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Original Article

Cytotoxicity of NeoMTA Plus, ProRoot MTA and Biodentine on human dental pulp stem cells

Sinem Birant [a*], Muazzez Gokalp [b], Yazgul Duran [b], Mine Koruyucu [c], Tunc Akkoc [b], Figen Seymen [c]

[a] Istanbul University-Cerrahpasa, Faculty of Dentistry, Department of Pedodontics, Istanbul Turkey
[b] Marmara University, Faculty of Medicine, Department of Pediatric Allergy-Immunology, Istanbul, Turkey
[c] Istanbul University, Faculty of Dentistry, Department of Pedodontics, Istanbul Turkey

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KEYWORDS
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Abstract *Background/purpose:* Dental pulp stem cells (DPSCs) play a crucial role in the tissue healing process through odontoblast like cell differentiation. The aim of this study was to evaluate the biocompatibility and compare the potential invitro cytotoxic effects of NeoMTA Plus, ProRootMTA and Biodentine on human dental pulp stem cells (hDPSCs).
Materials and methods: To assess the effects of NeoMTA Plus, ProRoot MTA and Biodentine extracts at 1st, 3rd and 7th d on hDPCs, cell populations was determined by flow cytometry using an Annexin V detection kit. The data were analyzed statistically using the Kruskal–Wallis test. A $p < 0.05$ was considered as statistically significant.
Results: All groups showed cell viability similar to that of the control group on 1st, 3rd and 7th d. Although Biodentine exhibited higher cell viability rates than the other material groups, no statistically significant differences were noted between the sampled days ($p > 0.05$).
Conclusion: All materials extracts are not cytotoxic and do not induce apoptosis in the hDPSCs. These results suggest that all the tested materials can lead to positive outcomes when used as reparative biomaterials.

* Corresponding author. Istanbul University-Cerrahpasa, Faculty of Dentistry, Department of Pedodontics, Cerrahpasa Campus, Kocamustafapasa Street No:34/E, Istanbul, 34098, Turkey.
E-mail address: sinembirant@istanbul.edu.tr (S. Birant).

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Introduction

Calcium silicate cements, such as Mineral trioxide aggregate (MTA) and Biodentine, have a wide and successful range of various clinical dental applications.[1,2] Tricalcium silicate cements are called dental biomaterials because of their physicochemical and biocompatibility. They have the ability to increase proliferation and odontogenic differentiation in human dental pulp cells in vitro.[3] MTA is predominantly formed of portland cement and comprises of tricalcium silicate, dicalcium silicate and bismuth oxide for radiopacifier.[4] MTA is used for perforation repair, root-end filling, direct pulp capping, partial or total pulpotomy methods due to its high sealing ability, biocompatibility, antibacterial activity, physical and chemical properties.[5] However, the use of MTA is reported to have some disadvantages due to the long material setting time, high material cost, poor handling characteristics and the potential for discoloration of dental tissues.[6,7] All the properties of MTA have been further developed to overcome the drawbacks of MTA and different bioactive endodontic cements have been introduced to the market. BioAggregate, Biodentine, BioRoot RCS, calcium—enriched mixture cement, Endo-CPM, Endocem, EndoSequence, EndoBinder, EndoSeal MTA, iRoot, MicroMega MTA, MTA Bio, MTA Fillapex, MTA Plus, NeoMTA Plus, OrthoMTA, Quick- Set, RetroMTA, Tech Biosealer and TheraCal LC have been changed by certain properties and manufactured by companies for different usage areas. It is claimed that these materials show similar properties to MTA and do not contain its disadvantages.[8—10]

Biodentine is a calcium silicate cement with dentin like mechanical properties, consisting of a powder and mixing liquid consisiting of water, calcium chloride (decreases the setting time) and a hydrosoluble polymer (water reducing agent), the powder part of which is composed of tricalcium silicate (Ca_3SiO_5) (main component), dicalcium silicate (second main component), zirconium oxide (radiopacifer) and calcium carbonate (filler component).[11,12] Although biocompatibility and dental applications of Biodentine are similar to MTA, it is reported that it is easier to use in clinical practise, shorter working time and does not cause discoloration in dental tissues due to the absence of bismuth oxide.[13]

NeoMTA Plus was developed with similar properties to MTA. It contains tricalcium silicate, dicalcium silicate, tantalum oxide, calcium sulfate and silica. Tantalum oxide (Ta_2O_5) was used instead of bismuth oxide as radiopacifier. NeoMTA Plus is indicated for direct pulp capping, indirect pulp, pulpotomy, root end filling, perforation repair and apexification. Bismuth oxide has been reported to cause discoloration in dental tissues due to contact with sodium hypochlorite solution.[14,15] It is stated that NeoMTA Plus does not cause discoloration in dental tissues, it has sufficient radiopacity and hydration with MTA and it shows the production of calcium hydroxide required to induce mineralized tissue formation.[15,16]

The aim of this study was to evaluate the biocompatibility and compare the potential invitro cytotoxic effects of NeoMTA Plus, ProRoot MTA and Biodentine on human dental pulp stem cells (hDPSCs).

