

Original Article

Effect of containing silica fume on cytotoxicity of white mineral trioxide aggregate

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ABSTRACT

Background: Mineral trioxide aggregate (MTA) has a high biocompatibility and its physical properties could be improved by adding the containing silica fume an amorphous silicon dioxide (condensed silica fume [CSF]). The aim of this study was to evaluate the cytotoxicity of MTA mixed with CSF on the viability of L929 mouse fibroblast cell using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay (MTT assay).

Materials and Methods: In this *in vitro* study white MTA was mixed with distilled water according to the manufacturer's instructions. Mixtures of White MTA with 10%, 15%, and 20% CSF by weight were prepared and mixed with distilled water. Cytotoxicity of mixtures was compared with MTT assay on L929 mouse fibroblast cell line after 24, 48, and 72 h. Differences in cytotoxicity were assessed by one-way analysis of variance (ANOVA).

Results: Mean \pm SD of vital cell counts cultured in MTA, MTA + 10% CSF, MTA + 15% CSF, and MTA + 20% CSF were $98\% \pm 6\%$, $97\% \pm 6\%$, $94\% \pm 4\%$, and $98\% \pm 4\%$, respectively. One-way ANOVA did not reveal any statistically significant difference between the groups ($P > 0.05$).

Conclusion: It may be concluded that addition of CSF to MTA may not influence its cytotoxicity.

Key Words: Cytotoxicity, fibroblasts, mineral trioxide aggregate, silicon dioxide

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INTRODUCTION

Mineral trioxide aggregate (MTA) was introduced by Lee *et al.* for repairs on experimentally produced root perforations.^[1] MTA is a remarkable root-end filling material, and there are two types of formula of ProRoot MTA: gray-colored and a tooth-colored formula. In comparison with amalgam or zinc oxide-eugenol, MTA has presented less leakage in leakage tests.^[2,3] Moreover, *in vitro* cytotoxicity^[4] and its biocompatibility were investigated when inserted into the bone and subcutaneous connective tissue.^[5,6] On the other hand, the applications of

MTA are as pulp-capping material,^[7] restoration of root perforation,^[8] block an apical in controlling the immature teeth (when the apics are open)^[9,10] and as filling materials in the root canal treatment.^[11] However, MTA is a biocompatible material in comparison to the traditional materials which were used in the root-end filling process and root repair. It is an expensive material and is difficult to use in the surgical site due to the small size of the root end.^[12] MTA could better show marginal adaptation when compared to

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the other materials such as intermediate restorative material (IRM) and super ethoxy benzoic acid.^[13] Healing of the surrounding tissues after using MTA as a root-end filling material is reported.^[14] Moreover, by adding condensed silica fume (CSF), physical properties of cement would be improved by two means: CSF acts as a filler and bridges between the existed pores in the cement and interacts with calcium hydroxide for the production of more solid volumes of calcium silicate hydrate gel.^[15] Regarding the similarity of MTA structure to the Portland cement, the addition of CSF could increase the strength of MTA. Compressing strength of materials which are used in the root-end filling process or perforation is important for the treatment success.^[16,17] In addition, root filling material must be biocompatible and nontoxic; as otherwise, an inflammatory reaction in the surrounding tissue of the root may be found. Therefore, evaluation of the toxicity would be as one of the approval steps for the end filling material to be used in the biological environment.^[18] Several studies have determined the great biocompatible potential of the MTA and its ability to stimulate the growth of cells.^[19-21] Since the addition of CSF may cause changes in the biological behavior of the MTA, performing the toxicity test is a matter of great importance. The aim of the present study was to evaluate the cytotoxicity of MTA mixed with CSF on L929 mouse fibroblast cells with 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide reduction assay (MTT assay).

MATERIALS AND METHODS

The mouse fibroblast cell line (L929) was obtained from National Cell Bank, Pasteur Institute, of Iran. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO, USA), and 100 IU/ml ampicillin and 100 µg/ml streptomycin (Sigma, USA). The cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C. After incubation for 1 week, the monolayer was harvested by trypsinization and the viability of cells was tested by trypan blue exclusion test. A cell suspension of 6000 cell/ml was seeded in each well, keeping one well as a control without any sample (negative control), and then maintained in an incubator for 96 h.

Four mixtures of MTA and CSF were prepared according to the manufacturer's protocol as follows and placed (3 mm thickness) within two 12-well

plates (CELLSTAR, Greiner Bio-One) and incubated under ultraviolet to set up the material at 37°C, 98% humidity for 24 h.^[22]

1. White root MTA (Tulsa Dental Products, Tulsa, OK, USA)
2. White root MTA + 10% w/w CSF
3. White root MTA + 15% w/w CSF
4. White root MTA + 20% w/w CSF.

