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Cytotoxicity and Histopathology of Novel Bioceramic Root Repair Materials Versus MTA Angelus: An in-vitro study

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Aim: The aim of this in vitro study was to evaluate the cytotoxic and histopathologic effects induced by NeoPutty, experimental Nano-Mineral Trioxide Aggregate (Nano-MTA) compared to MTA-Angelus on human gingival fibroblasts (HGFs).
Materials and Methods: Material extracts were prepared at (24, 48 and 72 hours) at different serial dilution (undiluted, 50%, 25%, 12.5%). Human Gingival Fibroblast post-treated with pure extract of each of the three tested materials were examined under light microscope to detect the presence of apoptosis.
Results: At all evaluation periods, all tested materials significantly decreased the viability percentage of the fibroblast compared to control group at (concentrations 100%,50%); while at concentrations 25%,12.5%), all materials were non-cytotoxic with no significant difference with the control group. At 24 & 48 hours (concentrations 100% and 50%), MTA Angelus presented the highest cytotoxicity significantly to the other groups. At 24h (concentration 100%), Nano-MTA showed the least cytotoxicity significantly to MTA Angelus & NeoPutty, while at (concentration 50%) there was no significant difference in cytotoxicity between Nano-MTA & NeoPutty. At 48h, there was no significant difference in cytotoxicity between NeoPutty and Nano-MTA. At 72h (concentrations 100% & 50%), MTA Angelus presented the lowest cytotoxicity significantly to NeoPutty with no significant difference with Nano-MTA. Apoptosis was shown with the three tested materials.
Conclusion: Within the limitation of the present study, NeoPutty and experimental Nano-MTA had favorable cell viability that could be comparable to the gold standard MTA Angelus.

Keywords: Cytotoxicity; experimental Nano-MTA; histopathology; MTA-Angelus; NeoPutty

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Introduction

Perforation is an artificial communication occurring between the root canal space and peridontium. Root perforations can be due to resorption and caries; in this case it is considered pathologically or iatrogenically during root canal treatment. If not repaired, it might be considered as major complication and might affect the treatment outcome. [1]

The ideal endodontic root repair material should be non-toxic and biocompatible. It should also be easily handled, radiopaque, insoluble, dimensionally stable with good sealing ability. [2] MTA is characterized by being biocompatible, possessing good sealing ability as well as its capability of promoting pulp and periradicular tissue regeneration.[3] The Mineral trioxide aggregate (MTA) is most commonly used root end filling material having various clinical applications, such as pulp capping and root perforations repair. [3] MTA is also used for apexification, pulpotomy and root resorption treatment.[4] However, MTA showed many drawbacks such as prolonged setting time and tooth staining with time. That' s why, novel bioactive endodontic cements have been evolved to obtain the best outcome. [5]

Endodontic bioceramics are biocompatible materials. They are not technique-sensitive as they are not susceptible to moisture or blood. They are dimensionally stable, with insignificant setting expansion and accordingly excellent sealing ability. In contact with tissue fluids, endodontic bioceramics release calcium hydroxide which reacts with phosphate in tissue fluids producing hydroxyapatite. This is the explanation of their inductive properties.[6]

NeoPutty (Avalon Biomed, Houston, USA) is a bioactive bioceramic root and pulp treatment material, combined with a non-aqueous, water-miscible carrier to reveal superior handling properties, permitting the formation of hydroxyapatite to generate the healing process. Tantalum oxide as a radiopacifier was incorporated instead of bismuth oxide, as the latter was claimed to be the cause of the discoloration produced by MTA[7]. There is no sufficient data about this material cytotoxicity, as it is recently released in market.

Nanotechnology is considered as a new evolution in all health aspects including the dental field. Nanotechnology is the manipulation of materials at the nanoscale (1–100 nm). [8] At the nanoscale, materials have unique properties. This is because of the large surface area to volume ratio.

