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Effect of active irrigation using shock wave-enhanced emission photoacoustic streaming on dental pulp stem cell viability

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ABSTRACT

Background: Shock wave-enhanced emission photoacoustic streaming (SWEEPS) is a novel irrigation activation method based on photoacoustic streaming. The aim of this study was to look into the impact of SWEEPS on the attachment and survival of dental pulp stem cells (DPSCs).

Materials and Methods: In this *in vitro* study, 34 standardized root segments were randomly allocated into two groups: SWEEPS and the conventional conditioning group. After the irrigation, human DPSCs were seeded on the internal walls of these samples, and the attachment and survival of 30 of them were assessed on different days. The remaining two samples were observed using a scanning electron microscope (SEM). Independent sample *t*-test, Mann–Whitney *U*-test, one-way ANOVA, Kruskal–Wallis, and two-way ANOVA were used for data analysis with the level of significance = 0.05.

Results: The viability of DPSCs was significantly greater in the SWEEPS group in comparison with the conventional conditioning group ($P = 0.029$). Both groups have shown a significant increase in the viability of DPSCs over time ($P = 0.0001$, $P = 0.003$). SEM results have shown a smear layer-free surface with firmly attached DPSCs in the SWEEPS group.

Conclusion: The results of this study indicated that active irrigation using SWEEPS could provide a superior surface in terms of viability and attachment of DPSCs compared to the conventional conditioning method.

Key Words: Lasers, mesenchymal stem cells, regenerative endodontics, root canal irrigants, smear layer

INTRODUCTION

Pulp necrosis prevents root formation in immature permanent teeth, making it harder to fulfill the aims of traditional root canal therapy while also leaving the root thin, brittle, and prone to fracture.[1,2] Regenerative endodontic procedures (REPs) are therapies that have recently attracted a significant amount of critical attention.[3,4] These treatment approaches aim to create an environment in the root canal that promotes pulp regeneration and root development.[5] Revascularization is the most extensively used approach for regenerative endodontics.[5,6] According to the modified protocol by

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Banchs and Trope,[7] revascularization consists of three essential aspects: complete root canal disinfection to prepare the root canal space for periradicular stem cells, dentin conditioning to release the growth factors embedded within it, and apical bleeding induction to acquire stem cells.

Eliminating the smear layer is another critical component of an efficient REP. Toxins and microbial byproducts in the smear layer's organic portion can significantly harm stem cells.[8] Based on the most recent American Association of Endodontists protocols, the standard irrigation technique for REPs is to utilize a low-concentration NaOCl (1.5%) with or without a final irrigant of 17% ethylenediaminetetraacetic acid (EDTA) (10 mL/canal, 5 min).[9] There are, however, insufficient data to support the effect of low-concentration NaOCl on smear layer elimination. Because prolonged use of EDTA might affect cell function and blood clot formation, it has the potential to harm cell attachment and tissue regeneration.[10,11] Furthermore, traditional needle irrigation appears inefficient in eliminating the smear layer.[12,13] As a result, active irrigation may be beneficial in these situations.

Various irrigation activation techniques have been proposed to improve chelating solutions' smear layer removal properties.[13] Based on previous studies, active irrigation is significantly more effective than conventional needle irrigation in smear layer removal.[14,15] Furthermore, active irrigation can be beneficial in REPs by increasing the release of growth factors, which are bioactive proteins embedded within the dentinal walls of the canal, and promoting the apical papilla cell adhesion to the root canals.[16-18]

Er:YAG laser can be utilized for a new approach in active irrigation called photon-induced photoacoustic streaming (PIPS), which employs a short pulse duration (50 μs) and low pulse energy of 10 or 20 mJ and pulse repetition rate of 15 Hz and produces a bubble at the fiber's tip that expands to its maximum volume before collapsing. The irrigants get agitated as a result of this occurrence due to the cavitation effect.[19] A novel shock wave-enhanced emission photoacoustic streaming (SWEEPS) modality, an improved version of PIPS, has recently been introduced. SWEEPS emits a second laser pulse rapidly after the first, causing a sequence of bubbles to develop at precisely the right moments, forcing existing bubbles to burst in the process. Compared to PIPS mode, it produces more powerful shock waves and improved photoacoustic streaming.[20-22] However, no prior research has been found to assess the effect of this active irrigation method on stem cell survival and adherence.

Therefore, the purpose of this study was to determine the influence of SWEEPS on the viability and adhesion of dental pulp stem cells (DPSCs). The null hypothesis was that using SWEEPS does not affect pulpal stem cells' viability and adhesion. This study's results are expected to contribute fresh insights into this area of regenerative endodontics.

