Journal of Lasers in Medical Sciences

J Lasers Med Sci 2022;13:e58

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2022.58

Combination of Dental Capping Agents With Low-Level Laser Therapy Increases the Cell Viability Percent of Stem Cells From Apical Papilla (SCAPs)

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Received: September 18, 2022
Accepted: December 3, 2022
Published online December 6, 2022

Abstract

Introduction: Dental pulp capping is a technique that is highly applicable in dental restorations. In this technique, a material is directly placed over the exposed pulp tissue, which promotes pulp healing and generates reparative dentin. Herein, we aimed to investigate the combined effect of different pulp capping agents, including *mineral trioxide aggregate* (*MTA*), *Emdogain* (*EMD*), calcium-enriched mixture (*CEM*), and low-level laser therapy (LLLT), on enhancing viability and proliferation of stem cells from apical papilla (SCAPs).

Methods: SCAPs were isolated from two immature third molar teeth through collagenase type I enzymatic activity. Isolated stem cells were then cultured with DMEM and α-MEM media enriched with 15% and 10% FBS respectively. After reaching 70%-80% confluency, the cells were seeded in a 96-well plate. Cell viability percent was assessed using the MTT assay after treatment with *MTA*, *EMD*, *CEM* and LLLT (λ=630 nm, 5 mW, 4 J/cm^2) alone and in combination for 24, 48 and 168 hours.

Results: Combination of MTA, CEM, EMD and LLLT resulted in significantly increased SCAPs viability as compared with other treatment groups. Increased SCAPs proliferation and viability were also observed in groups treated with the combination of MTA and CEM with EMD. However, the SCAPs survival rate in all defined time spans was reduced after treatment with MTA and CEM alone.

Conclusion: LLLT can be a stimulator of SCAPs cell viability when applied in combination with dental capping agents such as MTA, EMD and CEM, providing a therapeutic option for stem cell-based dental regeneration.

Keywords: MTA; CEM; Emdogain; Low-level laser therapy (LLLT); SCAPs.

Introduction

Dental pulp tissue, which is comprised of blood vessels and nerves, is responsible for homeostasis maintenance and protection against injurious factors.[1-4] Vital pulpotomy and direct dental pulp capping are employed as conventional treatments. Calcium hydroxide ($Ca(OH)_2$) has been mostly used as a dental-capping agent over the past decades.[5] However, it was proved to possess a number of drawbacks such as poor seal, cohesive strength, marginal leakage, and inadequate antibacterial capacity.[6,7] Therefore, designing novel and effective treatment strategies to overcome the disadvantages of conventional pulp-dressing agents (such as $Ca(OH)_2$) is of foremost importance.[8,9] Interestingly, *mineral trioxide aggregate* (*MTA*) contributes to hard tissue formation with less pulp inflammation as compared with Calcium hydroxide.[10] Another biologically active pulp-dressing agent is Emdogain known as EMD.[11,12] Additionally, calcium-enriched mixture (*CEM*) was first introduced as a root-end filling material which consists of sulfur trioxide (SO3), calcium oxide (CaO), silicon dioxide (SiO2), and phosphorus pentoxide (P2O5).[13,14]

Tissue engineering-based dental pulp regeneration employs three essential factors including cells, scaffolds, and bioactive molecules such as growth factors.[15] Intriguingly, the use of dental pulp-derived stem cells

Please cite this article as follows: Zafari J, Karkehabadi H, Nikzad F, Esmailnasab S, Abbasi Javan Z, Javani Jouni F. Combination of dental capping agents with low-level laser therapy increases the cell viability percent of stem cells from apical papilla (scaps). *J Lasers Med Sci.* 2022;13:e58. doi:10.34172/jlms.2022.58.

(DPSCs) has great potential for conducting research on various dental-associated mechanisms. The DPSC isolation protocol was first reported by Gronthos et al in 2000.[16] More importantly, stem cells from apical papilla (SCAPs) belong to the group of dental stem cells that facilitate root maturation and are characterized by their potency, plasticity, and versatility.[17,18] SCAPs are also able to support tissue survival during pulp necrosis.[17]

Low-level laser therapy (LLLT) has been found to possess regenerative potential, mainly due to its stimulatory effects on cell proliferation and differentiation.[19] Our hypothesis was that the combination of pulp-capping agents and LLLT radiation can be applied for the treatment of dental pulp diseases. Therefore, we attempted to investigate the effect of MTA, EDM, and CEM agents alone and in combination with LLLT on the cell survival rate of SCAPs.

