

## ORIGINAL RESEARCH

# An *in vitro* Evaluation of the Cytotoxicity of Varying Concentrations of Sodium Hypochlorite on Human Mesenchymal Stem Cells

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## ABSTRACT

**Aim:** To evaluate and compare the cytotoxicity of various concentrations of sodium hypochlorite on immortalized human bone marrow mesenchymal stem cells (MSCs).

**Materials and methods:** The 5.25 percent sodium hypochlorite (NaOCl) at concentrations of 0.5, 0.1, 0.025, 0.0125, and 0.005 mg/ml were used to assess the cytotoxic effect on MSCs. Immortalized human bone marrow mesenchymal stem cells (hTERT-MSCs) were exposed to NaOCl at 5 different concentrations. Cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and alamarBlue assays. The cell morphology changes were assessed with scanning electron microscopy (SEM) after exposure to 2, 4, and 24 hour incubation. The ethidium bromide/acridine orange (EB/AO) fluorescent stain was applied to the cells in the 8-chamber slides after they were incubated with the testing agents for 2 and 4 hours to detect live and dead cells. The observations were quantitatively and qualitatively analyzed.

**Results:** The cell viability study using MTT assay and AB assay showed significant reduction with varying concentration at 2 and 4 hours incubation period. The cell viability decreased with the higher percentage of NaOCl. The exposure time also revealed an inverse relation to the cell viability. The SEM analysis showed reduction in the number of cells and morphological alterations with 0.5 mg/ml at 2 and 4 hours compared to 0.025 mg/ml NaOCl. Destruction of the cells with structural alterations and lysis was evident under fluorescence microscope when the cells were exposed to 0.5 mg/ml NaOCl.

**Conclusion:** Within the limitations of this *in vitro* study it can be concluded that NaOCl is toxic to the human bone marrow MSCs. The cell lysis was evident with higher concentration of sodium hypochlorite. From the observations, it can be concluded that a lower concentration of NaOCl may be used as endodontic irrigant due to its cytotoxic properties. Further studies are man-

datory to evolve a consensus on the optimal concentration of sodium hypochlorite to be used as endodontic irrigant.

**Keywords:** Sodium hypochlorite, Cytotoxicity, Mesenchymal stem cells, Antimicrobial activity, Irrigation solutions.

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**Conflict of interest:** None declared

## INTRODUCTION

Biomechanical preparation during the root canal treatment involves cleaning and shaping procedures with endodontic instruments and irrigant solutions. Antimicrobial agents are used as irrigant for the successful elimination of bacterial contaminants from the root canal. The irrigant solutions remove dentine debris, dissolve organic materials, disinfect the canal system, and provide lubrication during biomechanical preparation. Sodium hypochlorite (NaOCl) is the most widely used irrigant in endodontic treatment because of its pronounced antimicrobial activity and the ability to dissolve organic soft-tissue in the root canal system.<sup>1-4</sup> Sodium hypochlorite is commonly used for irrigation of root canals in concentrations ranging from 0.5 to 5.25%. At higher concentrations, its solvent and antiseptic properties are greater, but so are its toxic effects.<sup>5,6</sup>

*In vitro* studies conducted on bovine pulp tissue with varying concentration of NaOCl showed rapid dissolution of the tissue with higher concentration.<sup>7,8</sup> Studies also showed that preheating of NaOCl resulted in greater solvent capacity of human pulp tissue.<sup>9,10</sup> The use of 5.25% NaOCl as endodontic irrigant has been supported for its desirable properties, such as effectiveness in dissolving organic tissue, greater antibacterial action, more alkaline pH, and shorter effectiveness time.<sup>11,12</sup>

One of the drawback of NaOCl is its contact with vital tissues which can produce severe inflammation and tissue necrosis, with complications ranging from minor discomfort to severe tissue necrosis and nerve damage.<sup>13</sup> The inadvertent introduction of NaOCl beyond the root canal system as well as spillage of NaOCl during dental procedures can also result

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in serious consequences including chemical dermatitis and chemical injuries to the eyes which may result in endophthalmitis and blindness.<sup>3,14</sup> The deleterious effects of NaOCl depends on the concentration and pH. The alkaline property enhances its tissue penetration causing deep and extensive tissue damage.<sup>15</sup> It causes liquefaction of the organic material by reacting with fatty acids and amino acid.<sup>16</sup>

Although, NaOCl has been used as the most popular agent for endodontic irrigation, there is no universal consensus regarding the concentration for optimal results without adverse reactions.<sup>1</sup> Sirtes et al<sup>10</sup> showed that 1% NaOCl is sufficient to dissolve the pulp tissue. Even though higher concentrations of NaOCl has better antimicrobial effect and tissue dissolving capacity it carries a risk of toxicity and tissue reaction.<sup>17</sup> Therefore, it is imperative to find out the optimal concentration of NaOCl that can be used as endodontic irrigant with no inadvertent action. Hence, the present study is conducted to evaluate the cytotoxicity of various concentration of NaOCl on human bone marrow mesenchymal stem cells.

