamethasone (Sigma Aldrich, St. Louis, MO, USA), and 50 mg/mL L-ascorbic acid. The cells were stabilized with 4% paraformaldehyde. After a period of 21 days, they were rinsed with phosphate buffered saline (PBS) and subjected to 1% alizarin red stain (Sigma Aldrich, St. Louis, MO, USA) at 37 °C for 30 min to identify the formed mineral nodules. An assessment was carried out via the SpectraMax M2 spectrophotometer at the 562 nm wavelength (Pereira et al. 2012).

Statistical analysis

Analysis of data was carried out using SPSS version 18 (SPSS Inc., Chicago, IL). The Kruskal–Wallis test and Mann–Whitney U post hoc test were implemented to analyze the data. For alizarin red staining, the data were interpreted as present/absent, and the chi-square test was used for analysis. The statistical significance was accepted at 0.05.

Results

Characterization of MgONPs

Figure 1 shows the XRD pattern of MgO nanoparticles synthesized from magnesium nitrate (II) trihydrate and pomegranate extract. The formation of synthesized MgO nanoparticle was confirmed by X-ray diffraction measurement. The diffraction peaks at 31.22°, 35.87°, 38.92°, 49.42°, 53.87°, 58.42°, 62.02°, 66.820°, 68.37° and 73.07° in Fig. 1 correspond to (100), (002), (200), (202), (020), (202),

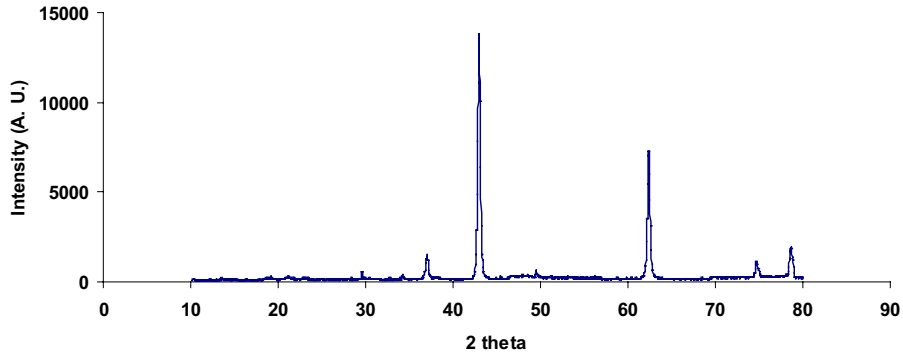

Fig. 1 Characterization of nanoparticles. A: XRD pattern of MgO nanoparticles, the XRD pattern of MgONPs synthesized from magnesium nitrate (II) trihydrate and pomegranate peel extract. (The formation of synthesized MgO nanoparticle was confirmed by X-ray diffraction measurement. The diffraction peaks at 31.22°, 35.87°, 38.92°, 49.42°, 53.87°, 58.42°, 62.02°, 66.820°, 68.37° and 73.07° in Fig. 1 correspond to (100), (002), (200), (202), (020), (202), (113), (022), (020), (311), and (004), respectively)