Experimental Nano-Mineral Trioxide aggregate (Nano-MTA) (NanoTech Egypt-Al Giza-Egypt) has an average powder size of less than 100nm.[9] Decreasing the particle size of the tricalcium silicate powders lead to better enhancement in its handling and setting properties. Smaller particles are characterized by better tubules penetration and faster hydration process than larger particles because of their higher surface-to-volume ratio.[10]

Although many studies[3,4,11,12,13] have assessed the biological effects of Mineral Trioxide aggregate (MTA), different biological properties of NeoPutty & experimental Nano- Mineral Trioxide aggregate (Nano-MTA) need to be studied.

This study aimed to examine the cytotoxic and histopathologic effects induced by NeoPutty (Avalon Biomed, Houston, USA) and experimental Nano-Mineral Trioxide Aggregate (Nano-MTA) in comparison with MTA-Angelus (Angelus, Londrina, PR, Brazil) on human gingival fibroblasts (HGFs).

Materials and Methods

Materials used in this study

1-**NeoPutty** (Avalon Biomed, Houston, USA) is an injectable, resin-free, bioceramic material. It is composed of: Tantalum oxide, tri and di calcium silicate, calcium aluminate, tricalcium aluminate, calcium sulfate, proprietary organic liquid and stabilizers.

2-**Experimental Nano-Mineral Trioxide aggregate** (Nano-MTA) was acquired from NanoTech Egypt (Giza-Egypt) as white powder. According to the manufacturers, it is soluble in dilute acidic medium, less than 100nm in size, spherical in shape and with similar composition to white mineral trioxide aggregate.[10] It is composed of: Tricalcium silicate, dicalcium silicate, tri-calcium aluminate, calcium sulfate, zeolite and strontium.

3-MTA-Angelus (Angelus, Londrina, PR, Brazil) composed of: tri-calcium silicate, dicalcium silicate, tricalcium aluminate, ferroaluminate tricalcium, calcium oxide & bismuth oxide as the radiopacifier.

Ethical consideration

The study was permitted and committed in accordance with the guidelines of the ethical committee in the National Research Centre under number: (34310112021)

Sample Size Calculation

Sample size was calculated referring to a preceding study[14]. Relying on this study, 3 per group was the minimally accepted sample size, when mean ± standard deviation of group 1 was 100.33 ± 1.53 while estimated mean difference with other group was 4, when the power was 80 % & type I error probability was 0.05. The independent t test was performed by using P.S. power 3.1.6.

Cytotoxicity evaluation

Preparation of the material extract

The NeoPutty repair material was presented as an injectable bioceramic material. The MTA-Angelus and the Nano-MTA mix were prepared following the manufacturers' instructions. Preparation of MTA-Angelus was done as follows: 1 spoon of powder with 1 drop of distilled water, and for the Nano-MTA: 1 spoon of powder with 3 drops of distilled water. Preparation of material extracts was performed according to ISO 10993-5:2009. For each material, three discs (n = 3) were prepared for each time point. The materials discs were prepared under aseptic conditions by placing the freshly mixed materials into a sterile cylindrical polyethylene tube (diameter, 5mm; height, 3 mm). Packing was done in 2 minutes. Getting rid of the excess materials of the tubes was accomplished by compressing with a glass slide. Polyethylene tubes containing the materials were kept in 5% CO2 &100% humidity incubator at 37º C for 6 hours to achieve complete setting. Then, removal of the materials in the form of discs was done. The materials discs were then sterilized with ultraviolet light for 1 hour. [15-17] Then discs were immersed into 1 mL Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) for extraction. They were incubated in a humidified atmosphere containing 5% CO2 for 24 hours. Then, we got rid of the discs. The supernatants were collected and filtered through a sterile 0.22-mm filter (Sigma-Aldrich, St Louis, MO), to assure the removal of any suspended particles from the materials in the extracts.[15-18]

Cell line

In this study, we used Human Gingival Fibroblast cells (ATCC® PCS-201-012™) obtained from the laboratory of the Tissue Culture Department of the Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt.

