ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
September 2021 • Vol. XXIV

Effect of Magnesium Oxide Irrigating Solution on Extracellular pH Changes Around Human Dental Pulp Stem Cells

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Abstract

Objective: The aim of the present study was to evaluate the effect of magnesium oxide as an irrigant preparedfrom nano or microparticle sizes on extracellular PH changes around human dental pulp stem cells.
Methods: Toevaluate PH changes around dental pulp stem cells, a composite of the cells with different irrigation solutions wereprepared and classified into six groups: Group I, 1.5 % sodium hypochlorite solution. Group II, 17% EDTA solution.Group III, 1.5 % sodium hypochlorite solution and 17% EDTA. Group IV, 5mg/L magnesium oxide (microparticles)solution. Group V,5mg/L magnesium oxide (nanoparticles) solution. Group VI was the negative control groupwithout any treatment. The cell cytotoxicity assay was performed using Methyl Thiazol Tetrazolium (MTT) Assayafter 1 hour, 24 hours and 72 hours of incubation with the tested irrigants. In addition, Assessment of pH ofhDPSCs treated with various solutions was performed. Data were statistically analyzed.
Results: With time, pH ofcells' culture decrease, and the percentage of cell's viability increase for all tested solutions.
Conclusions: Theincrease of % viability for hDPSCs cultured with different irrigation solutions is accompanied by decrease of theextracellular pH around the cells.
Keywords: dental pulp stem cells, PH, Irrigation

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Introduction

The effectiveness of endodontic treatment with pulp necrosis depends on the adequate disinfection of the root canals, which can be achieved through various irrigation solutions and the selective use of intracanal medications.1 Cleaning of cases with necrotic pulp, especially in immature teeth, requires the use of irrigation solutions with the least toxic effect on the stem cells required for pulp regeneration and continuation of root development.2

Recently, various types of stem cells have been isolated from the oral cavity, this includes dental pulp stem cells (DPSCs).3 DPSCs are mesenchymal stem cells with high self-renewal capacity that have the ability to differentiate into several cell types. Various types of root canal irrigants are available to be used for root canal disinfection during regenerative endodontic procedures (REPs). Many of these irrigants are cytotoxic. Sodium hypochlorite (NaOCl) is the most commonly used irrigant for this rationale. However, some authors have revealed that treating canal surfaces with NaOCl reduced the viability of stem cells due to its toxicity.4 Local and systemic pH variations can frequently occur under pathological conditions. Dental pulp is exposed to fluctuations in extracellular pH under several circumstances.

Antibacterial nanoparticles have been found to give a wide spectrum of antimicrobial activity and reduced susceptibility to induce antibacterial resistance in comparison to ordinary antimicrobials.5

Magnesium oxide nanoparticle (nMgO) is a metal based antibacterial nanoparticle that can be metabolized in human body. Against this background, this study aimed to further investigation of the effect of magnesium oxide nanoparticles (nMgO) as an irrigation solution on extracellular pH changes around human dental pulp stem cells.

Materials and Methods:

This study was approved by the Research Ethics Committee, Faculty of dentistry, Ain Shams University; with approval number (FDASU-Rec M 1021 11). All experiments were performed in accordance with the committee guidelines of the stem cells experiments.

- **Irrigants Preparation**

A.1. Preparation of 1.5% Sodium Hypochlorite Solution: Solution was prepared by dilution of 5% NaOCl solution. Solution preparation was done immediately before the experiment.

A.2. Preparation of Magnesium Oxide (microparticles and nanoparticles) Solutions: Solutions were prepared by Nanogate company, Cairo, Egypt. They were prepared by dilution of stock solutions in a concentration of 5 mg/L. Both solutions were stored at room temperature for no longer than 1 day before the experiments.5

Grouping of samples

All experimental procedures were done at stem cell unit, Global Research Laboratory, Cairo, Egypt. A composite of a previously characterized dental pulp stem cells and different irrigation solutions was prepared as follows:

Group I: 1.5 % sodium hypochlorite solution,
Group II: 17% EDTA solution,
Group III: 1.5 % sodium hypochlorite solution and 17% EDTA,
*GroupIV:*5mg/L magnesium oxide (microparticles) solution and
Group V: 5mg/L magnesium oxide (nanoparticles) solution.
Group VI: was the negative control group without any treatment (stem cells cultured in Dulbecco's Modified Eagle Medium). Then all groups were subdivided into subgroups according to the observation time (1, 24 and 72 hours).

Evaluation

The cell cytotoxicity assay was performed by Methyl Thiazol Tetrazolium (MTT) Assay Kit.6 hDPSCs were seeded in 96-well culture plate (8 x 103 cells/well) and incubated, for 24 hours, at 37 °C in an atmosphere of 5% CO2.

The culture media were replaced with the irrigation solutions according to the previously described classification, then the cells were incubated for three-time intervals (1 hour, 24 hours and 72 hours). MTT solution (1 mg/mL) was added to each well and the plate was incubated at 37 °C and 5% CO2 for 4 hours. Finally, the MTT solution was removed and 100 µL of Sulphur dodecyl sulphate-hydrochloric acid was added to the wells. Cell viability was determined by measuring the optical density at 570 nm on a spectrophotometer.