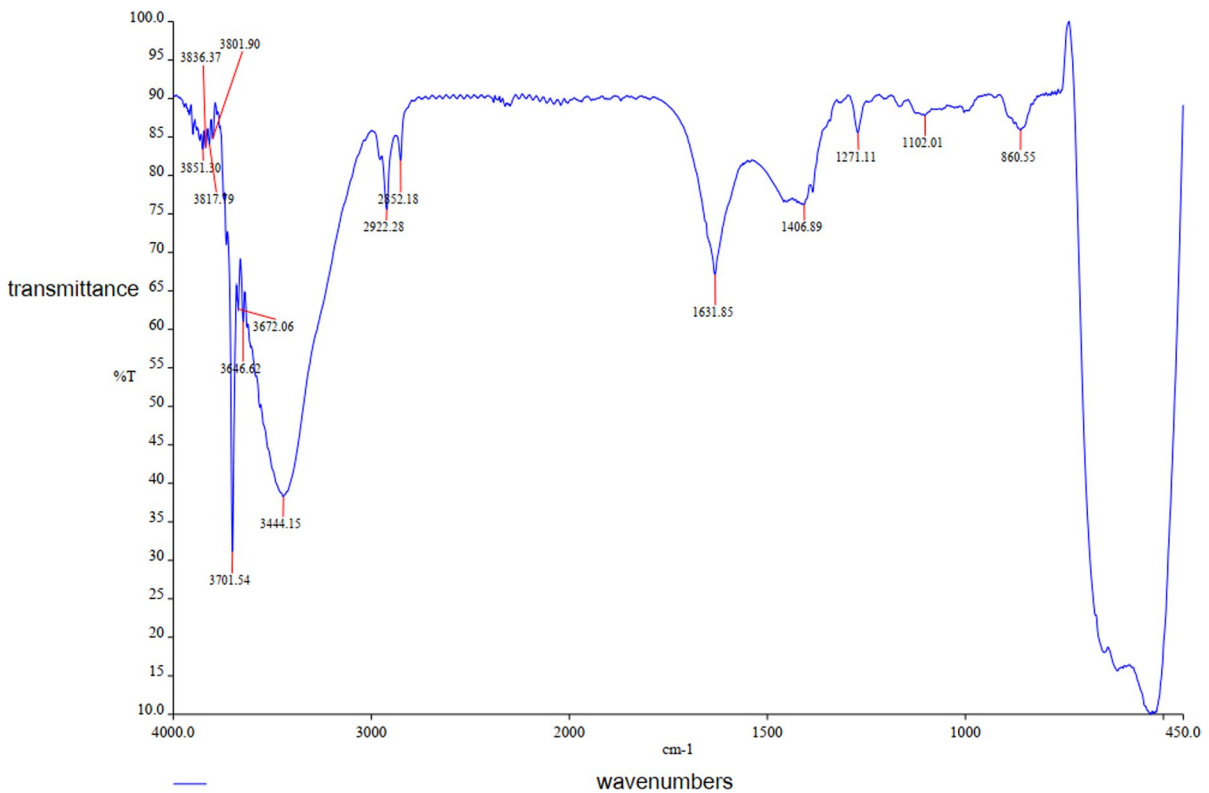

Fig. 2 Characterization of nanoparticles. FT-IR spectra of MgO nanoparticles, The FTIR spectra for the synthesized MgONPs. According to the FTIR spectra of MgONPs, the most significant absorbance peak was noted at 524 cm^{-1} is related to the stretching vibration of the Mg-O bond

(113), (022), (020), (311), and (004), respectively (30–32).

Figure 2 shows the FTIR spectrum of synthesized magnesium oxide nanoparticles. Based on the FTIR spectrum of nanoparticles, the most significant absorption peak in the region at 524 cm^{-1} is related to the stretching vibration of the Mg-O bond (20). Strong absorptions at 1041 cm^{-1} can be related to stretching vibrations related to C-O in the structure of the carboxylic group and flavonoids in the structure of the plant extract. The characteristic peak observed at 1382 cm^{-1} is due to C-N stretching vibration in the amine group [34]. The strong absorption band in the region of 1623 cm^{-1} is due to the bending vibration of the C=C bond. Absorption at 2917 cm^{-1} is also related to asymmetric and symmetric C-H stretching caused by phenolic compounds. Also, the broad band observed at 3402 cm^{-1} is due to the stretching vibrations of the hydroxyl group O–H (21).

The surface morphology of the synthesized MgO nanoparticles was studied by SEM and the results are presented in Fig. 3. The SEM image reveals that the synthesized nanoparticles exhibit quasi-spherical shapes with some degree of aggregation. The isolated cells had fibroblast-like morphology (spindle-shaped) with homogeneity (Fig. 4). Flow cytometric analysis using antibodies against CD45, CD34, CD90, and CD105 showed stromal surface markers on the cytoplasmic membrane of these cells. The results of flow cytometry showed the expression of a high proportion of the mentioned cell markers (Fig. 5).