Materials and Methods

The CEM (BioniqueDent, Tehran, Iran) and MTA (ProRoot MTA, Tulsa/Dentsply, OK) with a 10-mm diameter and 1-mm thickness were prepared in sterile laboratory conditions according to the manufacturer's instructions. These pulp-dressing agents were compressed in a paraffin wax mold for 10 min. at 37°C in a place with 96% humidity.

EMD (Straumamm , Swiss) solution (100 μg/mL) was also prepared by dissolving 30 mg/mL EMD gel in 0.7 mL sterile distilled water.

Two immature third molar teeth were obtained with informed consent. All experiments were reviewed and approved by the research committee of Hamadan University of Medical Sciences. The teeth were immediately rinsed with sterile phosphate buffered saline (PBS, Gibco, USA) and stored in it for further investigations. The isolation of SCAPs from apical papilla was performed through enzymatic digestion in 2 mg/mL collagenase type I solution (Worthington Biomedical, USA). Stem cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) enriched with 15% fetal bovine serum (FBS, Gibco, USA). Then the cells were re-cultured in the alpha-minimum essential medium (α-MEM) containing 10% FBS. One week after cell culture monitoring and changing the culture medium, the cells were subcultured and incubated until they were 70%-80% confluent.

SCAPs were seeded in a 96-well plate at a density of 5×10^3 cells/well. After overnight incubation, the cells were treated with pulp-capping agents and LLLT for 24, 48 and 168 hours. The treatment groups were designed as follows:

The first group of cells seeded in a 96-well plate was treated with MTA, MTA/EMD, MTA/EMD/LLLT, MTA/LLLT, CEM, CEM/EMD, CEM/EMD/LLLT, CEM/LLLT, EMD, EMD/LLLT and LLLT. The control group had no treatment.

It is important to note that for cell treatment with the combination of MTA or CEM with EMD, we first embedded MTA or CEM in EMD and then used the combination for cell treatment. More exactly, we used the EMD as a covering for MTA and CEM.

For laser treatment, SCAPs were seeded in a 96-well plate, with one empty well between the samples to avoid cross-irradiation between the samples. The irradiation was performed using a continuous-wave gallium aluminum arsenide diode laser (λ = 630 nm, 5 mW; P1 Dental Laser, Pioon, China) at 4 J/cm^2. The irradiation was carried out perpendicularly in contact with the bottom of the culture plate in a dark room. After 1-minute irradiation, the percentage of cell viability was calculated.

In each defined time point, the medium was replaced with 90 μL fresh MEM media containing 10% FBS plus 10 μL tetrazolium salt (MTT, Sigma Aldrich), followed by incubation at 37°C for 4 hours. Then 100 μL dimethyl sulfoxide (DMSO) was added to each well and incubated for 10 min. in a dark place. Finally, the absorbance of dissolved formazan was measured at a wavelength of 540-690 nm using the ELISA microplate reader (EMax® Endpoint).[20,21]

Statistical Analysis

Data were analyzed by Bonferroni *t* test using GraphPad Prism software version 5. All obtained data were expressed as mean ± SD of at least three experiments. Data with *P* values lower than 0.05 were viewed as statistically significant.

Results

The MTT assay was carried out to reveal the effect of MTA and CEM alone and in combination with LLLT and EMD on the cell viability rate of SCAPs.

The effect of MTA and its combination with EMD and LLLT on the SCAPs population has been represented in Figure 1. It is apparent from this figure that 168-hour treatment with the combination of MTA, EMD and LLLT caused an increase in the SCAPs viability percentage as compared to MTA + EMD and MTA + LLLT treatment groups. However, the most statistically significant difference was observed between MTA/EMD/LLLT and MTA alone groups ($P < 0.05$).

Figure 2 represents the effect of CEM and its combination with EMD and LLLT on the SCAPs viability percentage. As shown in this figure, 168-hour treatment with CEM, EMD and LLLT combination could remarkably provoke SCAPs survival in comparison with other treatments, especially the group of SCAPs treated with CEM alone ($P < 0.05$).

According to Figure 3A, in all time points, MTA induced the SCAPs viability rate more significantly than CEM. However, in all time spans, both MTA and CEM decreased SCAPs viability as compared to other

treatments. Furthermore, we found that laser alone, EMD alone, and the combination of EMD and LLLT surged the survival rate of SCAPs. Figure 3B shows the impact of different treatment groups at different time points. From this figure, it can be seen that CEM had a cell death-provoking effect in a time-dependent manner ($P < 0.05$). Nevertheless, there was no statistically significant change in the viability of SCAPs treated with MTA, EMD, laser, and combined EMD-laser.

