ORIGINAL RESEARCH

In Vitro Cytotoxic Evaluation of Mineral Trioxide Aggregate with Silver and Titanium Dioxide Nanoparticles

Teena Sheethal Dsouza[1], Mithra N Hegde[2], Vishakh Radhakrishna[3], Neevan Dsouza[4], Suchetha Kumari[5]

Abstract

Aim: The aim of the study was to evaluate the cytotoxicity of mineral trioxide aggregate (MTA) when mixed with either silver or titanium dioxide (TiO_2) nanoparticles on human lymphocytes.

Materials and methods: Human lymphocytes were exposed to white MTA or MTA in combination with silver or TiO_2 nanoparticles. The cytotoxicity was assessed using the MTT assay for freshly mixed pellets, at 24 hours, at 48 hours, and at 72 hours time periods. The results were statistically analyzed.

Results: There was no significant difference in the experimental groups with regard to cell viability. The addition of nanoparticles to MTA showed similar cell viability compared with MTA alone.

Conclusion: Mineral trioxide aggregate mixed with silver or TiO_2 nanoparticles showed similar biocompatibility to MTA alone.

Clinical significance: The cytotoxicity exhibited by silver or TiO_2 nanoparticles when mixed with MTA showed no significant difference and therefore, they can be used as a suitable additive to improve the antimicrobial efficacy of MTA.

Keywords: Cytotoxicity, Lymphocytes, Mineral trioxide aggregate, MTT assay, Nanoparticle, Silver, Titanium dioxide.

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Introduction

The introduction of mineral trioxide aggregate (MTA) in endodontics has satisfied most of the ideal properties of root-end filling material.[1–3] However, its limitations, such as long setting time and limited antibacterial activity have urged attempts to introduce newer substitutes and formulations.[2]

Metallic nanoparticles incorporated into dental materials have shown to improve the physicochemical, mechanical, and antibacterial properties.[4] Due to their high surface area to volume ratio and favorable properties, these nanoparticles have dawned as novel antimicrobial agents.[5] Silver nanoparticles have been added to composite resin and MTA to enhance the physical and antibacterial effects of the materials.[6,7] Nanosilver has also shown to serve as an excellent MTA additive against anaerobic endodontic-periodontal microbes.[8] Titanium dioxide (TiO_2) nanoparticles have been used in dentistry for a wide array of applications. It has been shown to improve the physical and antibacterial properties of restorative materials, such as glass ionomer cement (GIC) and root canal sealers.[9,10] One study on TiO_2 nanoparticles has also shown to shorten the setting time and improve the compressive and flexural strength.[11] However, in spite of the improved antibacterial results of these nanoparticles when added to MTA, the cytotoxicity of such combinations is a prime concern, due to the direct contact of these materials with periodontal tissues.[2]

The aim of this study was to evaluate and compare the cytotoxicity of MTA when combined with silver nanoparticles or TiO_2 nanoparticles on human lymphocytes.

Materials and Methods

Human lymphocytes were used in this study, which was obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were grown in Dulbecco Modified Eagle medium supplemented with 5% fetal bovine serum, 100 U mL^{-1} penicillin, 100 µL mL^{-1} streptomycin, and 2 mmol L^{-1} L-glutamine, at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Mineral trioxide aggregate pellet was prepared by mixing MTA with the nanoparticles.

The human lymphocyte cells were seeded into 96-well plates (2.7×10^4 cells) and incubated for 24 hours to allow adhesion. Then, nine MTA pellets of each experimental group were placed into the culture wells. Cells were treated in four groups.

Group I: no treatment (control group).

Experimental Groups

Group II: MTA mixed with distilled water.
Group III: MTA mixed with silver nanoparticles.
Group IV: MTA mixed with TiO_2 nanoparticles.

[1,2]Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, NITTE Deemed to be University, Mangaluru, Karnataka, India

[3,5]Central Research Laboratory, KS Hegde Medical Academy, NITTE Deemed to be University, Mangaluru, Karnataka, India

[4]Department of Humanities, Biostatistics, KS Hegde Medical Academy, NITTE Deemed to be University, Mangaluru, Karnataka, India

Corresponding Author: Teena Sheethal Dsouza, Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, NITTE Deemed to be University, Mangaluru, Karnataka, India, Phone: +91 9845732757, e-mail: drteena@nitte.edu.in

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Mineral trioxide aggregate pellets were tested at 0 hour (freshly mixed) after 24, 48, and 72 hours incubation for cytotoxicity by MTT assay. Spectroscopic absorbance (optical density) was measured at 630 nm using an ELISA microplate reader.

The results were evaluated by one-way analysis of variance. The mean differences between the groups were compared by Tukey honestly significant difference *post hoc* test. Differences were considered significant at $p < 0.001$ (Table 1).

Results

For freshly mixed samples, there was a statistically significant difference between the control group and the experimental groups. However, there was no significant difference between the experimental groups except between groups III and IV ($p < 0.001$) (Table 2).