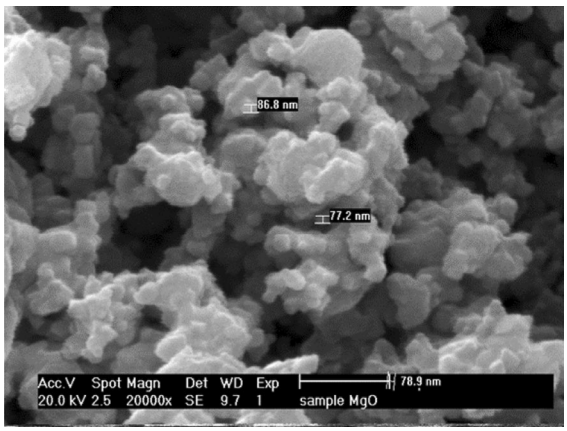

Fig. 3 Characterization of nanoparticles. SEM of MgO nanoparticles, The surface morphology of the synthesized CuONPs was inspected under the scanning electron microscopy (SEM). The synthesized nanoparticles exhibit quasi-spherical shapes with some degree of aggregation

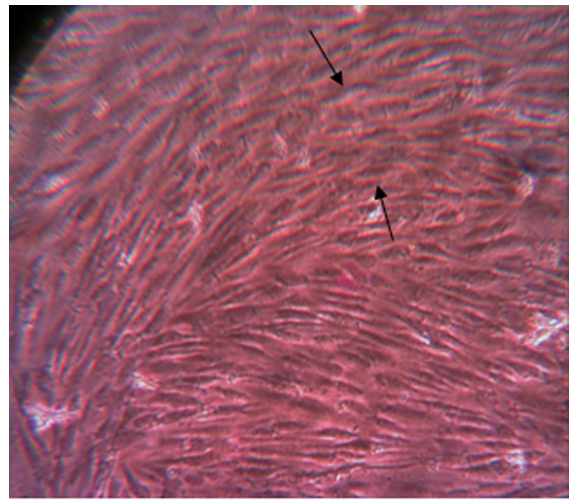

Fig. 4 The isolated cells had a fibroblast-like morphology (spindle-shaped) with optimal homogeneity, the fibroblast like cells were indicated with arrows (scale × 20)

In vitro assessment of cell viability

Results from Fig. 6 indicated that an elevated concentration of magnesium oxide nanoparticles led to a notable decrease in the survival rate of apical papilla stem cells, displaying a significant difference compared to the control group at both the 24 and 48-h intervals ($p < 0.05$). Tukey's test and a two-by-two comparison revealed that, after 24 h, concentrations of 1 and 10 µg/mL, and after 48 h at a concentration of 1 µg/mL, stem cell survival was comparable to the control. However, at higher concentrations, the survival of stem cells was significantly lower than that of the control group ($p < 0.05$).Therefore, in the continuation of the study, we used a concentration of 1 µg/mL of MgONPs to investigate the osteo/odontogenic differentiation.

Quantitative real-time PCR

According to the results of Fig. 7, after 24 and 48 h, the highest expression of all genes was observed in the LED + MgONPs group, then in the MgONPs group, and finally in the LED group ($P < 0.05$). The control group had the lowest expression of genes.