Cytotoxicity test

Fibroblasts was cultured in DMEM supplemented by 10% FBS, penicillin and streptomycin under standard cell-culture conditions (37 ºC, 100% humidity, 95% air, 5%CO2). The cells were seeded into the 96-

well plates (10^4 cells/ well) and incubated for 24 h in a humidified air atmosphere of 5% CO2 at 37º C to enable cell attachment before adding the extracts.[15,16] Ahead of the insertion in cell cultures, serial dilution of the extracts was done in DMEM supplemented with 10%FBS; (undiluted, 50% ,25%,12.5%).[11,16] Cells plated in 96-well plates were incubated with 100µL of each dilution and with the medium only without extract. The negative control was the fibroblasts cultured in DMEM only without any extract .[11,15,16]

The cell viability was recorded at 24h, 48h and 72h by the aid of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT is a tetrazolium dye that is reduced to an insoluble crystalline product (formazan) by specific mitochondrial enzymes (in the live cells).[19] At each time point, the MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added to the wells. and they were kept away from light and incubated at 37 °C for 4 h. An amount of 200 µL of isopropyl alcohol will be added to each well after getting rid of the MTT solution. In order to dissolve the dark blue crystals, the plate was kept under continuous agitation for 30 minutes. Then by a spectrophotometer (ShimadzuMultSpec-1501; Shimadzu Corporation, Chiyoda, Tokyo, Japan), the optical density (OD) was measured at 570 nm. Analysis was done for each condition in triplicate.[15,17]

The viability % was calculated in accordance with the following equation

$$\text{Viability \%} = \frac{\text{Mean optical density (OD) of test dilution}}{\text{Mean optical density (OD) of negative cell control}} \times 100$$

Viability % was plotted against the concentration of extracts. [20]

Based on the ISO 10993-5, if cell viability percentage recorded is above 90%, it denotes non-toxicity, while if between 60-90%, 30-60% and less than 30%, it denotes mild, moderate and severe toxicity, respectively. [12]

Histopathological profile

Hematoxylin and Eosin staining

Fifty microliters of Human Gingival Fibroblast cell cultures treated with undiluted extract of each one of the three tested materials for 24 hours forming three groups; one group for each material in addition to untreated cell culture representing the control group were dispensed on clean slides, (3 slides for each group).

We dried the slides by using air. They are then fixed with methanol and rehydrated in alcohol.

Washing of the slides was done in distilled water for 5 minutes. They were then immersed in filtered hematoxylin (HE) stain for 3 minutes and washed twice with distilled water. The slides were then dipped for 5 seconds in filtered eosin stain before washing with distilled water. The slides were then dipped in xylene, mounted with Canada balsam; and left to dry. For each slide, ten microscopic fields were photomicrographed at the power of 100 by a digital camera (Canon, Japan), that was connected to a light microscope. Images were carried to the computer system for analysis. Field selection was done on the basis of the presence of the highest number of apoptotic cells. The photomicrographs were qualitatively evaluated according to the presence of apoptosis morphological features.[20]

Statistical Analysis

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed parametric (normal) distribution.

One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with

IBM® SPSS® Statistics Version 20 for Windows.

Results

Cytotoxicity evaluation

The three tested materials reduced the viability percentage of the fibroblast in a concentration dependent matter as the viability increased relatively to decreasing the concentration. Treatment of fibroblasts with NeoPutty, Nano-MTA and MTA Angelus at concentrations 100% & 50% significantly decreased the viability percentage of the fibroblast compared with the control group (untreated cells) at the three evaluation periods ($p<0.0001$). Meanwhile, at concentrations 25% & 12.5%, no significant difference was recorded between the three test materials and the control group at the three evaluation periods ($p>0.0001$).

At 24h of incubation & concentration 100%, MTA Angelus presented the highest cytotoxicity (moderate cytotoxicity) to fibroblasts with a significant difference to NeoPutty & Nano-MTA; $P<0.05$. NeoPutty showed moderate cytotoxicity with a significant difference to Nano-MTA (mild cytotoxicity); $P<0.05$. At concentration 50%, MTA-angelus presented also the highest cytotoxicity but with mild cytotoxicity to the fibroblasts and with a significant difference to NeoPutty & Nano-MTA; $P<0.05$, while no significant difference was found in cytotoxicity between NeoPutty and Nano-MTA (mild cytotoxicity); $P>0.05$.