After 72 hours of incubation, the difference between the experimental groups was not statistically significant ($p < 0.001$) (Tables 3 and 4).

Discussion

Metallic nanoparticles have been used in dentistry since recently in various restorative materials; they have been proven to contribute largely to the enhancement of the bacteriostatic and bactericidal properties.[1]

The addition of nanoparticles to MTA and other calcium silicate-based cement has been shown to improve its antibacterial activity. Samiei et al. observed that MTA mixed with silver nanoparticles enhanced antimicrobial effects against various microbial species.[7] The antibacterial and physicochemical properties of TiO_2 nanoparticles have also been well-documented. Mouthrinses containing TiO_2 nanoparticles are be effective against *Streptococcus mutans* and *Streptococcus sanguis*.[12] However, the clinical success of a biomaterial largely and solely depends on the reaction of the host tissue to the material. The biocompatibility of MTA alone has been reported to be favourable with dental pulp and fibroblast cells in previous literature.[13,14] The cytotoxic reactions that occur in the host tissues with the addition of these nanoparticles needs to be evaluated. The cytotoxicity of the experimental groups was analysed using the MTT assay. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) depends upon the capacity of mitochondrial dehydrogenase enzyme to convert the water-soluble tetrazolium salt into dark blue formazan crystals. Cell viability depends on the quantity of formazan that is produced in the reaction.[15] Greater the optical density value, the greater the survival of lymphocytes and less toxic is the material. The cell lines that were used in this study were human lymphocytes. The advantage of using these cell lines is that they are karyotypically normal human cells and claim to be standard indicators for any systemic burden by exposure factors.[16] Therefore, human lymphocytes were chosen in the present study.

In our study, for the freshly mixed samples, the cell viability of MTA mixed with silver or TiO_2 nanoparticles showed a statistically significant difference. However, the cytotoxicity exhibited by the set cement experimental groups with silver and TiO_2 nanoparticles at 72 hours, was similar to that of MTA alone. This is in accordance with the study by Samiei et al., in which the incorporation of TiO_2 nanoparticles to MTA showed to have no negative effect on the biocompatibility of MTA.[1] However, there are certain contradictory

Table 1: For freshly mixed cement pellets analysis of variance (ANOVA)

	n	*Mean*	*SD*	*Minimum*	*Maximum*
1	9	0.391291	0.0655917	0.2912	0.4749
2	9	0.248958	0.0545548	0.1722	0.3038
3	9	0.211788	0.0163765	0.1949	0.2350
4	9	0.378183	0.1050941	0.2311	0.5164
Total	36	0.307555	0.1028419	0.1722	0.5164

$F = 15.881$; $p < 0.001$

Table 2: *Post hoc* test

				95% confidence interval	
(I) group	*(J) group*	*Mean difference (I–J)*	*p value*	*Lower bound*	*Upper bound*
1	2	0.1423328	0.001	0.055259	0.229407
	3	0.1795028	0.001	0.092429	0.266577
	4	0.0131078	0.977	−0.073966	0.100182
2	3	0.0371700	0.658	−0.049904	0.124244
	4	−0.1292250	0.002	−0.216299	−0.042151
3	4	−0.1663950	0.001	−0.253469	−0.079321

Table 3: Set cement pellets after 72 hours

	n	*Mean*	*SD*	*Minimum*	*Maximum*
1	9	0.391291	0.0655917	0.2912	0.4749
2	9	0.286215	0.0558910	0.2270	0.3928
3	9	0.225848	0.0317397	0.1775	0.2778
4	9	0.219333	0.0214186	0.1834	0.2479
Total	36	0.280672	0.0832305	0.1775	0.4749

Table 4: *Post hoc* test

				95% confidence interval	
(I) group	*(J) group*	*Mean difference (I–J)*	*p value*	*Lower bound*	*Upper bound*
1	2	0.1050761	0.001	0.044857	0.165295
	3	0.1654428	0.001	0.105223	0.225662
	4	0.1719578	0.001	0.111738	0.232177
2	3	0.0603667	0.049	0.000147	0.120586
	4	0.0668817	0.025	0.006662	0.127101
3	4	0.0065150	0.991	−0.053704	0.066734

studies in previous literature, which indicate a discrete use of nanoparticles for medical and dental applications. A study by Tabari et al. concluded that higher concentration and longer duration of exposure with TiO_2 nanoparticles caused increased cell death.[17] Shantiaee et al. tested the biocompatibility of nanosilver coated gutta-percha after 24 hours and found it to favorable; with the lowest cytotoxicity among the experimental groups after 1 week.[18]

Though the improved antibacterial activity and other physical properties of the filling materials incorporated with these nanoparticles are reported in previous works of literature the biocompatibility of these combinations is a major issue. Further biological tests, such as cell viability and genotoxicity tests must be conducted to use these materials in clinical applications.

Conclusion

Under the limitations of this study, MTA combined with silver or TiO_2 nanoparticles showed favorable cell viability. Thus, these nanoparticles could be potential additives to enhance the biological and physicochemical properties of MTA without altering its biocompatibility.

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