MATERIALS AND METHODS

Specimen preparation

This *in vitro* study was approved by the Islamic Azad University of Medical Sciences Ethics Committee (IR.IAU.DENTAL.REC.1400.048). Thirty-four recently extracted, single-rooted, single-canal, noncarious human anterior teeth removed for periodontal reasons were collected after the sample size was determined based on previous research[17] with a statistical power of 80% and the probability of making a type one error of 0.05. After cleaning the surface of the teeth with a curette, to maintain the root length at 12 mm, the teeth were decoronated with a diamond disc. The length of a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was measured after being inserted into the root canals and its tip was observed at the apical foramen. The working length was obtained by deducting 0.5 mm from this length. Instrumentation of the roots was done up to the #25/8% size of Neolix (Neolix SAS, Chatres-La-Foret, France) to the working length.

Irrigation protocol

Prepared roots were longitudinally grooved on the outer aspect of the buccal and lingual surfaces without entering the root canals before irrigation of the samples. Following that, these 34 roots were randomly separated into two groups, each with a distinct irrigation protocol:

Group A (SWEEPS group): The SWEEPS final irrigation protocol recommended by Olivi and Divito[20] was employed, which is as follows: "two cycles of 17% EDTA (Morvabon, Iran) activated by SWEEPS for 30 s each, followed by rinsing with distilled water activated by SWEEPS for 30 s, then three cycles of 5% NaOCl (Morvabon, Iran) activated by SWEEPS for 30 s each, and a resting time of at least 30 s."

Group B (conventional conditioning group): "1 mL of 1.5% NaOCl for 5 min, followed by 3 mL of phosphate-buffered saline (PBS) for 3 min (1 mL/min), followed by 1 mL of 17% EDTA for 5 min, followed by 3 mL of PBS for 3 min (1 mL/min) as a final rinse"[17] without any activation.

In Group A, the irrigants were activated using the Auto SWEEPS modality on an Er:YAG laser (LightWalker Fotona, Ljubljana, Slovenia) with a flat-end fiber tip (SWEEPS 600; Fotona) inserted 2.5 mm into the access cavity and held stationary and activated the irrigation with the following parameters: 20 mJ per pulse, 20 Hz, and 0.3 W. The interval between the pulses varied randomly from 250 to 600 μs.

After irrigation, the teeth were divided into halves using a mallet and a chisel and the half with the most visible parts of the internal wall was preserved and the other half was discarded.

Cell culture and viability assessment

Human DPSC line (DPS-13, IBRC-C10896) (Iranian Biological Resource Center, Iran) were cultured in Dulbecco's Modified Eagle Medium (DMEM; BIO-IDEA, Tehran, Iran)/Nutrient Mixture F-12 (DMEM/F12), with added 20% fetal bovine serum and 2 mM L-glutamine and preserved at 37°C with 5% CO_2. After three passages, DPSCs (1×10^4 cells/sample) were seeded on the internal walls of the ultraviolet sterilized (260 nm, 300 mJ/cm^2, 4 min, mercury lamp) root segments. Each group was then separated into three subgroups ($n = 5$) to determine viability at 1, 4, or 7 days.

The viability of the DPSCs, at each period, was evaluated utilizing the Mosmann's tetrazolium toxicity (MTT) assay. After adding 5 mg/mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], samples were incubated for 4 h at 37°C and 5% CO_2. The formazan crystals were dissolved in dimethyl sulfoxide. After the extraction of mediums from each well, the absorbance value was measured using a microplate reader (SPECTROstar Nano, BMG LABTECH, Offenburg, Germany) at wavelengths of 570 nm.

Scanning electron microscope

Two root segments from each group on the 7th day were selected for scanning electron microscope (SEM) evaluation. After the fixation of the samples using 2.5% glutaraldehyde for 30 min, root fragments were dried with hexamethyldisilazane after being dehydrated in a graded ethanol series. After that, the coating of the samples was done with a thin coating of gold, and the morphology of the DPSCs was examined with a SEM (MIRA 3, TESCAN, Brno, Czech Republic) at 5000X, 10,000X, and 15,000X.

Statistical analysis

After the data's normality was evaluated using the Shapiro–Wilk test, the independent sample *t*-test (for homogeneous data) and Mann–Whitney *U*-test (for nonhomogeneous data) were used to evaluate the difference between the groups in each observational period. One-way ANOVA and Kruskal–Wallis were used to assess DPSC's viability in each group over time followed by pairwise comparison using *post hoc* tests. Two-way ANOVA was used to analyze the overall DPSC's viability between the two groups. For all statistical analyses, SPSS (SPSS for Windows, version 16, SPSS Inc., Chicago, IL, USA) was used. The level of significance was set at $\alpha = 0.05$.