Material and methods

Isolation and passaging of hDPSCs

Human dental pulp tissue were isolated from extracted third molars from healthy three adult patients aged from 18 to 25 years at the Faculty of Dentistry Oral and Maxillofacial Surgery, Istanbul University, Turkey. All experiments were performed with the approval of the Ethics Committee of the Faculty of Dentistry (no: 2017/39). After extraction, the teeth were transported in dulbecco's phosphate-buffered saline (DPBS; Gibco, Grand Island, NY, USA) containing 1% penicillin and 1% streptomycin (Gibco) to the laboratory in the Department of Pediatric Allergy/Immunology, Marmara University Research Hospital. The teeth were split open then the pulp tissues were aseptically removed and isolated under sterile conditions.

The pulp tissues were divided into 1 mm^3 pieces by applying the mechanical seperation method with the scalpel. After micromechanical digestion, colllagenase solution was prepared using 1 ml of DPBS (Gibco) and 0.003 g Type 1 collagenase (Gibco). Next, 2 ml of the collagenase solution was then added to micromechanically decomposed tissues, following which enzymatic digestion was started. After enzymatic and mechanical disintegration, the tissues were placed in 15 ml sterile Falcon tubes. Pipetting was performed in dulbecco's modified eagle medium (DMEM; Gibco) to ensure homogenization of the tissues, which were then placed in the incubator for 45 min to 1 h at 37 °C and %5 CO_2. After the tubes were removed from the incubator, the cells were centrifuged at 1500 rpm for 5 min. The cells pellets were obtained, and the supernatant was aspirated. The cells were transfered to a T-25 cm^2 flask containing 4 ml of culture medium consisting of DMEM (Gibco), suplemented with %15 fetal bovine (FBS; Gibco), %1 penicillin/streptomycin (Gibco). The culture medium was changed every 3 d, and the proliferation and spreading of the cells im the flask were monitored at regular intervals with an invert microscope (EVOS-AMG, Thermo Fisher Scientific, Waltham, MA, USA). The hDPSCs were seperated with %0.25 trypsin—EDTA (Gibco) when they attained %70—80 confluence. The characterization of cell cultures reaching the third passage was identified for specific surface markers and multiple differentiation potential using flow cytometry.

Flow cytometry analysis

The cell surface antigen expressions of the third-passage hDPSCs were analyzed after the cells were incubated with antibodies for human CD73 phycoerytrin (PE), CD90 PE, CD146 fluorescein isothiocyanate (FITC), CD29 allophycocyanin (APC), CD105 PE, CD45 FITC, CD34 PE, CD14 PE, CD25 APC, and CD28 PE (BD Biosciences, CA, USA) at room temperature in the dark.

The control antibodies were phycoerythrin-conjugated or fluorescein isothiocyanate-conjugated and allophycocyanin-conjugated Mouse IgG1 and Mouse IgG2 (BD Biosciences, San Diego, CA, USA). The flow cytometry outcomes were

examined using a flow cytometer (BD, FACSCalibur, San Jose, CA, USA).

Differentiation of stem cells

In order to characterize mesenchymal stem cells (MSCs) in vitro according to International society for cellular therapy (ISCT) criteria, their trilenage differentiation including osteogenic, chondrogenicand adipogenic was evaluated. The ostogenic, chondrogenic and adipogenic differentiation potentials of the cells were analyzed using the differentiation kits [Stem Pro Osteogenesis Differentiation Kit, (Thermo Fisher Scientific), Stem Pro Adipogenesis Differentiation Kit (Thermo Fisher Scientific), and Stem Pro Chondrogenesis Differentiation Kit (Thermo Fisher Scientific)]. The cells were plated in 6 well plates (5×10^4) cell/well) and the differentiations mediums were applied on the cells according to the manufacturer's instructions. The differentiation medias were changed three times per week.The calcium deposits and extracellular matrix mineralization were confirmed with Alizarin Red (Sigma—Aldrich, St. Louis, MO, USA) staining assay performed after 28 days of the osteogenic stimulation. After 14 days the cell cultures were stained with Alcian Blue (Sigma—Aldrich) to assess the chondrogenic differentiation potentials of the cells and evaluated the presence of chondrocyte-like cells and proteoglycans. The Oil Red (Sigma—Aldrich) staining was used to determine the adipogenic differentiation potential of the cells on the 14th day after the application of the differentiation media. The staining cells were evaluated using a binocular microscope (Olympus, BH2-RFCA, Olympus, Tokyo, Japan).