In addition, Assessment of pH of hDPSCs treated with various solutions at three-time intervals (1 hour, 24 hours and 72 hours) was performed.7 The pH meter was calibrated against standards and adjusted for the temperature effect. The indicator electrode and the reference electrode were immersed in the solutions, the small potential difference between the two half-cells was measured by a very sensitive voltmeter and the hydrogen ion activity was read. The obtained data were collected; tabulated and statistically analyzed.

Results:

Effect of pH change of the culture media on the viability of hDPSCs cultured in different solutions

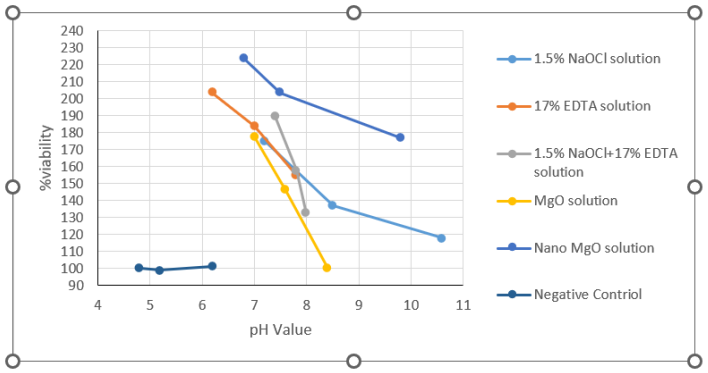
a Person's correlation analysis was conducted to correlate the pH versus percentage viability (Table 1). Results showed that, as the % viability of cells increase for all tested solutions, the pH of cells' culture decrease. No significant difference was reached for any of the tested solutions, unless for MgO (microparticles) solution, a significant difference was detected ($p<0.05$).(Figure 1)

Table (1): Correlation analysis for pH pattern obtained from hDPSCs cultured with different solution and % viability of the cell

		1hr	24hrs	72hrs	Pearson r	p-value
1.5% NaOCl	pH	10.6	8.5	7.2	-0.94802	0.206 [ns]
	%Viability	118.1%	137.1%	174.9%		
17% EDTA	pH	7.8	7	6.2	-0.99802	0.069 [ns]
	%Viability	155%	183.9%	203.6%		
1.5%NaOCl+ 17% EDTA	pH	8	7.8	7.4	-0.9919	0.08[ns]
	%Viability	132.2%	157.9%	189.9%		
MgO	pH	8.4	7.6	7	-0.99969	0.016 [s]
	%Viability	100.3%	146.1%	177.5%		
Nano- MgO	pH	9.8	7.5	6.8	-0.97373	0.146 [ns]
	%Viability	177.1%	203.3%	223.9%		
Negative Control	pH	6.2	5.2	4.8	0.558207	0.623 [ns]
	%Viability	101.3%	99%	100.5%		

ns: non-significant, s: significant ($p \leq 0.05$)

Figure (1): XY (Scatter) illustrating negative correlation between pH of culture media and % Viability of hDPSCs in all the tested groups



Discussion:

A successful consequence of root canal treatment depends on efficient disinfection of the root canals, including the use of irrigation solutions, which differ in their antibacterial and cytotoxic properties.8

Cytotoxicity of root canal irrigants is crucial due to their close contact with vital tissues. With the development of regenerative endodontics, we must be concerned with the preservation of host cells in addition to the decline of microorganisms.9,10

Permanent dental pulp stem cells (pDPSCs) were the first stem cells to be isolated and recognized, which have distinctive features as mesenchymal stem

cells, signifying plastic adherence and clonogenicity.11

NaOCl is the most used agent for chemical disinfection in endodontic treatment, including REPs. It has a superior antibacterial efficacy and tissue dissolution capacity. However, it was observed to have toxic effects on stem cells.12

Several studies have proposed that EDTA is the most stem cell-friendly irrigant and suggested its use during regenerative endodontics.12

Nanomaterials offer exceptional physicochemical characteristics. Increased surface to volume ratio and increased number of atoms present close to the surface compared with micro-/macrostructures are proposed to promote the distinct properties of nanomaterials.13 Nano-Magnesium oxide is a metal oxide that has been displayed antibacterial activity against gram- positive and gram-negative bacteria. In addition, it has been described that nano-MgO particles present antibacterial activity against spores, and viruses.5

In the present study we evaluated the effect of magnesium oxide irrigating solutions prepared from nano and microparticle sizes on the extracellular pH changes of DPSCs' culture.

To investigate the effect of pH change of the culture media on the viability of hDPSCs cultured in different irrigation solutions, a Person's correlation analysis was conducted to correlate the pH versus percentage viability. Results showed a negative correlation between the pH and % viability of cells for all tested solutions. There wasn't significant difference between any of the tested solutions, unless for MgO (microparticles) solution, in which a strong negative correlation was obtained. On the contrary, Hirose et al.14 observed different results. They found that decreased pH levels were accompanied by cell death. This difference in results with our study might be attributed to the use of different solutions or subjecting cells to lower ranges of pH levels.

Conclusion:

The increase of % viability for hDPSCs cultured with different irrigation solutions is accompanied by decrease of the extracellular pH around the cells.

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