Preparing the extraction

Culture media with or without serum could be used as an extraction vehicle, according to the guidelines of ISO 10993:9-5:4.2.2.^[23] In this study, 4 ml of complete DMEM with serum was added to each well of plates and kept at 37°C for 24 h.^[24] After 24 h, the extractions were filtered by filter 0.22 µm (Schleicher and Schwell), and finally, 8 ml of extraction was made for each material (negative control group). Moreover, distilled water was used as an extraction vehicle in the positive control group. After adjusting the pH of extractions, the media were removed from the plates, and 150 µl of extraction was loaded into the 96-well plates. Then, the plates were incubated at 37°C with 98% humidity and 5% CO₂.^[25]

3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide reduction assay

Cell viability was assessed using an MTT assay kit (Sigma-Aldrich, USA). Cells were incubated with 150 µl of MTT solution (5 mg/ml MTT in phosphate-buffered saline) at 37°C for 4 h, 98% humidity, and 5% CO₂. MTT-containing medium was removed and 150 µL 0.04 mol/L HCl in isopropanol was loaded to each well. Plates were shaken for 10 min at room temperature to dissolve precipitated formazan crystals. Then, 100 µL from each well was placed into 96-well ELISA plates [Figure 1] and supernatant optical density (OD) values were measured at 570 nm using an ELISA plate reader (Anthos 2020, Australia). The MTT assay was performed in triplicate and repeated twice.

Statistical analysis

After calculating the mean ± standard deviation of OD, a one-way analysis of variance was performed to compare the effect of the material on cell viability in negative and positive control groups after 24, 48, and 72 h, and *P* < 0.05 was considered statistically significant.

RESULTS

The results of the statistical analysis showed that the viability of the cells was not significant between the groups in three measured times ($P > 0.05$). The mean viability percentage of the cells is shown in Table 1.

DISCUSSION

The main components of the MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide. Besides these oxides, other inorganic oxides are also responsible for the physical and chemical properties of MTA. MTA is a powder that contains hydrophilic particles and hardens in the presence of water. In this study, CSF with different percentages (10%, 15%, and 20%) was added to the MTA, and its cytotoxic effect was investigated by MTT assay. In the metallurgical process, adding the CSF could improve the physical properties of the cement. In the previous studies, adding 10%–20% CSF to the Portland cement was reported.^[26]

In this study, MTT assay was performed on L929 cell line as reported by Haglund *et al.* They investigated the effects of four root-end filling materials (MTA, IRM, amalgam, and retroplast) on the cell

growth, cell morphology, and interleukin (IL)-1 β and IL-6 production in mouse fibroblasts and macrophages.^[27] Gorduysus *et al.* in 2007 compared the cytotoxicity (by MTT assay), apoptosis/necrosis, and apoptotic process in human periodontal ligament (PDL) fibroblasts which were treated with White ProRoot MTA, Diaket, Endion, and CYMED 8410. Effect of MTA after 24, 48, and 72 h showed no significant differences in MTT reduction and viable cell number in comparison to controls. However, exposure of PDL fibroblasts to three materials (Diaket, Endion, and CYMED 8410) after 24, 48, and 72 h showed a significant cytotoxicity with MTT and a decrease of viable cell number in comparison with controls (from $P < 0.05$ to $P < 0.001$).^[18] These results showed high biocompatibility of MTA like our results. Other studies have examined the biocompatibility of the MTA by evaluating its effect on MG63 cell line,^[28] SaoS2 cell line,^[29] and human osteoblasts.^[30] In this study, DMEM was used according to other studies, but with different concentrations of ampicillin and streptomycin.^[20] Several studies corroborated the use of MTA in the root-end treatment.^[21,31,32] In line with these findings, our results suggested that using MTA alone or mixed with CSF represented no significant differences in the viability of cells.

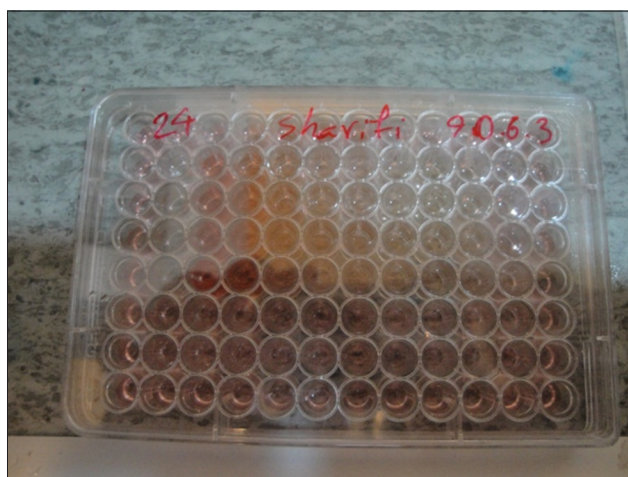


Figure 1: 96-well ELISA plates containing samples.

Table 1: Mean \pm standard deviation of viability percentage of the cells

Treatment Time	MTA	MTA+10% CSF	MTA+15% CSF	MTA+20% CSF	P
24 h	98 \pm 6	97 \pm 6	94 \pm 4	98 \pm 4	0.06*
48 h	95 \pm 5	96 \pm 7	94 \pm 7	90 \pm 7	0.09*
72 h	93 \pm 7	90 \pm 7	89 \pm 5	87 \pm 6	0.55*

*No significant ($P > 0.05$). MTA: Mineral trioxide aggregate; CSF: Condensed silica fume

CONCLUSION

No significant differences were found between the effect of MTA and MTA mixed with CSF (10%, 15%, and 20%) on the viability of cells via MTT assay during the 24, 48, and 72 h exposure. It is suggested that a mixture of MTA and CSF be used in endodontic treatments.

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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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