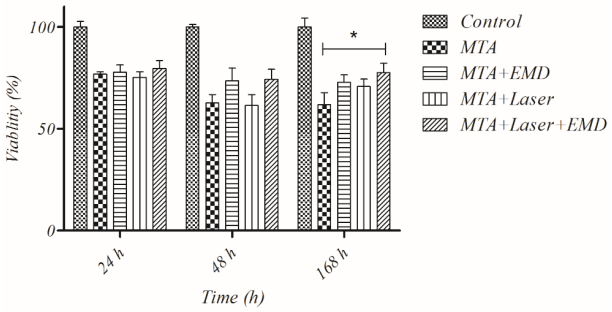

Figure 1. The Cell Viability Percent of SCAPs After 24, 48 and 168 Hours of Treatment With MTA, MTA/EMD, MTA/Laser and MTA/Laser/EMD Relative to Untreated Control; Measured by MTT Assay. The obtained values are presented as mean ± SD of at least three replications, (* *P* value < 0.05).

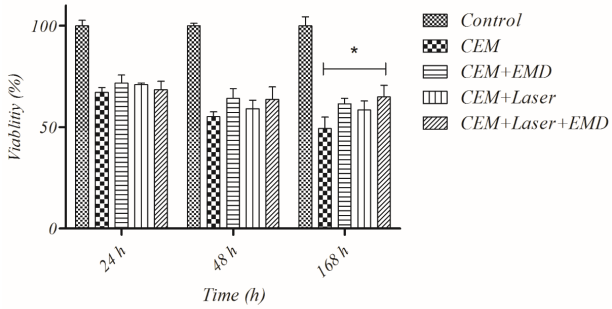

Figure 2. The Cell Viability Percent of SCAPs After 24, 48 and 168 Hours of Treatment With CEM, CEM/EMD, CEM/Laser and CEM/Laser/EMD Relative to Untreated Control; Measured by MTT Assay. The obtained values are presented as mean ± SD of at least three replications, (** *P* value < 0.01, *** *P* value < 0.001).

The effect of the combined MTA and CEM as well as their combination with EMD and LLLT on SCAP cell viability during 24-hour, 48-hour and 168-hour treatments has been shown in Figures 4, 5 and 6 respectively. According to Figure 4, the survival rate of SCAPs treated with MTA/CEM decreased in comparison with other treatment groups. However, there was no statistically significant difference between treatment groups.

Figure 5 illustrates that the administration of MTA/CEM-EMD and MTA/CEM-EMD-laser combination for 48 hours could potentially increase SCAPs viability percentage as compared with other treatments.

Figure 6 shows that SCAPs treatment with MTA/CEM-EMD-laser for 168 hours led to a noticeable increase in the cell viability and population compared to other treatment groups.

Discussion

Over the past decades, a myriad of commercial products have been developed as direct dental pulp-capping agents.[22] Briefly, dental pulp-capping is a therapeutic modality that leads to enhanced pulp vitality through placing a dental biomaterial directly over a damaged dental pulp. More precisely, the ultimate goal of using dental pulp-capping agents is to stimulate undifferentiated dental pulp stem cells to form reparative tertiary dentine.[23] SCAPs are dental stem cells that are relatively easily obtainable and have a broad range of applications in tissue regeneration processes. This in vitro study designed an environment to expose SCAPs to pulp-dressing materials alone and in combination with LLLT and measure the cell viability rate.

In this study, we found that the application of combinational therapy with the use of dental-capping materials and LLLT could effectively increase SCAPs viability. We observed that MTA treatment alone had a negative impact on SCAPs viability. This finding is in consistent with a recent study, which reported that direct administration of MTA impairs cell viability of stem cells from human exfoliated deciduous teeth through inducing