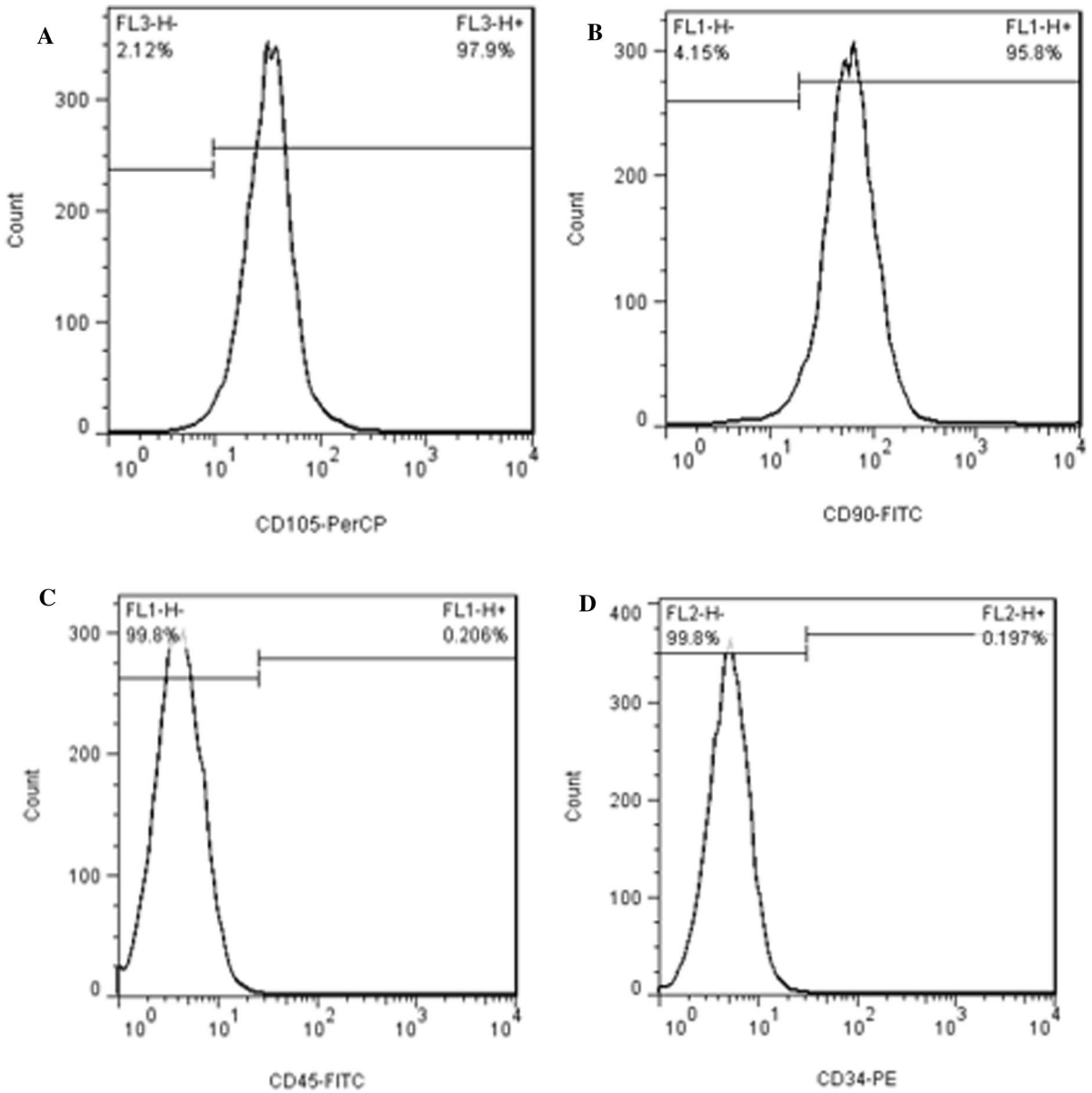

Fig. 5 Flow cytometric results indicated a relatively high expression of the mentioned surface markers (**A**) Expression of CD105-PerCP marker, (**B**) CD90-FITC marker, (**C**) CD45-PE marker, (**D**) CD34-FTIC

Alizarin red S staining

ARS indicates calcium deposition in the extracellular matrix. According to Fig. 8, after 24 and 48 h, the density of ARS increased in all experimental groups compared to the control group, and LED + MgONPs and MgONPs groups showed the highest density. In other words, exposure to MgONPs and LED irradiation increased bone mineralization.