## MATERIALS AND METHODS

The 5.25 percent NaOCl at concentrations of 0.5, 0.1, 0.025, 0.0125 and 0.005 mg/ml were used to study the viability of exposed human bone marrow MSCs. The cell morphology studies were performed with 0.5 and 0.025 mg/ml of NaOCl.

### Cell Culture

The protocol of Simbula et al<sup>18</sup> with some modifications, was followed in this study. Immortalized human bone marrow mesenchymal stem cells (hTERT-MSC-C1), at the 25th passage, were used for all experiments in this study. These cells were a kind gift from Professor Moustapha Kassem, Odense University Hospital, Denmark.<sup>19</sup> The cryopreserved cells were rapidly thawed, transferred into a T-75 flask (BD Falcon™, NJ, USA) and cultured in Dulbecco's modified Eagle's medium (Gibco®), which was supplemented with glutamine (Gibco®), 1% penicillin-streptomycin (Gibco®), 10% fetal bovine serum (FBS Gibco®), and 1% non-essential amino acids (HyClone®), in a humidified atmosphere of 5% CO<sub>2</sub>/ 95% O<sub>2</sub> at 37°C. At 70% confluence, the medium was aspirated, and the cells were washed with phosphate buffered saline. Three milliliters of prewarmed 0.05% trypsin/EDTA (Gibco®) was added to the flask, and the cells were incubated for 1 minute. After gentle tapping, cell detachment was checked under an inverted light microscope (Observer A1, Zeiss®, Gottingen, Germany), and 12 ml of culture media was then added to the flask to neutralize the enzymatic effect of trypsin. For cell counting, two samples (10 µl) were taken from the cell suspension after appropriate

mixing. The samples were placed in the upper and lower chambers of a Neubauer hemocytometer counting chamber (Paul Marienfeld GMBH & Co. KG.), and cells were counted manually under an inverted microscope (10× magnification). Cells were seeded on different plates and slides, as discussed below, for each part of the study. All procedures were performed under a class II laminar flow hood (LabGard ES 425 Biological Safety Cabinet, NuAire®).

### MTT Cell Viability Assay

Cells were seeded in 96-well plates (clear, flat bottom, polystyrene TC-Treated 96-well microplates) at a concentration of 1×10<sup>4</sup> cells/well and were then incubated for 24 hours to allow cell adherence to the bottom of the wells. Culture media were then aspirated from each well and replaced with 150 µl of sterile NaOCl solution. Eight wells were used as replicates for each group.

Sodium hypochlorite groups was subdivided into the following:

1. NaOCl (0.5) group
2. NaOCl (0.1) group
3. NaOCl (0.025) group
4. NaOCl (0.0125) group
5. NaOCl ( 0.005) group.

Each group was incubated for the following periods: 2, 4 or 24 hours. Then, 10 µl of the MTT reagent (Cayman Chemical Company, Ann Arbor, MI, USA) was added to each well. Thereafter, 96-well plates were further incubated for 3 hours at 37°C. Finally, the solution from each well was aspirated, and 150 µl of dissolving agent was added to dissolve the formazan precipitate. The absorbance of each sample was measured with a microplate reader (Epoch Microplate Spectrophotometer, BioTek®) at a wavelength of 570 nm. The data were gathered using Gen<sup>5</sup> Data Analysis Software (BioTek®, USA). The experiment was repeated twice for each group at each interval.

### AlamarBlue (AB) Cell Viability Assay

The same groups that were used for the MTT assay (mentioned above) were used for the AB assay with the same intervals. The test agents were added to each well. After 2, 4 and 24 hour incubation periods, 10% AB reagent (Serotec®, Oxford, UK) was added to each well. After the plates were further incubated for 4 hours, the fluorescence of each well was measured at wavelengths of 530/25 and 590/35 nm excitation/emission using a fluorescence reader (BioTek®). The data were gathered using the Gen5 Data Analysis Software (BioTek®, USA). The experiment was repeated twice for each group at each interval.

## Cell Morphology Assessment

Sodium hypochlorite group was subdivided into the following:

1. NaOCl (0.5) group
2. NaOCl (0.025) group

Cell morphology was evaluated with SEM after 2 and 4 hours of exposure to the test solutions. Briefly,  $8 \times 10^4$  cells/well were seeded on glass cover slides ( $1 \times 1.5$  cm) in 6-well plates (CellStar<sup>®</sup>, Carrollton, TX) overnight. The next day, the cells were exposed to the test solutions. Two milliliters of each sterile irrigation solution was added to the slides in each well. Control untreated cells were maintained in culture medium. Immediately after adding the test solutions, the cells were examined under an inverted LM (10 $\times$  magnification). At the end of the incubation periods (2 and 4 hours), the solutions in each well were aspirated. The slides were washed with PBS and were then fixed with 2.5% glutaraldehyde in 0.1 M Sodium cacodylate buffer (pH 7.2) at room temperature. Thereafter, the specimens were washed with 0.1 M sodium cacodylate buffer (pH 7.2).