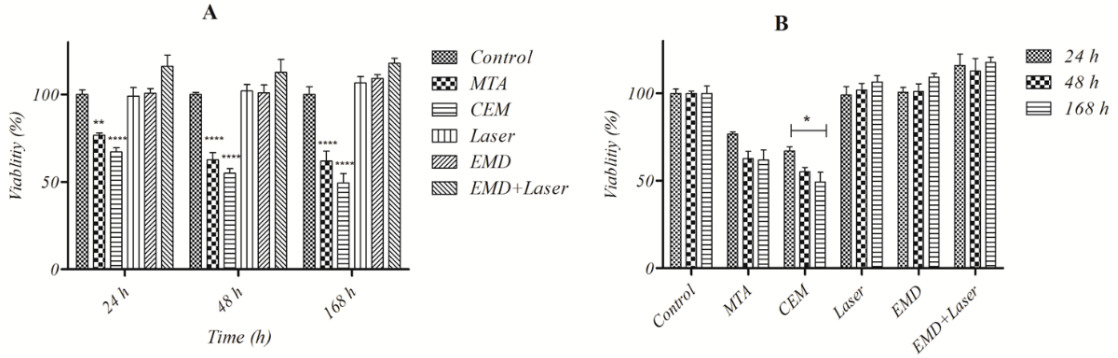

Figure 3. (A) The viability percent of SCAPs after 24, 48 and 168 h of treatment with MTA, CEM, laser, EMD and EMD/laser relative to untreated control; measured by MTT assay. (B) MTA, CEM, laser, EMD and EMD/laser compared to their untreated control. The obtained values are expressed as mean ± SD of at least three repetitions, (** *P* value < 0.01, **** *P* value < 0.0001).

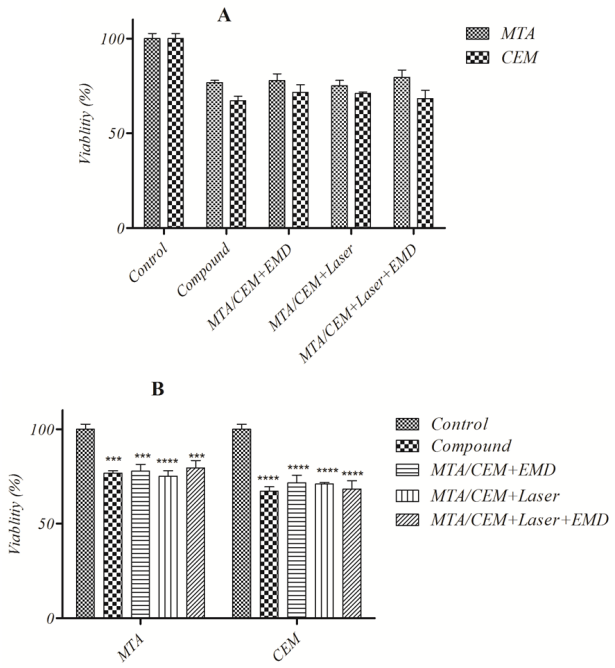

Figure 4. (A and B) The Viability Percent of SCAPs after 24 Hours of Treatment With MTA-CEM Compound, MTA-CEM/EMD, MTA-CEM/Laser and MTA-CEM/Laser/EMD Relative to Untreated Control; Measured by MTT Assay. The obtained values are presented as mean ± SD of at least three replications, (* *P* value < 0.05, ** *P* value < 0.01, *** *P* value < 0.001, **** *P* value < 0.0001).

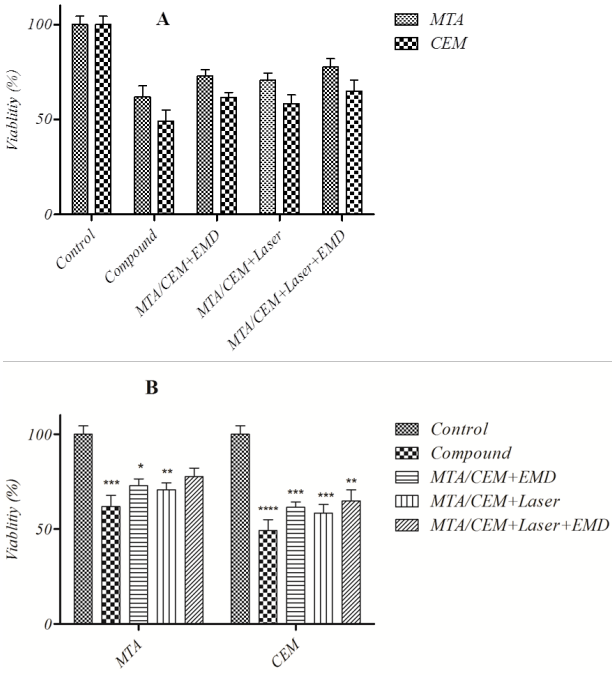

Figure 6. (A and B) The Viability of SCAPs After 168 Hours of Treatment With MTA-CEM Compound, MTA-CEM/EMD, MTA-CEM /Laser and MTA-CEM /Laser/EMD Relative to Untreated Control; Measured by MTT assay. The obtained data are presented as mean ± SD of at least three replications, (* *P* value < 0.05, ** *P* value < 0.01, *** *P* value < 0.001, **** *P* value < 0.0001).