Preparation of materials

The materials used in study and their contents are shown in Table 1. Complete dulbecco's modified eagle medium (CDMEM; Gibco) was used in the control group in this study.

ProRoot MTA (Dentsply Tulsa Dental, Hohnson City, Germany) Biodentine (Septodont, Saint Maurdes Fosses, France) and NeoMTA Plus (Avalon Biomed, Bradenton, FL, USA) were mixed according to the manufacturer's instructions under sterile conditions; then they were added to the molds (diameter 5.0 mm and height 3.0 mm) (n: 16 samples per group). After materials underwent polymerization, each discs were removed using sterile forceps, and the all discs were exposed to ultraviolet (UV) light for 30 min to prevent contamination.

The direct contact test was used to obtain material extraction fluids. Since the surface/medium ratio was 3 cm^2/ml for disc shaped samples with a thickness of more than 1 mm specified in ISO (International Organization for Standardization) 10993-12[17] standards while preparing the release fluids of solid form materials, the discs of each material were placed into 15 ml of DMEM and incubated for 24 h at 37 °C in a humidified 5% CO_2 environment. The material solutions were passed through the 0.22 μm filter twice to remove the remaining small particles, and the material extracts were obtained for the cell viability tests (ISO 19993-5).[18]

Cell viability tests

Cell viability was determined using a flow cytometry assay. Human dental pulp stem cells (hDPCs) were plated onto 48-well plates and the culture medium was added. After 24 h, the culture medium was removed. The extraction materials were enumerated by someone outside the researcher team and randomly applied to the cells. The cells were exposed for 1 day, 3 days, and 7 days obtain the experimental material extracts (ProRoot MTA, NeoMTA Plus, Biodentine).

The Annexin V-FITC Apoptosis Detection Kit (BD Biosciences) was used to read the effects of material extracts on the viable, necrotic, early and late apoptotic cell ratios. The five cell viability experiments repeated independently from each other, and the average of the obtained values was evaluated.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 22 (IBM SPSS Inc.). The Kruskal—Wallis test was used to study the differences among the groups and days in the non-

Table 1 Composition of materials evaluated.

Materials	Manufacturer	Composition
ProRoot MTA	Dentsply Tulsa Dental, Hohnson City, Germany	Powder: Portland cement, bismuth oxide, calcium sulfate dihydrate, tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite Liquid: Water
NeoMTA Plus	Avalon Biomed, Bradenton, Florida, USA	Powder: Tricalcium silicate, dicalcium silicate, tantalum oxide Liquid: Water, proprietary polymers
Biodentine	Septodont, Saint Maurdes Fosses, France	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, iron oxide, zirconium oxide Liquid: Calcium chloride, hydrosoluble polymer
CDMEM	Gibco, Grand Island, NY, USA	%10 FBS, %1 penicillin/streptomycin, DMEM (Dulbecco's Modified Eagle Medium)

CDMEM, complete dulbecco's modified eagle medium; DMEM, dulbecco's modified eagle medium.

normally distributed data. $p < 0.05$ was considered as the significance level.

Results

The confluent cells in the plates exhibited a spindle-shaped fibroblast-like morphology. From the first passage to the third passage, the confluent structures of the cells and their contact with each other increased (Fig. 1A, B, C).

Then, the cells in the third passage were examined to determine their immunnophenotype and differentiation potentials. The cells demonstrated a positive expression for mesenchymal stem cell (MSC) markers (CD105, CD146, CD90, CD29, CD73) (Fig. 2A); the staining for hematopoietic stem cell markers was negative (CD45, CD34, CD25, CD28,CD14) (Fig. 2B).