At 48h of incubation & concentration (100%,50%) MTA Angelus presented the highest cytotoxicity to fibroblasts with a significant difference to both NeoPutty & Nano-MTA; $P<0.05$. There was no significant difference in cytotoxicity between NeoPutty and Nano-MTA; $P>0.05$.

At 72h of incubation, at concentrations (100%,50%), MTA Angelus presented the lowest cytotoxicity to the fibroblasts (mild cytotoxicity) with significant difference to NeoPutty; $P<0.05$ and with no significant difference to Nano-MTA; $P>0.05$. There was no significant difference between NeoPutty & Nano-MTA (mild cytotoxicity) at both concentrations; $P>0.05$.

At the three evaluation periods (24 h, 48 h and 72 h), there was no significant difference between the three materials at concentrations (25%, 12.5%); $P>0.05$. The three tested materials were non-cytotoxic at these concentrations.

Table 1: The mean values & and standard deviation (SD) of vitality% of different groups at 24 hours

	24 hours							
	Conc.100%		Conc.50%		Conc.25%		Conc. 12.5%	
	M	SD	M	SD	M	SD	M	SD
Control	99.31% a	0.01581	99.31% a	0.01581	99.31% a	0.01581	99.31% a	0.01581
NeoPutty	56.02% b	0.01581	85.08% b	0.08366	99.3% a	0.0269	99.24% a	0.08444
Nano-MTA	62.9% c	0.1581	85.06% b	0.01581	99.29% a	0.05471	98.23% a	0.0577
MTA	47.02% d	0.01581	75.21% c	0.01581	99.33% a	0.01581	98.28% a	0.03049
p-value	< 0.0001***		< 0.0001***		0.399ns		0.1063ns	

P-value ≤ 0.05; significant. ns; non significant as P >0.05. Different letters in the same column designate significant difference, similar letters indicate no significance.

Table 2: The mean values & and standard deviation (SD) of vitality% of different groups at 48 hours.

	48 hours							
	Conc.100%		Conc. 50%		Conc. 25%		Conc .12.5%	
	M	SD	M	SD	M	SD	M	SD
Control	100.1% a	0.01581	100.1% a	0.01581	100.08% a	0.015811	100.08% a	0.015811
NeoPutty	70.29% b	0.1024	87.07% b	0.1436	100.09% a	0.015811	100.1% a	0.015812
Nano-MTA	70.3% b	0.1581	87.02% b	0.01581	100.1% a	0.015812	100.093% a	0.00114
MTA	51.93% c	0.01581	73.98% c	0.01581	100.096 a	0.013417	100.095% a	0.00114
p-value	< 0.0001***		< 0.0001***		0.2226ns		0.0663ns	

P-value ≤ 0.05; significant. ns; nonsignificant as P>0.05 Different letters in the same column designate significant difference, similar letters indicate no significance.

Table 3: The mean values & and standard deviation (SD) of vitality% of different groups at 72 hours

	72 hours							
	Conc.100%		Conc. 50%		Conc. 25%		Conc .12.5%	
	M	SD	M	SD	M	SD	M	SD
Control	100.67% a	0.015811	100.7% a	0.01581	100.67% a	0.015811	100.67% a	0.015811
Neoputty	68.92% b	0.015811	82.19% b	0.03564	100.66% a	0.016589	100.652% a	0.008368
Nano-MTA	68.96% bc	0.028283	82.21% bc	0.005478	100.67% a	0.016734	100.63% a	0.031146
MTA	68.98% c	0.008368	82.24% c	0.03412	100.67% a	0.013038	100.65% a	0.020737
p-value	< 0.0001***		< 0.0001***		0.7455ns		0.1553ns	

P-value ≤ 0.05; significant. ns; nonsignificant as P>0.05. Different letters in the same column designate significant difference, similar letters indicate no significance.