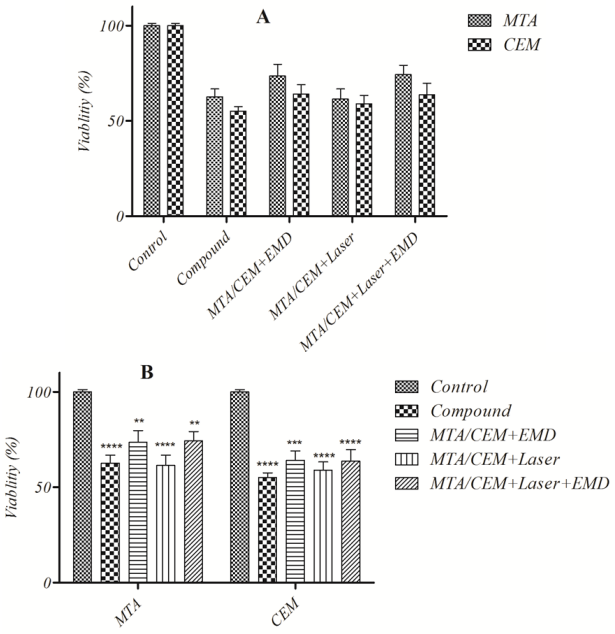

Figure 5. (A and B) The Viability of SCAPs After 48 Hours of Treatment With MTA-CEM Compound, MTA-CEM/EMD, MTA-CEM /Laser and MTA-CEM /Laser/EMD Relative to Untreated Control; Measured by MTT Assay. The obtained data are expressed as mean ± SD of at least three repetitions, (* *P* value < 0.05, ** *P* value < 0.01, *** *P* value < 0.001, **** *P* value < 0.0001).

cell apoptosis.[24] A possible explanation for these results might be the fact that MTA contains calcium hydroxide, which is associated with high calcium ion release and sustained high pH.[25]

It seems that these results challenge the biocompatible effects of MTA. It is interesting to note that in vitro studies regarding the effect of MTA have revealed that cells response to the material based on many factors including cell types and study deign, frequency of changing the cell culture media, use of fresh or cured material, concentration of the MTA in cell culture media and use of MTA extract or direct application of it.[26]

Our findings showed that the viability of SCAPs was increased after exposure to the combination of MTA, LLLT and EMD. It seems that the addition of laser and EMD to the pulp-capping agents is the turning point in increasing SCAPs viability as compared to each treatment alone. Recent studies have demonstrated potent effects of LLLT in modulating stem cell proliferation and differentiation.[27,28] Interestingly enough, LLLT-induced stem cell proliferation has been reported in adipose tissue stem cells,[29] bone marrow stem cells,[30] and DPSCs.[31,32] These results provide further support for the hypothesis that applying LLLT plus dental capping agents like MTA, CEM and EMD yields synergistic effects on the induction of stem cell proliferation. It is also worth noting that according to an earlier study, biostimulation with the aid of LLLT is capable of activating stem cells.[33] Furthermore, we found that CEM decreased SCAPs viability, while combined CEM, EMD and LLLT led to a significant increase in the SCAPs viability percentage, indicating that

CEM might be cytotoxic in early application. However, the use of EMD and LLLT in combination with CEM could distinctly decline CEM-mediated cytotoxicity. Our results demonstrated that the use of laser irradiation and EMD combined with dental capping agents like MTA and CEM gave rise to higher SCAPs survival rate in comparison to each treatment alone. It is encouraging to compare this result with findings of a previous study, which reported proliferative effects of LLLT on human dental mesenchymal stem cells including DPSCs, SCAPs, periodontal ligaments and exfoliated deciduous teeth.[19] Increased synthesis of DNA, RNA, ATP, reactive oxygen species and nitric oxide after laser irradiation have been proposed as mechanisms of LLLT-induced cell proliferation.[34,35] Of note, LLLT has been considered an effective factor in tissue regeneration as it can affect chemical mediators and reduce local inflammation.[36]

Conclusion

The main goal of the current study was to determine the effect of dental pulp-capping materials with LLLT on dental pulp stem cell viability. One of the most significant findings to emerge from this study was increased cell viability after administration of our proposed combination therapy. In this study, there was a paucity of samples and thus, caution must be applied, as the findings might not be transferable to clinical settings. Therefore, more research is required to determine the efficacy of the proposed combination therapy suggested in this study.

Conflict of Interests

All authors declared that they have no known financial or personal conflict of interest.

Ethical Considerations

This research was approved by the research ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.REC.1399.001).

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