The hDPCCs showed the potential for osteogenic, chondrogenic, and adipogenic differentiation after stimulation with the differentiation medium. When osteogenic differentiation was evaluated, the presence of mineralized calcium nodules stained in an orange-red color were detected (Fig. 3A). In the chondrogenic differentiation, proteoglycan and chondrocyte-like cells stained a blue-turquoise color were observed (Fig. 3B). In the adipojenic differentiation, the presence of oil droplets stained in a red color was observed (Fig. 3C).

After exposing the three experimental material extracts and the control group to DMEM on the 1st, 3rd, 7th days, the flow cytometry graphics of the cells were obtained (Figs. 4–7, respectively). The lower left (LL) part of the flow cytometry graphs shows the live cell ratios, the lower right (LR) part shows the early apoptotic cell ratios, the upper right (UR) part shows the late apoptotic cell ratios, and the upper left (UL) part shows the necrotic cell ratios. The averages of the datas obtained as a result of analysis with flow cytometry were indicated in the relevant areas on the graphs as percentages.

In the ProRoot MTA group, cell viability percentage on the 7th day was found to be mathematically higher than the other days when the live cell ratios were compared with the days in flow cytometry graphs. The early apoptotic cell ratios were analyzed as high on the 1st day. While the late apoptotic cell ratios were found to be the highest on the 3rd day, the highest rates of the necrotic cells were determined on flow cytometry graphics on the 7th day (Fig. 4).

In the NeoMTA Plus group, cell viability percentage on the 3rd day was found to be mathematically lower than the other days when the live cell ratios were compared with the days in the flow cytometry graphs. The percentages of early apoptotic, late apoptotic and necrotic cells were calculated to be higher on the 1st, 3rd and 7th days, respectively, similar to the flow cytometry analysis of the ProRoot MTA group (Fig. 5).

In the Biodentine group, when the live cell ratios were compared with the flow cytometry graphs, the cell viability on the 7th day was found to be mathematically higher than the other days. The percentages of early apoptotic, late apoptotic and necrotic cells were calculated to be higher on the 1st, 3rd and 7th days, respectively, similar to the flow cytometry analysis of the ProRoot MTA group and NeoMTA Plus group (Fig. 6).

In the Control group, when the live cell ratios were compared with the flow cytometry graphs, the cell viability on the 1st day was found to be mathematically higher than the other days. While early and late apoptotic cell ratios were found to be the highest on the 7th day, the highest rates of necrotic cells were determined on flow cytometry graphics on the 3rd day (Fig. 7).

The cell viability in all tht experimental groups did not show a significant difference when the 1st, 3rd and 7th days were compared ($p > 0.05$) (Table 2). When the experimental and control groups were compared, the control group had the highest cell viability on the 1st and 3rd days and the Biodentine group had the highest percentage of viable cells on the 7th day, but there was no statistically significant difference between the groups based on time times ($p > 0.05$) (Table 2).

The early apoptotic cell rates are showed in Table 3. The lowest early apoptotic cell rates were observed in the CDMEM and Biodentine groups, respectively, but there was no statistically significant difference between the groups in terms of early apoptotic cell rates based on times ($p > 0.05$) (Table 3). Moreover, there was no statistically significant difference in the early apoptotic cell rates in each group based on time ($p > 0.05$) (Table 3).

Comparisions of the late apoptotic cell rates between the groups and the late apoptotic cell rates based on days are shown in Table 4. Although the lowest late apoptosis rates in were in the CDMEM group on the 1st and 3rd days and the Biodentine group on the 7th day, no difference

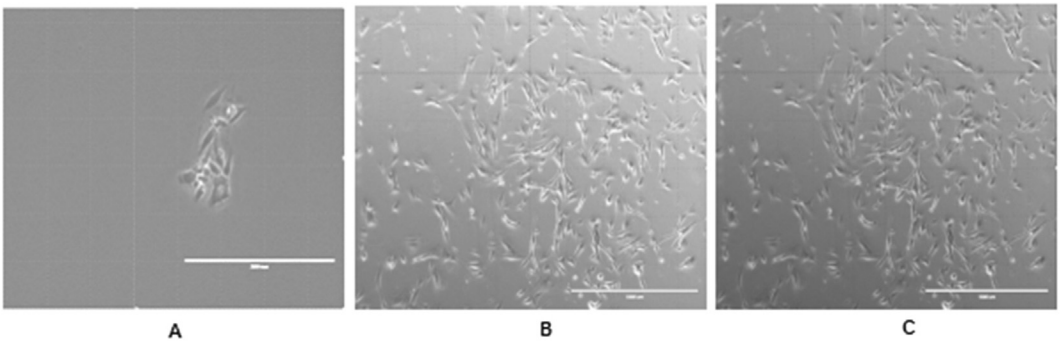

Figure 1 Morphological appearance of hDPSCs, (x10); A) hDPSCs on 1st passage B) hDPSCs on 2nd passage C) hDPSCs on 3rd passage.