After fixation, the specimens were treated with 1% osmium tetroxide for 1 hour. Then, they were washed with distilled water and dehydrated using graded ethyl alcohol, in concentrations of 50, 70, 80, 90 (5 minutes each), 95 (twice, 15 minutes each), and finally, 100% absolute alcohol (twice, 30 minutes each). The specimens were dried using a critical point dryer with CO<sub>2</sub> (SADRI-PVT-3B). Slides were mounted on copper stubs with double adhesive tape and were then gold sputter coated to a thickness of 5 to 7  $\mu$ m. The specimens were then observed and photographed using a JSM-6360 LV scanning electron microscope.

Two evaluators assessed the photomicrographs. The cell morphology was described according to the following criteria: the shape of the cell (normal or abnormal compared with the control), attachment to the subsurface, attachment to other cells, cytoplasmic surface extensions (blebs or microvilli), and cell wall integrity. Roundness of the cells, the presence of blebs, or detachment of the cells indicated greater cell injury.<sup>20,21</sup>

## Live/Dead Analysis

The NaOCl group, was subdivided into the NaOCl (0.5) and NaOCl (0.025) groups. Two solutions were selected for the live/dead analysis. A modified Eagle's medium (MEM) without phenol red (Gibco<sup>®</sup> Gaithersburg, MD) was used for this experiment. Briefly, cells were seeded in three 8-chamber slides (Lab-Tek<sup>®</sup>) at a concentration of  $1.5 \times 10^4$  cells/well and were then incubated for 24 hours to allow cell adhesion to occur. The culture medium in each well was replaced with 300  $\mu$ l of each solution and then

incubated for 2 hours. Finally, 10  $\mu$ l of EB/AO fluorescent dye was added to each chamber, and the fluorescence of the cells was analyzed under a fluorescent inverted microscope (ECLIPSE Ti, Nikon, Tokyo, Japan), with 10 $\times$  magnification. Images were captured with imaging software (NIS-Elements, Nikon, Tokyo, Japan). The EB/AO fluorescent dye was prepared by mixing 15 mg acridine orange with 50 mg ethidium bromide powder that was first dissolved in 1 ml 95% ethanol and then diluted in 49 ml of distilled water (DW).

The images were evaluated by two evaluators according to previously described criteria. Under the green filter, an intact green nucleus, which is comparable to the control, indicates a viable cell, whereas a green fragmented nucleus indicates early apoptosis. A red nucleus indicates a ruptured cell wall, whereas a red intact nucleus indicates necrosis, and a fragmented red nucleus indicates late apoptosis under the red filter.<sup>22,23</sup>

## Statistical Analysis

The results of the MTT and AlamarBlue assays were calculated as percentages relative to the control (100% = no toxicity). The data were analyzed using SPSS Pc+ version 21.0 statistical software. Descriptive statistics (mean and standard deviation) were used to describe continuous outcome variables. Student's t-test for independent samples was used to compare the mean values of two groups. A one-way analysis of variance, followed by a multiple comparison Tukey's test, was used to compare the mean values of the three groups. A p-value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

### MTT Assay

The MTT assay was conducted on four concentrations of NaOCl and control group with cultured hTERT-MSCs in 96-well plates. Each group was incubated for 2, 4 and 24 hours. The cell viability for each group is presented as percentage of the control group at each time point (Table 1 and Graph 1). When the cells were exposed to NaOCl for 2 hours, the cell viability at the four concentrations (0.5, 0.1, 0.0125 and 0.005 mg/ml) decreased significantly to 39.4, 60.2, 71.9 and 82.6%, respectively ( $p < 0.05$ ). However, the cell viability of the (0.025) group was 93.9%, which was not significantly different from the control ( $p > 0.05$ ). When the exposure time increased to 4 hours, the cell viability at the four concentrations (0.5, 0.1, 0.0125 and 0.005 mg/ml) decreased significantly to 35.5, 62.4, 82 and 85.7% respectively ( $p < 0.05$ ). However, the cell viability of the (0.025) group was 96.4%, which was not significantly different from the



**Table 1:** The cell viability percentage for various concentrations of NaOCl at 2, 4 and 24 hours after exposure using MTT assay

Groups	Percentage viability		
	2 hours	4 hours	24 hours
Control	100	100	100
0.5 NaOCl	39.4	35.5	22.2
0.1 NaOCl	60.2	62.4	34.7
0.025 NaOCl	93.9	96.4	33
0.0125 NaOCl	71.9	82	33.6
0.005 NaOCl	82.6	85.7	36.8

control ( $p > 0.05$ ). After the 24 hours exposure time, at all concentrations (0.5, 0.1, 0.025, 0.0125 and 0.005 mg/ml), the cell viability decreased significantly to 22.2, 34.7, 33, 33.6 and 36.8%, respectively ( $p < 0.05$ ).