Discussion

The biocompatibility of endodontic repair material is of major importance due to its intimate contact to the pulp and periodontal cells.[13]The cell viability assays are the most common way to evaluate the dental materials biocompatibility that come into contact with the surrounding tissues. [21, 22] Cell viability of the tested materials was determined either quantitatively or qualitatively; quantitatively by MTT assay depending on its ability to convert the yellow water-soluble tetrazolium salt MTT into dark blue formazan crystals in presence of mitochondrial dehydrogenase enzymes in living cells. The MTT method is a simple, rapid and precise method.[3] Also, it was achieved qualitatively by comparing the morphology of an untreated control and the materials'exrtacts treated cell cultures using the optical microscope.

This study was performed on human gingival fibroblasts which are the main cells of the gingiva and the periodontal ligament. This was done to simulate the clinical conditions, as there is a close relation of endodontic cements to the periodontal tissues. It was shown that, the gingival fibroblasts (GF) and periodontal ligament fibroblasts (PDLF) were similar in morphology and proliferation rates. Moreover, PDLF and GF derived from the same patient showed similarity in protein production pattern.[23] Choosing human gingival fibroblasts presented the additional advantage of reducing bias concerning species origin. [11]

We used the non-diluted extracts and different dilutions of the extracts following the ISO 10993-5:2009. After application of the material in the tissue, the soluble components of the material are continuously eliminated by the extracellular fluids. In accordance with ISO standards, cell viability tests can be assessed after 24 hours, 48 hours and 72 hours to evaluate the long-term toxic effect of the materials.[24]

Studies that evaluated the cytotoxic effect of MTA on human periodontal ligament fibroblasts[25,26] and human gingival fibroblasts[27] came to the conclusion that MTA possess the least cytotoxic effect on human cells. Although, the cytotoxicity of MTA was investigated in many researches, the cytotoxicity of NeoPutty and experimental Nano-MTA needed to be confirmed.

Cell viability depends on the nature of the tested materials, extract concentration and the surrounding medium pH.[28] This present study showed dose dependent effects of the three tested materials. The concentration of extracts affected the cell viability; the higher the concentration of the extract, the more cytotoxic it was. At high concentrations (100% and 50%), all evaluated materials; at the three evaluation periods significantly decreased the viability percentage of the fibroblasts compared with the control group (untreated cells). In contrast, at low concentrations (25% and 12.5%), the three materials did not decrease the viability percentage of the fibroblasts at the three evaluation periods.

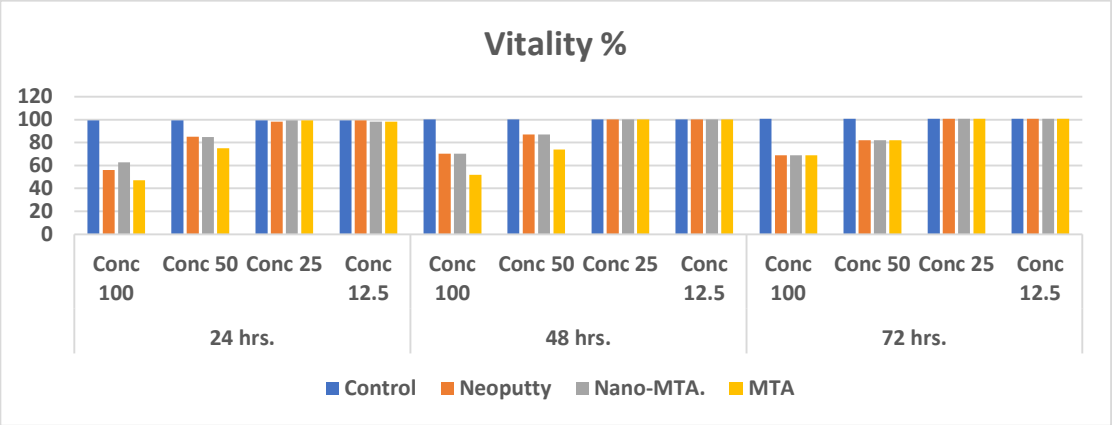

Figure 1: Bar chart representing vitality% of different groups at different evaluation periods