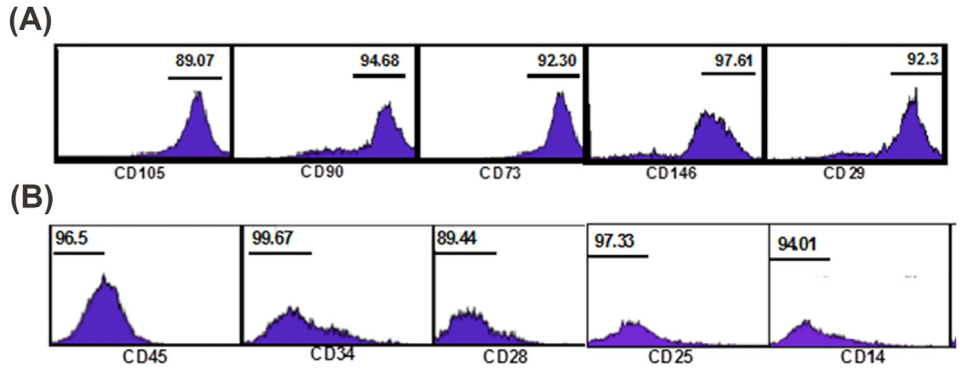

Figure 2 (A): Representative flow cytometry analysis of mesenchymal cell surface markers on hDPSCs. (B): Representative flow cytometry analysis of hematopoietic cell surface markers on hDPSCs.

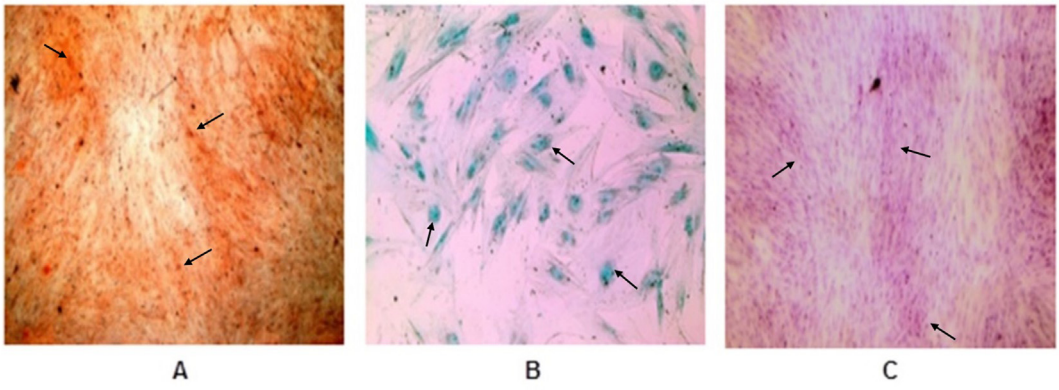

Figure 3 Differentiation analysis in hDPSCs; A) Differentiation of hDPSCs into osteoblasts was confirmed by Alizarin Red staining, (x40); B) Differentiation of hDPSCs into chondrocytes was confirmed by Alcian Blue staining, (x40); C) Differentiation of hDPSCs into adipocytes was confirmed by Oil Red O staining, (x40).

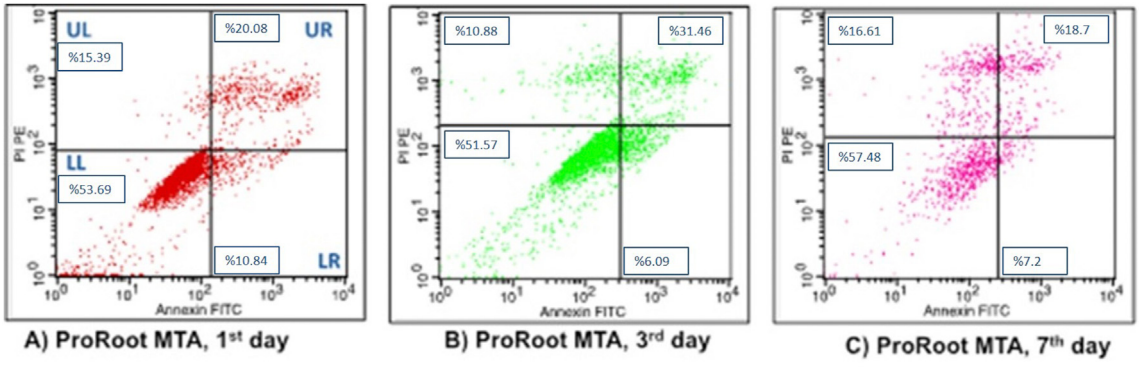

Figure 4 Flow cytometry analysis of hDPSc after incubation in ProRoot MTA for different time intervals. (A) 1 day; (B) 3 days; (C) 7 days.

was found between the days and the groups ($p > 0.05$) (Table 4).