Discussion

In this study, we investigated the effect of magnesium oxide nanoparticles and LED irradiation on survival

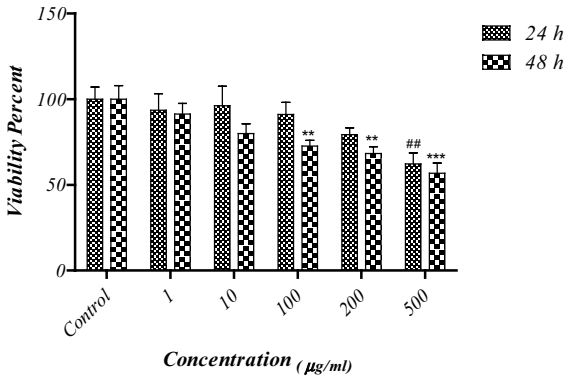

Fig. 6 Cell viability of the groups at 24 and 48 h. ***and ###: P < 0.001.** and ##: P < 0.01. *and #: P < 0.05. The higher concentrations of magnesium oxide nanoparticles resulted in a significant reduction in the survival rate of apical papilla stem cells compared to the control group at both 24 and 48 h ($p < 0.05$). Tukey's test and a two-by-two comparison indicated that at concentrations of 1 and 10 µg/mL after 24 h, and at 1 µg/mL after 48 h, stem cell survival was comparable to the control. However, at higher concentrations, stem cell survival markedly decreased, significantly differing from the control group ($p < 0.05$)

and odontogenic/osteogenic differentiation of SCAPs. Due to their anatomical placement and critical function in regenerative endodontic treatments, SCAPs were the suitable choice for this research. (Theocharidou et al. 2017). Furthermore, SCAPs demonstrated a dramatically increased rate of multiplication, tissue regeneration ability, and a larger amount of STRO-1 positive cells compared to dental pulp stem cells (DPSCs) (Karkehabadi et al. 2022; Karkehabadi et al. 2023b; Radio et al. 2006).

Materials that are measured to contain at least one dimension in the range of 1–100 nm are classified as nanomaterials. Apart from their distinctive properties, these materials may also cause a certain degree of cytotoxicity. Numerous approaches have been proposed concerning the cytotoxicity linked to nanomaterials. It is theorized that exposure to nanoparticles can cause a rise in oxidative stress and upset calcium homeostasis intracellularly, ultimately leading to cellular destruction, apoptosis, and cell cycle disruption (Huang et al. 2017). To minimize the level of cytotoxicity caused by exposure to nanoparticles in this study, we initially exposed SCAPs to different concentrations of nanoparticles with the MTT assay to select the maximum cell survival after 24 and 48 h. MTT is a widely used technique to measure the survival of cells, and to assess cell toxicity (Chang et al. 2014a, b; Karkehabadi et al. 2018). According to the results of the MTT assay in Fig. 4, with the increase in the concentration of magnesium oxide nanoparticles, after 24 and 48 h, the survival of SCAPs decreased, and the highest survival of cells was observed at a concentration of 1 µg/mL. Consequently, a concentration of 1 µg/mL was utilized in the following study to evaluate the result of nanoparticles and LED irradiation on odontogenic/osteogenic differentiation.

Results similar to these were seen in a separate study that evaluated different concentrations of MgONPs in the form of a cell scaffold; it was observed that the proliferation rate of bone marrow mesenchymal stem cells at a concentration of under 1 µg/mL was similar to the control sample (Wetteland et al. 2016). Due to discrepancies in particle size and cell type, the safe concentration may differ between studies (Gurunathan et al. 2019).

The Real-Time Quantitative Reverse Transcription PCR results in Fig. 5 showed that the MgONPs + LED group had the highest gene expression of odontogenic/osteogenic markers, followed by the MgONPs group. These findings are comparable to experiments in which the odontogenic differentiation of DPSCs was notably increased when magnesium phosphate (MgP) was incorporated into the cell scaffold structure. (Ahmed et al. 2023; Farag et al. 2022; Qu et al. 2014). The Mg ions were thought to play a role in the activation of ALP (alkaline phosphatase) and the progression of the crystallization and pattern formation processes of the inorganic mineral phase, which were the basis for these results(Salem et al. 2021). In accordance with the current results, a study which looked into the impact of MgONPS on the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), noted that MgONPs enhance the expression of genes and proteins associated with osteogenesis differentiation. These results were found to be caused by the activation of canonical Wnt/β-catenin and BMP signaling pathways that were activated by MgONPs(Babuska et al. 2022; Bozorgi et al. 2021; Raghav et al. 2022; Wetteland et al. 2016).