**AlamarBlue (AB) Assay**

The AB assay was conducted for the NaOCl and control groups using cultured hTERT-MSCs in 96-well plates. The cell viability for each group is presented as the percentage of the control group (Table 2 and Graph 2). When the cells were exposed to NaOCl, at all concentrations (0.5, 0.1, 0.025, 0.0125 and 0.005 mg/ml), the cell viability decreased significantly to 13, 17.4, 34.3, 70.5 and 68.1% respectively, after 2 hours. The cell viability further decreased to 10.2, 17.8, 30.9, 26.7 and 51.6% respectively, after the 4 hours incubation and to 4.6, 5.7, 4.4, 4.1 and 3.8% respectively, after 24 hours ( $p < 0.05$ ).

**Cell Morphology**

*SEM Observations*

According to the SEM analysis, the cell number decreased relative to the control after a 2 hours exposure to 0.5 mg/ml NaOCl (Figs 1A and B). The remaining cells were shrunk, with a thread-like or round shape (Figs 1E and F). Cells became detached from the subsurface, and cell-to-cell attach-

**Table 2:** The cell viability percentage for various concentrations of NaOCl by alamarBlue assay at 2, 4 and 24 hours of exposure

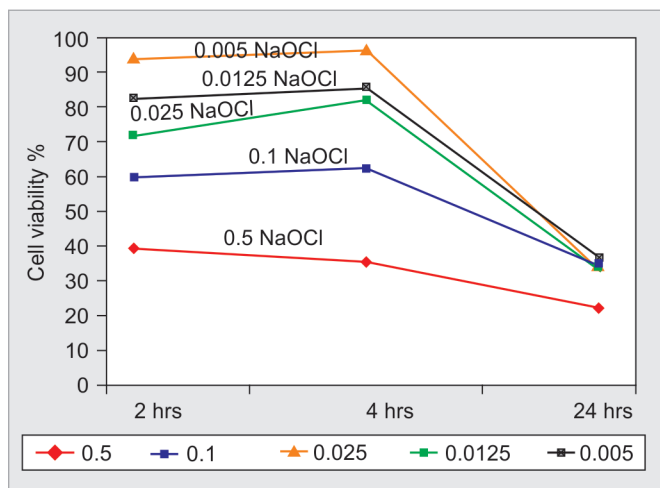
Groups	Percentage viability		
	2 hours	4 hours	24 hours
Control	100	100	100
0.5 NaOCl	13	10.2	4.6
0.1 NaOCl	17.4	17.8	5.7
0.025 NaOCl	34.3	30.9	4.4
0.0125 NaOCl	70.5	26.7	4.1
0.005 NaOCl	68.1	51.6	3.8

ments were lost. Lysosomal secretions emerging from the cell can be observed, and the nucleus was extruded through the ruptured cell wall, which is clearly demonstrated in (Fig. 1F). The morphological changes at a 4 hour exposure were similar to those at 2 hours (Figs 2A and B) but with more intense destruction (Figs 2E and F).

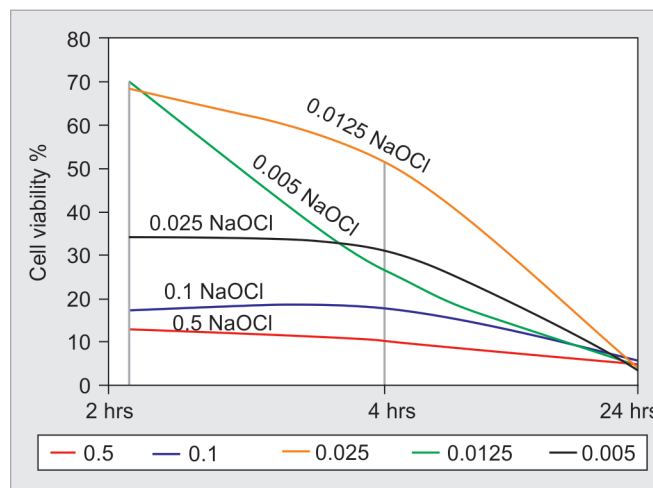
After a 2-hour exposure to (0.025 mg/ml) NaOCl, cells appeared irregular with variable shapes (Figs 1C and D). Cells were attached to the subsurface with no cell-to-cell attachments. The cell wall appeared intact with some blebs. After 4 hours, the few surviving cells were rounded and shrunken (Figs 2C and D). Furthermore, the cell wall was completely distorted and had some blebs; the attachments to the adjacent cells and to the substrate were lost; and many lysosomal secretions were evident between the cells (Figs 2C and D).