For all the groups, no significant difference in the percentage of necrotic cells was observed based on time ($p > 0.05$) (Table 5). Furthermore, no statistically significant difference was found between the groups in terms of the necrotic cell rates ($p > 0.05$) (Table 5).

Discussion

Mesenchymal stem cells (MSC) can be isolated from many different tissues. MSCs show fibroblast-like spindle morphology and have the potential to differentiate into various cells. They are also reported to carry some specific cell surface markers (CD73, CD90 and CD105).[19,20]

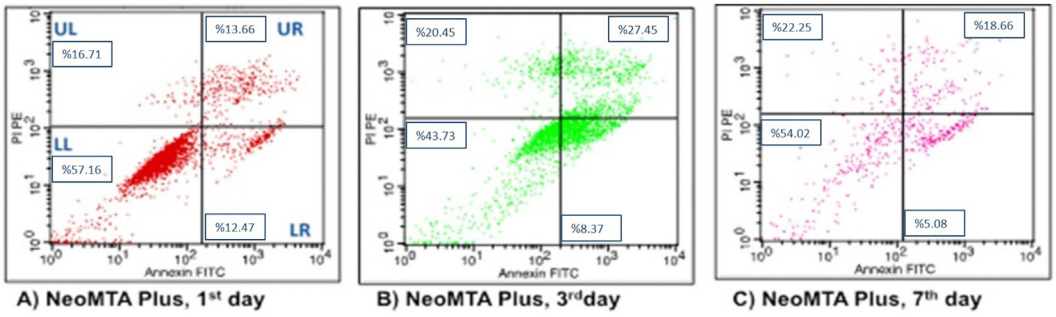

Figure 5 Flow cytometry analysis of hDPSc after incubation in NeoMTA Plus for different time intervals. (A) 1 day; (B) 3 days; (C) 7 days.

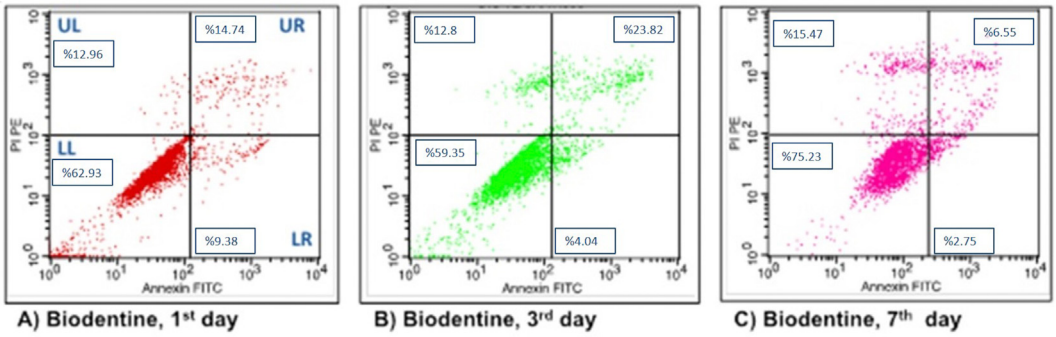

Figure 6 Flow cytometry analysis of hDPSc after incubation in Biodentine for different time intervals. (A) 1 day; (B) 3 days; (C) 7 days.