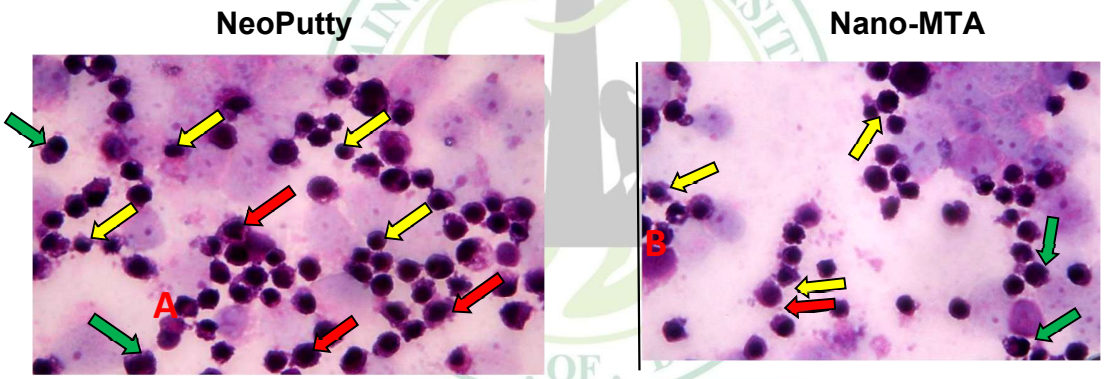

Figure 2: photomicrograph (H & E) of: A) NeoPutty group presenting shrunken nuclei in shrunken apoptotic cells (Yellow arrows) and irregular cell membranes (Red arrows), peripheral condensation of chromatin (Green arrow). B) Nano-MTA group presenting shrunken nuclei in shrunken apoptotic cells (Yellow arrows) and irregular cell membranes (Red arrows), peripheral condensation of chromatin (Green arrow), magnification 100X.

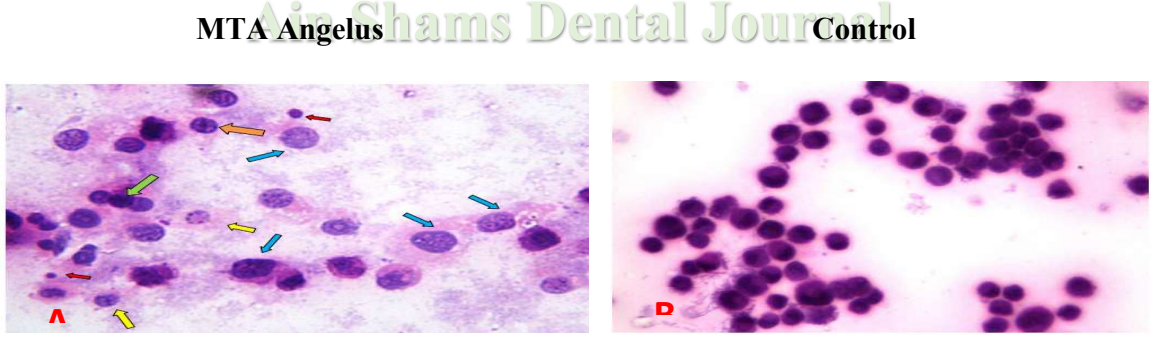

Figure 3: A photomicrograph (H & E) of : A) MTA Angelus presenting necrotic swollen cells and swollen nuclei with mixed euochromatin and heterochromatin and ruptured cell membranes (Blue arrows).
Shrunken apoptotic cells (Green arrows) with peripheral condensation of chromatin (Orange arrow). Secondary necrotic cells with peripheral condensation of chromatin and ruptured cell membranes (yellow arrows). Apoptotic bodies (Red arrows) . B) control group presenting regular cells with hyperchromatic nuclei and nuclear pleomorphism, magnification100X.