**Live/dead Analysis with EB/AO Staining**

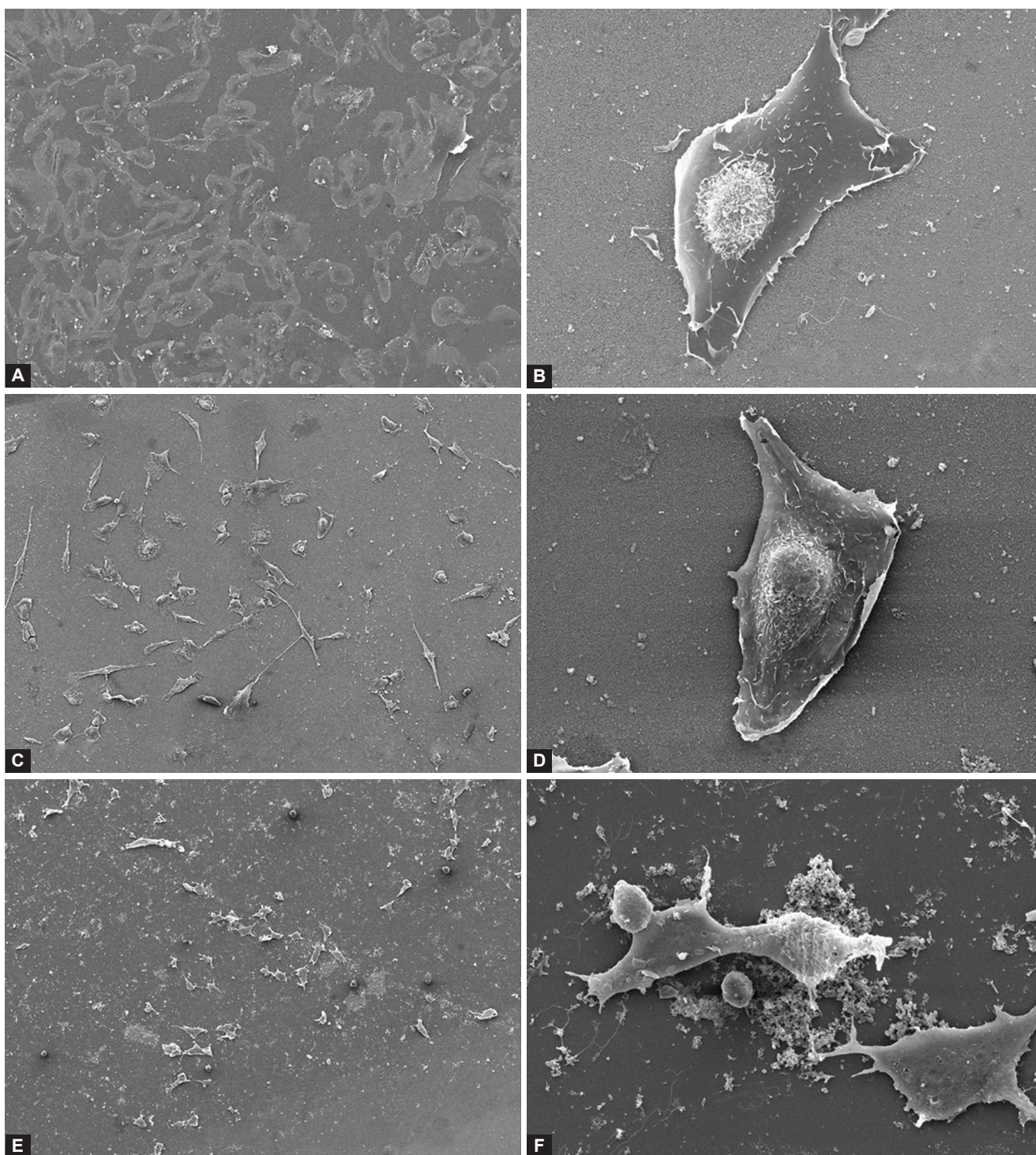
Cultured human bone marrow hTERT-MSCs were exposed to two concentrations (0.5 and 0.025 mg/ml) of NaOCl for 2 hours. The EB/AO stain was applied to the cells, which were then examined under an inverted fluorescence microscope. The images for each group display live cells in green and dead cells in red. The untreated cells had intact, well-defined nuclei, which were green under the green filter, as shown in Figure 5A. Under the red filter, only the cell surface showed



**Graph 1:** The cell viability percentage for various concentrations of NaOCl at 2, 4 and 24 hours after exposure using MTT assay



**Graph 2:** The cell viability percentage for various concentrations of NaOCl by AlamarBlue assay at 2, 4 and 24 hours of exposure



**Figs 1A to F:** Human bone marrow hTERT-MSCs exposed for 2 hours to 0.5 and 0.025 mg/ml of sodium hypochlorite: (A) control  $\times 100$ , (B) control  $\times 1000$ , (C) 0.025 mg/ml NaOCl  $\times 100$ , (D) 0.025 mg/ml NaOCl  $\times 1000$ , (E) 0.5 mg/ml NaOCl  $\times 100$ , (F) 0.5 mg/ml NaOCl  $\times 1000$

red fluorescence, not the nucleus, which indicated an intact cell wall (Fig. 3 B).