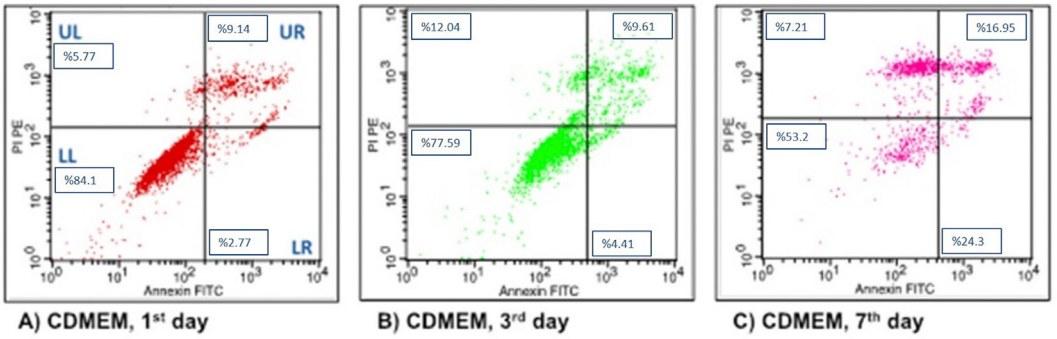

Figure 7 Flow cytometry analysis of hDPSc after incubation in CDMEM (control group) for different time intervals. (A) 1 day; (B) 3 days; (C) 7 days.

The minimum criteria for the identification of MSCs have been developed by the International Cellular Therapy Association. Accordingly, MSCs should adhere to plastic surfaces under standard culture conditions and express CD105, CD73 and CD90, no expression of CD45, CD34, CD14 and CD11b. MSCs should be able to differentiate into different cells such as osteoblasts, adipocytes and chondroblasts in vitro.[21]

In this study, the cultured hDPSCs were characterized by detecting specific surface antigens. The cells isolated from the pulp tissue showed a morphologically fibroblast-like spindle structure, and also potently expressed CD105, CD146, CD90, CD29 and CD73 markers immunophenotypically. Furthermore, these cells showed potential for adipogenic, osteogenic and chondrogenic triple differentiation. These cells cultured with these properties have proved to have MSC character.[22]

The evaluation of biocompatibility is very important because MTA-based materials are used in vital pulp therapies in the last couple decades. Dental pulp stem cells (DPSCs) play a crucial role in the tissue healing process through odontoblast like cell differentiation. DPSCs are clonogenic and capable of self-renewal and multifaceted differentiation. DPSCs respond to tooth injury by differentiating, proliferating, differentiating and adhering to odontoblast-like cells to replace lost odontoblasts,

Table 2 Evaluation of cell viability among 3 different days and among 4 different groups.

	Cell Viability			p
	1st Day	3 rd Day	7 th Day	
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
Biodentine	62.93 ± 31.45 (62.7)	59.35 ± 33.08 (63.8)	75.23 ± 13.38 (76.6)	0.926
ProRoot MTA	53.69 ± 30.22 (53.7)	51.57 ± 29.87 (53.3)	57.48 ± 19.75 (57.6)	0.912
NeoMTA Plus	57.16 ± 28.13 (57.1)	43.73 ± 23.31 (36.2)	54.02 ± 19.53 (49.5)	0.472
CDMEM	84.1 ± 1.8 (84.9)	77.59 ± 6.09 (77.7)	53.2 ± 36.34 (52.9)	0.393
p	0.091	0.452	0.608	

CDMEM, complete dulbecco's modified eagle medium; SD, standard deviation.
*Kruskal Wallis Test *p < 0.05.*

Table 3 Evaluation of early apoptotic cell ratios among 3 different days and among 4 different groups.

	Early Apoptotic Cell Ratios			p
	1st Day	3 rd Day	7 th Day	
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
Biodentine	9.38 ± 15.34 (2.5)	4.04 ± 4.87 (2.5)	2.75 ± 4.21 (0.9)	0.794
ProRoot MTA	10.84 ± 17.54 (3.1)	6.09 ± 4.73 (6.2)	7.2 ± 12.17 (1.6)	0.904
NeoMTA Plus	12.47 ± 18.68 (4.9)	8.37 ± 9.82 (5.4)	5.08 ± 4.29 (4.9)	1.000
CDMEM	2.77 ± 1.29 (2.5)	4.41 ± 2.49 (4.9)	24.3 ± 25.64 (24.6)	0.665
p	0.993	0.907	0.311	

CDMEM, complete dulbecco's modified eagle medium; SD, standard deviation.
*Kruskal Wallis Test *p < 0.05.*

Table 4 Evaluation of late apoptotic cell ratios among 3 different days and among 4 different groups.