These results agreed with the previous ones[11, 12, 29], as in Yoshino et al study [11], where MTA expressed cytotoxic effect in undiluted extracts at 24 h and 72 h. Ma et al[29] also observed that at 3 days, MTA extracts possessed a dose-response effect on cell toxicity in vitro. Jaberiansari et al[12] found that the proliferative effect of three MTA formulations present in the market naming ProRoot MTA, MTA Angelus and Nano-MTA increased by decreasing their concentration.

Calcium silicate hydrate and calcium hydroxide are released as a result of MTA hydration. The high pH of MTA is referred to calcium hydroxide .[28,30] Different types of MTA possess different alkalizing capabilities and are responsible for the significantly pH increase.[31]

In the mineralization process, the high pH of MTA is responsible of the activation of alkaline phosphatase and the neutralization of the acids secreted by osteoclasts.[32]

The higher cytotoxic effect of higher concentrations of MTA angelus (100%, 50%) to the fibroblasts at 24h and 48h of incubation more than NeoPutty & Nano-MTA could be attributed to this alkaline pH. MTA angelus presented the lowest cytotoxicity at 72h incubation period significantly to NeoPutty at concentrations 100%, 50%. Consistent with our study, Ma et al[29] found that at 3 days, gingival fibroblasts exposed to MTA exhibited the highest cell viability. Also, Samara et al[33] proved that over 72-hour period, PDL fibroblasts cells viability increased in the presence of MTA. The lower cytotoxicity of MTA at 72 hours, could be attributed to the overtime decrease in pH of MTA that leads to decrease in cell damage and increasing cell proliferation[34].

Even though Nano-MTA has almost the same composition as white MTA, Nano-MTA presented significantly lower cytotoxicity to the fibroblasts than MTA angelus at 24h and 48h of incubation at high concentrations (100%, 50%). Even at 72h of incubation, no significant difference was found between Nano-MTA and MTA at different concentrations. This may be because Nano-MTA induced less alkalinity due to its reduced setting time which can be attributed to the greater surface area of the nano particles that sped up the reaction of the powder and the liquid and accelerated the hydration process. This led to less time taken during the reaction to increase the pH.[35,36] These results did not agree with Jaberiansari et al[12] where Nano-MTA was more cytotoxic than MTA angelus on dental pulp stem cells in all evaluation periods. This may be due to different type of cells & also different types of Nano-MTA used in both studies. Non diluted extracts of Nano-MTA had mild cytotoxic effects at all incubation periods which agreed with Torshabi et al [37] at 24h of incubation and did not agree with them at 72h where Nano-MTA showed moderate cytotoxicity, Torshabi et al[37] examined Nano-MTA at 24h and 72h only.

NeoPutty showed significantly less cytotoxicity than MTA angelus at concentrations 100% and 50% at 24h and 48h of incubation. This finding may be justified not only by the high pH induced by MTA angelus at these concentrations and incubation periods but also by bismuth oxide absence in the composition of Neoputty; as Neoputty contains tantalite as radiopacifier. A study revealed the supposably cytotoxic effect of bismuth oxide on the human osteoblast like cells when present with dicalcium silicate cement compared with it without the radiopacifier.[38] Moreover, bismuth oxide, when added to Portland cement increased the cytotoxicity in human dental pulp cell .[39]

Till now, there is limited scientific evidence regarding the biological properties of NeoPutty.

NeoPutty is a resin free, ready to use bioactive material that promote the formation of hydroxyapatite and enhance healing. (40)

Sun et al[41] evaluated the biocompatibility of NeoPutty & the commercially available EndoSequence BC RRM Putty (ES Putty) on human dental pulp stem cells (hDPSCs) and human periodontal ligament fibroblasts (hPDLFs), before and after complete setting. Biocompatibility results showed relatively more cytotoxic effect of both putties than the bioinert teflon negative control. NeoPutty was less cytotoxic than ES putty before setting, especially with hDPSCs. After complete setting, both putties exhibited more favorable cytotoxicity profiles. Another study by Lozano-Guillen et al[7], reported that NeoPutty had similar biocompatibility as its forerunner NeoMTA Plus and the commonly used MTA Angelus towards hDPCs. These two studies indicated favorable cytocompatibility of NeoPutty which agreed with the results of our study. Also, they agreed with Joanna et al [42] where the MTT assay showed that viability percent was higher in NeoPutty as compared to MTA at most of concentrations at 24h.