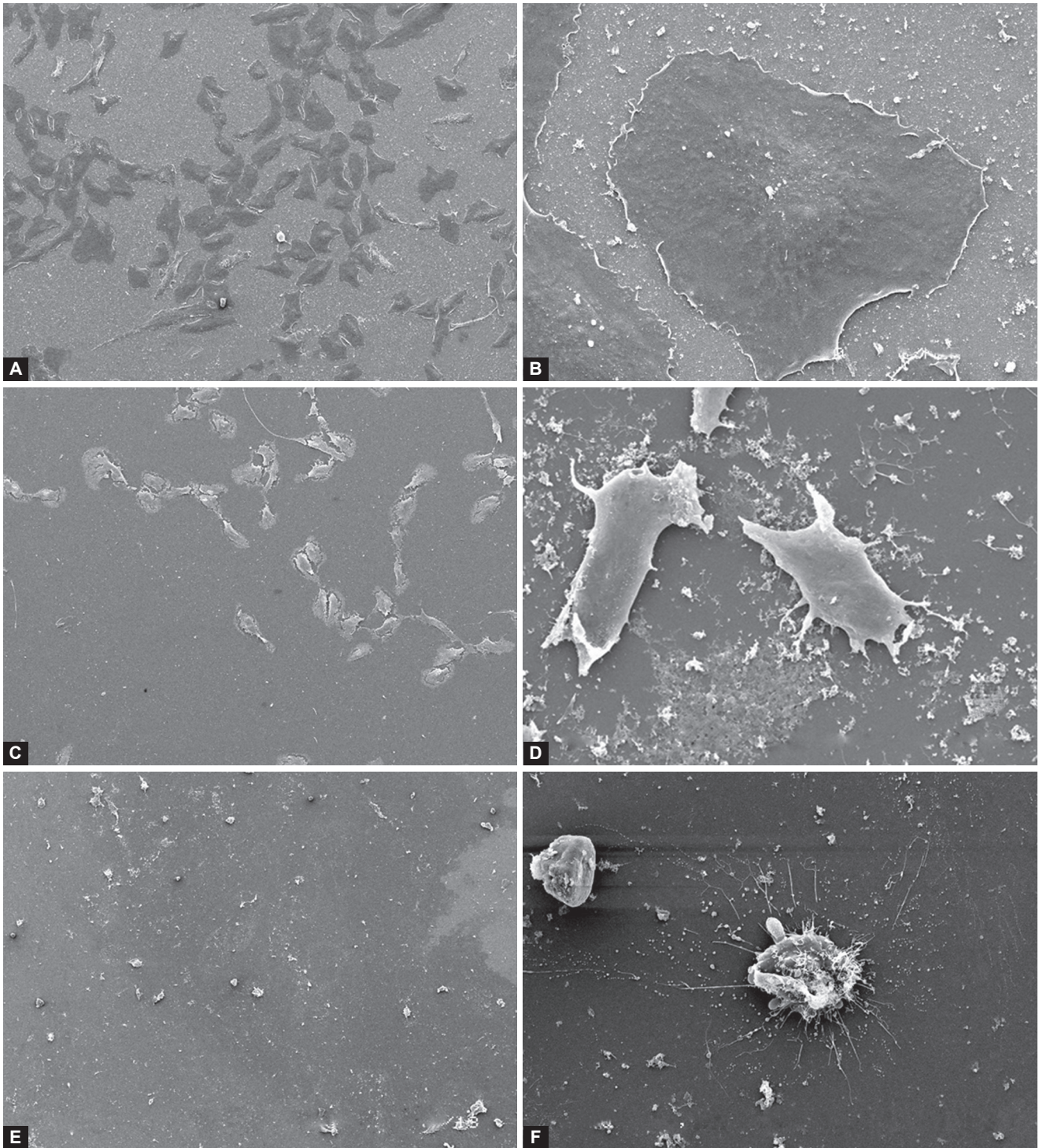
When the cells were exposed to 0.5 mg/ml NaOCl, no cell structures, such as the nucleus or cell membrane, were observed; cell lysis was evident, and the remnant material was positive for both green and red fluorescence (Figs 3C and D). When the cells were exposed to (0.025 mg/ml) NaOCl, the cell number was comparable to that of the control group, and the nuclei had intact green fluorescence (Fig. 3E).

However, the cell surface appeared pale red, and only a few bright red nuclei were observed (Fig. 3F).

## DISCUSSION

The study was conducted to assess the *in vitro* cytotoxicity of the varying concentrations of NaOCl on human bone marrow MSCs. These cells have previously been suggested as a good model for toxicological testing.<sup>24</sup> The MSCs that were used in this study were immortalized by the ectopic