Studies have revealed that magnesium deficiency causes a rise in osteoclast formation, causing a decrease in bone mass, abnormal bone growth and a weakened skeletal structure. (Belluci et al. 2013;

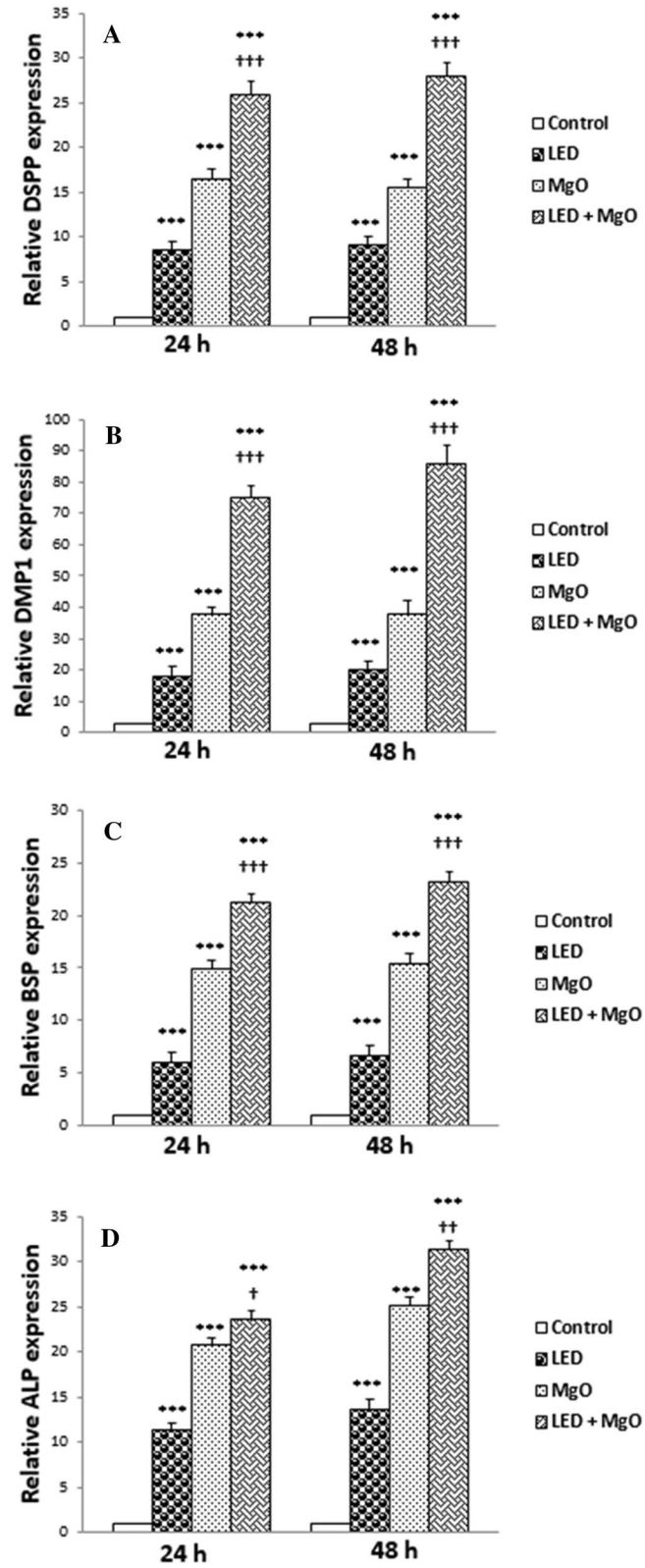

Fig. 7 The expression profile of SCAPs treated with LED, MgONPs, and LED + MgONPs. The relative mRNA expressions of genes were compared among the three groups and also with the control group (undifferentiated SCAPs). **A**: DSPP, **B**: DMP1, **C**: BSP, **D**: ALP, after 24 and 48 h, the highest expression of all genes was observed in the LED + MgONPs group, then in the MgONPs group, and finally in the LED group ($P < 0.05$). The control group had the lowest expression of genes