RESULTS

Cell viability

The viability of DPSCs increased significantly over time in both the SWEEPS and conventional conditioning groups ($P = 0.0001$, $P = 0.003$). The cell viability of the SWEEPS group was greater than the other group on all days; however, the difference was only significant on day 4 ($P = 0.003$). The SWEEPS group had greater overall cell viability than the conventional conditioning group ($P = 0.029$). The results of each group are shown in Table 1.

Scanning electron microscope observations

SWEEPS application exhibited complete smear layer removal and open dentinal tubules. The conventional protocol removed the smear layer at some degrees, while some dentinal tubules were still obstructed.

The DPSCs seem to be firmly attached by their cytoplasmic extensions to the clean dentinal surface of the SWEEPS group samples. In contrast, the DPSCs on the conventional conditioning group's samples

Table 1: Results of each group on each day

Irrigation protocol	Mean±SD			Difference (*P**)
	Day 1	Day 4	Day 7	
SWEEPS	0.21±0.05	0.74±0.42	0.71±0.19	0.0001†
EDTA	0.18±0.06	0.33±0.09	0.67±0.12	0.003
Difference (*P**)	0.321	0.003‡	0.590	

*Significance level=0.05; †Nonparametric test (Kruskal–Wallis); ‡Nonparametric test (Mann–Whitney). SWEEPS: Shock wave-enhanced emission photoacoustic streaming; SD: Standard deviation; EDTA: Ethylenediaminetetraacetic acid

were round and poorly attached. Figure 1 shows SEM images of samples in both the groups.

DISCUSSION

Since stem cell survival and attachment are crucial in REPs, researchers must investigate the impact of each treatment phase, such as irrigation, dentin conditioning, and scaffold selection, on these issues to establish a feasible REP protocol.[23] In this study, we have evaluated the effect of an irrigation activation technique on DPSC's survival and morphology.

It has long been proved that using 17% EDTA as an irrigant promotes the release of growth factors.[24,25] These growth factors can significantly improve stem cell recruitment in a REP.[26] The use of 17% EDTA alongside NaOCl as irrigants has been shown to enhance the release of growth factors such as transforming growth factor-beta-1.[27] Recently, in a study conducted by Aksel *et al*.,[17] an optimized EDTA conditioning protocol was suggested, which is used in the conventional conditioning group of our research. In addition, activation of the irrigation protocol has been demonstrated to have a favorable influence on growth factors produced from the dentin matrix, stem cell survival, and migration in prior research by Aksel *et al*.[17] and Widbiller *et al*.[18]

In some studies, photoacoustic streaming has been mentioned as an effective method for active irrigation.[28-30] The Er:YAG laser was employed in this study to generate shock waves in spatially constrained canals using a novel SWEEPS modality. During SWEEPS, a second laser pulse is administered right before the bubble of the first laser pulse collapses. The second laser pulse's rapid expansion of the second bubble increases the pressure on the first bubble, which accelerates its collapse and causes shock waves to be released. Shock waves are also created by bursting secondary cavitation bubbles that grow throughout the canal's length during laser-induced irrigation.[22] SWEEPS irrigation has been proven to result in considerably improved flushing action,[20] as well as higher irrigant penetration into dentinal tubules owing to increased pressure generation,[31] without increasing the risk of apical extrusion.[32]

In the current study, the SWEEPS group has shown significantly better results than the control group regarding DPSCs' viability. This outcome