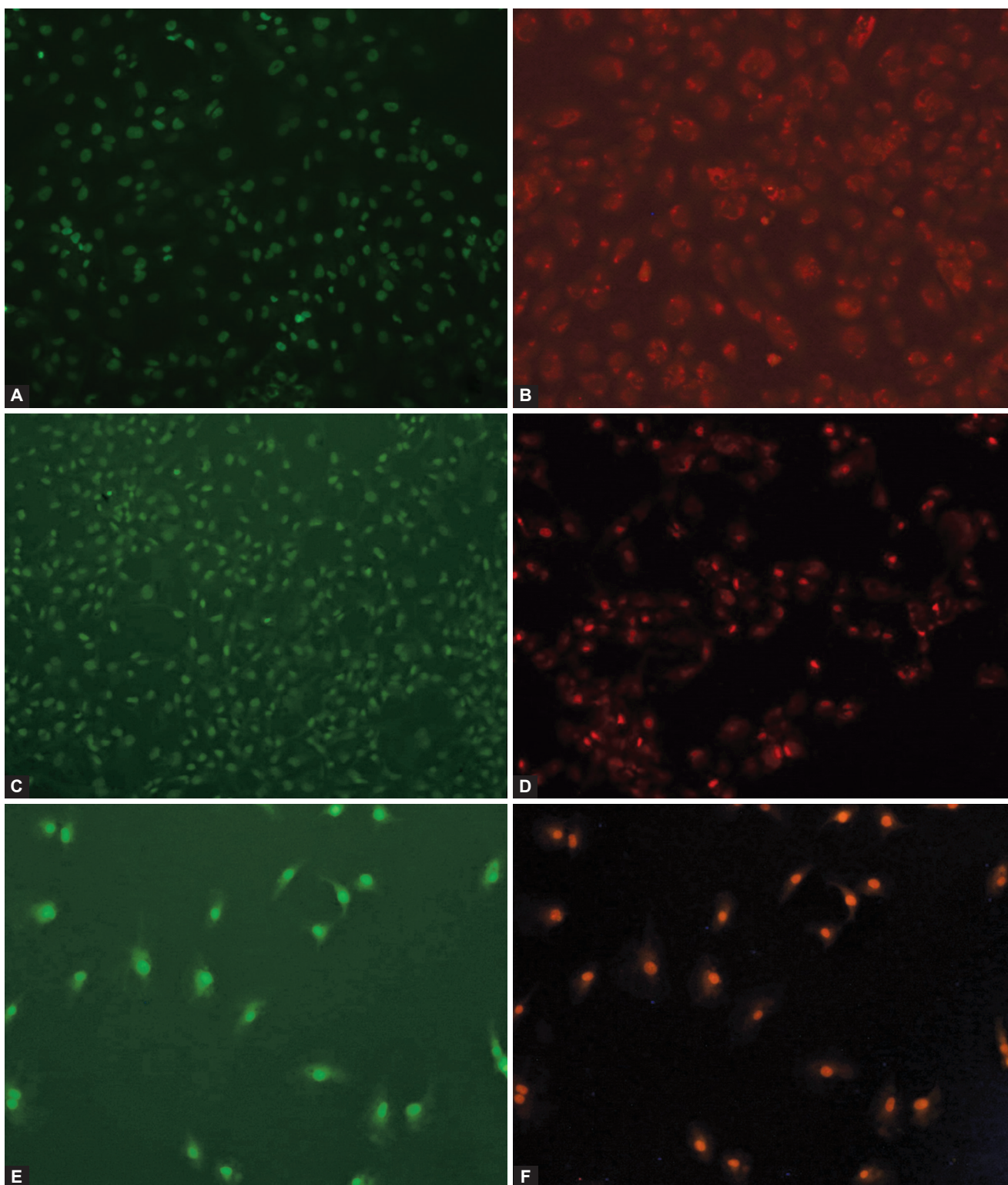


**Figs 2A to F:** Human bone marrow hTERT-MSCs exposed for 4 hours to 0.5 and 0.025 mg/ml of sodium hypochlorite: (A) control  $\times 100$ , (B) control  $\times 1000$ , (C) 0.025 mg/ml NaOCl  $\times 100$ , (D) 0.025 mg/ml NaOCl  $\times 1000$ , (E) 0.5 mg/ml NaOCl  $\times 100$ , (F) 0.5 mg/ml NaOCl  $\times 1000$

expression of human telomerase reverse transcriptase (h TERT); this immortalization increased the life span of the cells<sup>19</sup> and maintained their stem-like properties.<sup>25</sup> Previous investigations have reported that immortalized cells can be used as a test model for dental materials.<sup>26</sup> Primary cells are always considered a better option for *in vitro* studies; however, primary cells are available in limited numbers and show batch-to-batch variation, unlike immortalized cells, which are available in unlimited number and are easily maintained.<sup>19,24</sup>

Aqueous NaOCl solution is widely used in dental practice as a root canal irrigant. Sodium hypochlorite possesses an excellent tissue dissolving capability as well as a pronounced antimicrobial efficiency against a broad spectrum of pathogens: Gram-positive and Gram-negative bacteria, fungi, spores and viruses. In addition, NaOCl has the ability to inactivate or neutralize lipopolysaccharides and has the unique property to disrupt or to remove biofilms.<sup>27,28</sup> Although NaOCl has excellent properties as a root canal