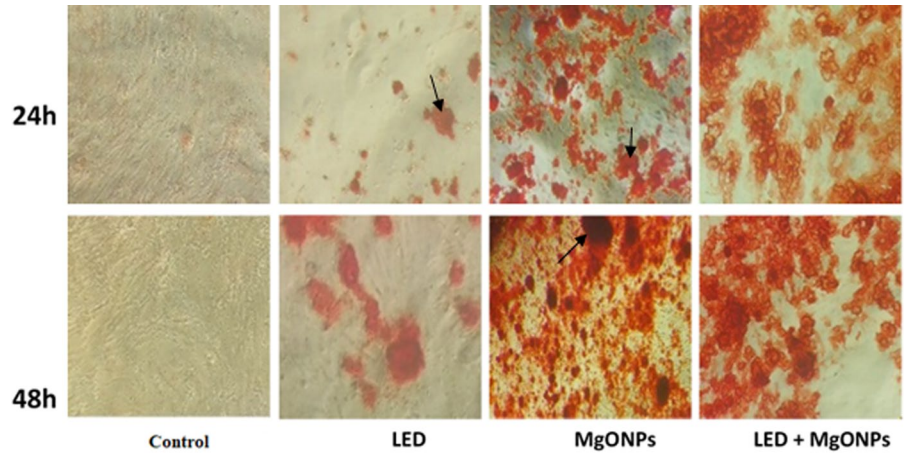

Fig. 8 Alizarin red staining of treated SCAPs, the density of ARS increased in all experimental groups after 24 h and in MgONPs + LED and MgONPs groups after 48 h compared with the control group. The black arrows indicated the red nodule like staining which express calcium deposition

Martiniakova et al. 2022; Xing et al. 2022). Supplementation of magnesium to phosphate glasses and ceramics boosts the osteogenesis associated bioactivity of scaffolds (Gu et al. 2022; Martiniakova et al. 2022).

According to the study of Yu et al.(Yu et al. 2017), The addition of Mg and Zn ions on the titanium surfaces of dental implants promotes the adhesion and proliferation of rat bone marrow mesenchymal stem cells. In addition, these ions promoted bone formation, angiogenesis and osseointegration by augmenting the amounts of OCN (osteocalcin) and ALP (alkaline phosphatase) (Yu et al. 2017). The results of the current study are in agreement with the positive effects of magnesium ion on bone formation as demonstrated in aformentioned studies. As opposed to the present study, a low concentration of magnesium nanoparticles was incorporated to reduce the cytotoxicity and to benefit from the nano structure.

As Fig. 5 demonstrated, the effect of MgONPs on gene expression was enhanced by LED irradiation, with maximal success in the combination of MgONPs and LED irradiation. LLLT has been determined to be of assistance in the treatment of many diseases. It has been ascertained that following treatment with low level laser therapy, a rise in mitochondrial activity, ATP production, DNA and RNA synthesis has been observed. In addition, the synthesis of reactive oxygen species (ROS) and the release of nitric oxide (NO) elevates, hence bringing about an upsurge in cell proliferation and survival. Low-Level Laser Therapy has been proven to be successful in diminishing pain, inflammation and promoting wound healing. It increases the migration, proliferation and survival of stem cells and the expression of proteins and stimulates cell differentiation of progenitor cells (Abrahamse 2012).

In accordance with the current findings, LLLT has been seen to induce an upturn of bone formation, angiogenesis and fibroblasts proliferation (Khadra et al. 2004). Furthermore, low level laser irradiation has been seen to improve bone regeneration and reduce inflammation levels (Pretel et al. 2007).

Research that included the utilization of both LLLT and mesenchymal stem cells (MSCs) for bone repair demonstrated that combining these two resulted in a heightened level of new bone formation in comparison to MSCs utilized by themselves (Fekrazad et al. 2015). Research has demonstrated that the application of LLLT in cell culture results in an increase in proliferation and differentiation of stem cells, greater secretion of growth factors, and heightened levels of calcium and alkaline phosphatase enzyme activity (Eduardo et al. 2008; Kim et al. 2009).