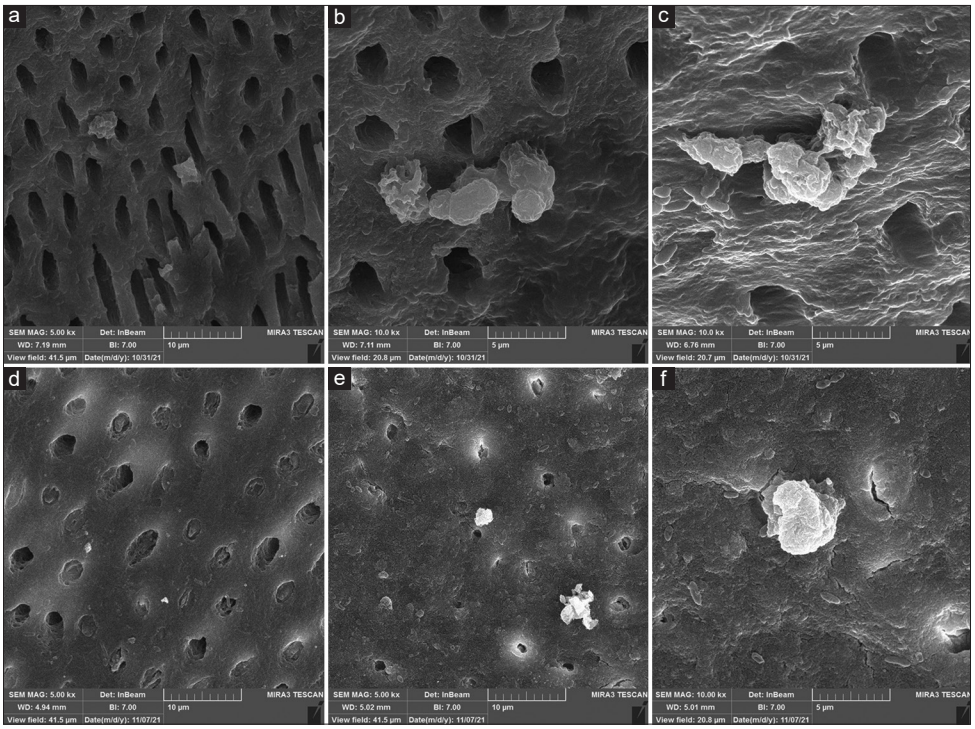

Figure 1: Scanning electron microscopic images of the samples. (a-c) Samples irrigated using shock wave-enhanced emission photoacoustic streaming showing complete smear layer removal with open dentinal tubules (×5k and ×10k and ×10k) and (b and c) Firm attachment of dental pulp stem cells (DPSCs) to the dentinal surface using cytoplasmic extensions. (d-f) Samples conditioned using conventional conditioning protocol showing partial removal of smear layer with some obstructed dentinal tubules (×5k and one x5k is extra and ×10k). (f) Round and poorly attached DPSCs to the dentinal wall. SEM: Scanning electron microscope.

is somewhat in accordance with what Wu *et al.* suggested,[33] who found the highest number of stem cells of the apical papilla in the group consisting of NaOCl + EDTA + PIPS (EDTA). These results might be due to the better smear layer removal in the groups using photoacoustic streaming because removing the smear layer, which is consisted of toxic remnants, and exposing the dentin surface to the DPSCs may enhance stem cell attachment and survival, while the smear layer's existence may cause failure in regenerative endodontic therapies.[8,34,35] However, no significant difference was reported regarding the smear layer removal using PIPS in the study by Wu *et al.*[33] The lack of shock waves in PIPS activation was attributed to friction in a restricted environment, which dramatically increases bubble oscillation periods and hence does not generate shock waves.[36] In addition, The MTT assay demonstrated that the number of cells on dentin surfaces increased with time, regardless of the type of treatment. These results are in line with those of Özdal-Kurt *et al.*,[37] and it can be attributed to stem cells' proliferation throughout time.

SWEEPS produces a smooth surface with open dentinal tubules, which have been described as a site for cytoplasmic processes to extend.[38] The contact area of cells in cell morphology can be used as a predictor of cell affinity. With their elongated cell bodies and lamellipodia, flat cells often have a strong attachment to the surface, whereas round cells can be thought of as having a weaker attachment.[39] The SWEEPS group's samples showed elongated cell bodies and prolonged cytoplasmic processes in the SEM findings, which may be related to greater cell viability [Figure 1]. Moreover, in SWEEPS, the laser tip only needs to be placed in the pulp chamber; therefore, it does not need particular canal enlargement and preparation.[40] This is of utmost importance, especially in immature teeth, which have fragile dentinal walls and are prone to fracture.[41,42]

It is conceivable that a variety of restrictions could have affected the outcomes. One of them is the constrained number of samples. An additional possible source of concern in this research was the absence of a final rinse in the protocol of SWEEPS[20] to remove the residual EDTA, which has a direct negative influence on blood clot formation.[11] Having said that, this study has been one of the first attempts to assess the impact of laser-activated irrigation on the viability of DPSCs and can serve as a foundation for future studies that will help us establish a higher degree of precision on this subject.

CONCLUSION

The findings of this study indicate that employing the SWEEPS procedure may be advantageous for regenerative endodontic therapies. The results of this study must be interpreted with great care given the small number of samples. Designing a SWEEPS final irrigation protocol that is acceptable for regenerative endodontic treatment will require further research. The findings of this study may be useful in developing an optimum irrigation technique for REPs.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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