**Figs 3A to F:** Live/dead analysis of human bone marrow hTERT-MSCs under a fluorescence microscope ( $\times 10$ ): (A) untreated cells with the green filter, the nucleus appears intact, which indicates a viable cell, (B) with the red filter, the nucleus appeared dark, and only the external surface of the cells was red, indicating that the cell wall is intact, (C and D) treated with 0.025 mg/ml of sodium hypochlorite fluorescence microscope ( $\times 10$ ), (E and F) treated with 0.05 mg/ml of sodium hypochlorite under a fluorescence microscope ( $\times 10$ )

irrigant it exert manifold toxic effects such, as hemolysis, skin ulcerations, inhibition of neutrophil migration, damage to endothelial and fibroblast cells, facial nerve demyelination and tissue necrosis.<sup>13,29-31</sup> The cytotoxic effect of NaOCl on vital tissues resulting in hemolysis is well documented, and its use warrants proper care.<sup>32</sup>

Sodium hypochlorite solutions are used in concentrations varying from 0.5 to 6.15%.<sup>4,33</sup> The tissue dissolution capability, the antimicrobial efficiency as well as the toxicity of NaOCl are dependent on the concentration of the solution, its pH, osmolarity, nature of contact and the duration of exposure.<sup>6,11,34</sup> In the present study, we found that the

toxicity of NaOCl showed a direct correlation to the concentration used. Both cell viability assays showed similar results. This is in agreement with the earlier studies on the toxicity of NaOCl.<sup>18,35,36</sup> The NaOCl toxicity was attributed to high pH, which interferes with the cytoplasmic membrane integrity.<sup>37</sup> According to the AB assay findings in the present study, the cell viability decreased significantly when the cells were exposed to higher concentrations of NaOCl at 2 and 4 hours. These findings showed that the toxic effect of an agent would increase gradually with time and with higher concentrations. This observation is in agreement with those of previous studies, confirming that toxicity is concentration- and time-dependent.<sup>38</sup>

The sensitivity of the cell viability vary according to the assay used. Hence, in this study, we used both MTT assay and AB assay in order to avoid false positive or a false negative results. Earlier reports showed that the AB assay is slightly more sensitive than the MTT assay. However, both assays rely on enzymatic metabolism, which may be inhibited or induced by the testing agent.<sup>39</sup> Hence, it is desirable to use more than one assay for cytotoxicity studies.

The cells exposed to 0.5 mg/ml NaOCl have cytoplasmic shrinkage or ruptured membranes, which are typical characteristics of necrosis.<sup>40</sup> The EB/AO staining of the NaOCl (0.5) group displayed remnant material that may be a result of cell lysis, which reflects both green and red, and no cell structure could be observed. However, the 0.025 NaOCl group displayed surviving cells with irregular and variable shapes under the SEM, which remain attached to the subsurface after a 2 hours exposure.

According to Galluzzi et al,<sup>41</sup> cell death can be classified into four different types, based on morphological characteristics: apoptosis (Type 1), autophagy (Type 2), necrosis (oncosis, Type 3), and mitotic catastrophe. Each mode of cell death has its own function; necrosis is considered a method of inducing an inflammatory response when it is needed, whereas apoptotic cells *in vivo* are rapidly phagocytosed without inducing an inflammatory response, which is considered a mechanism for avoiding immune activation.<sup>42</sup> Cell necrosis is a passive form of cell death that is characterized by cytoplasmic swelling, rupture of the plasma membrane, release of cell components into the extracellular medium, swelling of cytoplasmic organelles, and some condensation of nuclear chromatin.<sup>40,43</sup> The mode of cell death accountable with NaOCl is due to necrosis.

The observations of the study clearly indicated that the concentration of NaOCl is the deciding factor inducing cytotoxicity. This observation is in agreement with other studies which showed a correlation to the concentration, pH and duration of tissue exposure to NaOCl.<sup>27,44</sup> Milano et al<sup>45</sup> in an *in vitro* study showed that the time taken for pulp

dissolution time ranged from 20 minutes to 2 hours with different NaOCl concentrations (0.5, 1, 2.5 and 5.25%). We also observed maximum toxic effect within 2 to 4 hours after exposure to NaOCl.

This *in vitro* study has the following limitations: it was conducted on cultured cells, and the results represent only the response of these cells in isolation, without taking into account the host defense mechanism for detoxification. Furthermore, in a clinical setting, the solutions are always delivered to root canals, which are surrounded by dentine, and are then extruded to the periapical area. Previous studies have reported that the cytotoxic effect of irrigants can be neutralized by dentine.<sup>36,46</sup> We placed the solutions directly onto the cells, and no attempts were made to deliver these solutions through root canals to mimic the clinical scenario.

## CONCLUSION

Within the limitations of this study, it can be concluded that exposure of NaOCl to human bone marrow MSCs showed cytotoxic effect. The toxicity showed a direct correlation to the concentration and the duration of exposure. From the observations, it can be concluded that a lower concentration of NaOCl may be used as endodontic irrigant due to its cytotoxic properties. Further studies are mandatory to evolve a consensus on the optimal concentration of NaOCl that can be used as endodontic irrigant.

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