There were no significant differences between NeoPutty & experimental Nano-MTA at all test conditions except at concentration 100% after 24 hours of incubation, where Nano-MTA showed less cytotoxicity with a significant difference to NeoPutty. This agreed with Lozano-Guillen et al[7] who reported that the undiluted NeoPutty and NeoMTA Plus extracts showed a decreased cell viability. They attributed the cause to the enormous intracellular amont of Ca^{2+} and the highly alkaline culture medium that caused mitochondrial dysfunction and consequently a reduced cell viability.

The three tested materials showed comparable cell viability in relation to the control group at low concentrations (25%, 12.5%) at the three evaluation periods of time. The calcium silicate-based materials as more diluted showed increased cytocompatibility in previous in vitro studies[7]

The photomicrographs of hematoxylin & eosin (HE) stained slides of human gingival fibroblast cell cultures treated with undiluted extracts of each of the three tested materials for 24 hours showed the presence of morphological features of apoptosis which were not present in non-treated control group. Cells treated with MTA exhibited early signs of apoptosis which include shrunken cells and chromatin condensation. Shrunken cells are small cells with compacted organelles and condensed cytoplasm. Chromatin condensation is the most characteristic feature of apoptosis. MTA treated cells showed also late apoptotic features; secondary necrotic cells with peripheral condensed chromatin, disrupted cell membranes and apoptotic bodies. Apoptotic bodies are produced during a process called budding by separation of cell fragments.[43] Cells treated with NeoPutty and Nano-MTA showed diminished apoptotic cells with contracted nuclei, non-uniform cell membranes and peripheral thickening of chromatin. The two materials showed less morphological features of apoptosis than MTA. Apoptotic cells appeared to be less with Nano-MTA than NeoPutty. It should be highlighted that these morphological results were descriptive and non-statistical observations that depended on the evaluation of the researchers. However, these types of bioassays are frequently used and complemented by quantitative biological assays.[7] These morphological features

suggested superior initial biocompatibility of Nano-MTA and NeoPutty over MTA and superior initial biocompatibility of Nano-MTA over the other both materials. These findings were consistent with the MTT assay results.

These results went in accordance with Tsai et al[44] who observed apoptosis of dental pulp stems cells in direct contact with MTA and with joanna et al[40] who observed more apoptotic behaviors of fibroblast cells in direct contact with MTA than NeoPutty.

The cause of the initial cytotoxic effect of the materials might be due to the elevated pH of the cements that led to adjacent cells degradation .[3] As advocated by many studies, initial release of calcium-ions, pH variations and presence of ionic and toxic components could affect the survival of cells. [45] Also it was found that, when using MTA, superficial necrosis was commonly observed. This is due to the alkalinity on adjacent tissues.[46] This study had potential limitation, being an in vitro experiment, the results reflect the response of the cultured cells without the involvement of the host defense mechanism.

Therefore, further clinical supporting evidence is needed to evaluate the long-term cytotoxicity effect of the tested materials.

Conclusions

Within the limitations of this study, it can be concluded that:

- Cytotoxicity of all tested materials is dose dependent.
- Cell viability of undiluted extracts of the materials at 72 hours was exceeding that at the 24 hours. This could be interpreted by the time dependence of the cell viability of the tested materials.
- NeoPutty and experimental Nano-MTA had favorable cell viability that could be compared to the gold standard MTA Angelus. These results may act as preliminary evidence for their use as root repair materials.

Declarations

Funding

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The study was permitted and committed in accordance with the guidelines of the ethical committee in the National Research Centre under number: (34310112021)

Competing interests

The authors declare that there is no conflict of interest.

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