An additional study revealed that LED irradiation on Mg-based and Zn-doped bioceramic scaffolds could enhance the odontogenic differentiation and biomineralization of hDPSCs (Theocharidou et al. 2017).

The results of the present study concerning the positive effect of LED irradiation in regard to odontogenic/osteogenic differentiation was not far from what was expected, yet the observation of better results in the MgONPs + LED combination group compared to LED irradiation alone, could point to a synergistic effect of the two in regenerative endodontics.

According to Fig. 6, the density of ARS has increased in all experimental groups within 24 and

48 h, compared to the results of the control group. The greatest density was seen in the MgONPs and MgONPs + LED groups. In summary, MgONPs had a positive effect on bone mineralization. In this situation, MgONPs have been used to enhance calcium deposition in BMSCs (Wetteland et al. 2016).

Limitations and avenues for future studies

Time Constraints: The present study faced limitations in terms of time constraints, impacting the depth of analysis and the exploration of more extended time points. Future studies should allocate adequate time to ensure a comprehensive understanding of the long-term effects.

Financial Constraints: Financial limitations hindered the inclusion of certain analyses, such as Western blot tests for assessing synthesized proteins and signaling pathways. Future research with expanded resources could delve into these molecular aspects for a more thorough investigation.

Short Interval Irradiation: Due to financial considerations, the study utilized shorter intervals (24, 48 h) for stem cell irradiation. Future investigations should explore diverse time intervals, aligning with the broader spectrum suggested in systematic reviews (6–48 h), to discern nuanced effects (Borzabadi-Farahani 2016; Marques et al. 2016).

Cell Expansion Challenges: The study emphasized the necessity of cell expansion to obtain sufficient quantities for clinical applications, particularly given the limited number of stem cells in the apical zone of the tooth. Future research should address strategies for efficient and rapid cell expansion.

Mechanistic Understanding: While the study examined the impact of nanoparticles and LED irradiation on SCAP proliferation and differentiation, the exact underlying mechanisms remain unclear. Future studies should delve into the molecular mechanisms involved, contributing to a more comprehensive understanding of these regenerative processes.

Functional Analysis: The study did not conduct functional analyses, such as assessing cell migration, homing, and apoptosis. Future research should incorporate these functional aspects to provide a holistic evaluation of the effects of LED and nanoparticles on SCAPs.

Clinical Translation: The study highlighted the challenge of providing cells for injured tissue promptly. Future research should focus on bridging the gap between laboratory findings and clinical applications, exploring the translational potential of SCAP-based regenerative therapies.

Diversity in Nanoparticle Effects: Future studies could investigate the diverse effects of different nanoparticles, considering variations in size, composition, and surface properties. This would contribute to a nuanced understanding of the role of nanoparticles in stem cell responses(Patil et al. 2022; Tizu et al. 2022).

In summary, addressing these limitations and exploring the outlined avenues for future studies will contribute to advancing the field, fostering a more robust understanding of the therapeutic applications of Stem Cells from the Apical Papilla (SCAPs) in conjunction with LED irradiation and nanoparticles.

Conclusion

The current results suggest that the combination of magnesium oxide nanoparticles and LED irradiation in the culture medium of stem cells from the apical papilla plays a crucial role in promoting the survival and osteogenic/odontogenic differentiation of these cells.

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Data availability The complete documentation of participant enrolled in this study belongs to the corresponding author, Roshanak Abbasi, and is available only upon reasonable request.

Declarations

Competing interest The authors have no relevant financial or non-financial interests to disclose. The authors have no financial or proprietary interests in any material discussed in this article.

Ethics approval and consent to participate The study was performed according to the ISSCR Guidelines for Stem Cell

Research and Clinical Translation, and was approved by the ethics committee of the Hamadan University of Medical Sciences (IR.UMSHA.REC.1402.660). The procedure of this study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent All procedures were explained to the patient that participated in this study. Participant consented to the use of the extracted teeth for research purposes prior to extraction at the department of oral and maxillofacial surgery, Hamadan University of Medical Sciences, and signed an informed consent form for this purpose.

Consent for publication Not applicable.

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