	Late Apoptotic Cell Ratios			p
	1st Day	3 rd Day	7 th Day	
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
Biodentine	14.74 ± 11.93 (12.7)	23.82 ± 26.72 (14.4)	6.55 ± 3.7 (6.8)	0.551
ProRoot MTA	20.08 ± 10.84 (16.3)	31.46 ± 27.29 (28.7)	18.7 ± 18.68 (14.2)	0.694
NeoMTA Plus	13.66 ± 5.23 (12.7)	27.45 ± 19.03 (29.7)	18.66 ± 12.57 (19.2)	0.368
CDMEM	9.14 ± 1.77 (9.3)	9.61 ± 3.6 (10)	16.95 ± 13.62 (17.1)	1.000
p	0.197	0.707	0.530	

CDMEM, complete dulbecco's modified eagle medium; SD, standard deviation.
*Kruskal Wallis Test *p < 0.05.*

resulting in the formation and secretion of tertiary dentin, leading to dentin bridge formation. Therefore, these materials must be biocompatible with the pulp cells.[23–29]

According to ISO standards, cell viability tests can be evaluated at the end of the 24, 48 and 72 h. Although the cell viability test periods are generally stated as 1st day and 3rd day in the literature, the cell viability was evaluated at the end of the 7th day in this study in order to evaluate the long-term toxic effects of the materials.[22]

Chang et al. evaluated the biocompatibility of Biaggregate (BA), Micromega MTA (MMTA), ProRoot MTA (PMTA) and intermediate restorative material (IRM) on human pulp cells by MTT test at 1, 7 and 14 days PMTA, BA and MMTA showed equal biocompatibility, whereas IRM had a cytotoxic effect on cells when compared these materials.[24]

Luo et al. showed the effects of 4 different concentrations of Biodentine solutions (Biodentine 0,02 mg/ml; Biodentine 0,2 mg/ml; Biodentine 2 mg/ml; Biodentine 20 mg/ml) on hDPSCs at the end of days 1, 3, 5 and 7th days with MTT test and also they evaluated the cell viability of these experimental groıps on pulp cells at the end of 24 h with Brdu test. They demonstrated that BD 0,2 and BD 2 groups increase the proliferation of hDPSCs, but the highest concentration of BD 20 group by reducing the cell proliferation by cytotoxic effect.[30]

Rodrigues et al.[31] studied the effects of MTA Plus and MTA Angelus on human pulp stem cells. They evaluated the

Table 5 Evaluation of necrotic cell ratios among 3 different days and among 4 different groups.

	Necrotic Cell Ratios			p
	1st Day	3 rd Day	7 th Day	
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
Biodentine	12.96 ± 21.97 (2.6)	12.8 ± 9.04 (11.2)	15.47 ± 11.91 (12)	0.368
ProRoot MTA	15.39 ± 24.76 (4.5)	10.88 ± 5.49 (9.1)	16.61 ± 7.53 (17.8)	0.368
NeoMTA Plus	16.71 ± 23.44 (7.4)	20.45 ± 15.28 (21.1)	22.25 ± 10.48 (21.4)	0.584
CDMEM	5.77 ± 1.09 (6.3)	12.04 ± 7.74 (10.5)	7.21 ± 1.01 (7.1)	0.135
p	0.706	0.812	0.191	

CDMEM, complete dulbecco's modified eagle medium; SD, standard deviation.
Kruskal Wallis Test $*p < 0.05$.

cell viability by MTT test and indicated that neither experimental group had a cytotoxic effect on cells. They also examined apoptotic and necrotic cell ratios by flow cytometry. The apoptptic cell ratios in both experimental groups were similar to each other and positive control group (DMEM). They stated that MTA Plus and MTA Angelus did not induce apoptosis, but there was a small increase in necrotic cells. They also reported that this small increase in the rate of the necrosis did not affect the overall rate of dead cells (apoptosis + necrosis).[31]

Jung et al.[32] assesed the viability of MTA, Biodentine and Bioaggregate on HDPCs by using MTT test and reported that the effects of these materials on cell viability were similar.[32]

Although there are many studies investigating the effects of materials such as MTA and Biodentine on the pulp cells, there are limited studies about NeoMTA Plus which is a new material which is releases to eliminate the disadvantages of MTA.

In the previous studies, it was demonstrated that MTA cements have not cytotoxic effects on dental pulp stem cells. Cell viability and bioactivity tests are important to evaluate cellular damage and biological effect of new biomaterials.[33] In the present study, the effects of NeoMTA Plus, which is a new material besides MTA Angelus, ProRoot MTA on human dental pulp stem cells were investigated.

Tanomaru-Filho et al.,[34] researched the biocompatibility of the NeoMTA Plus, MTA Angelus and the tricalcium silicate cement containing the tantalum oxide with MTT test. They formed the materials in different dilutions and measured the cell viability after 24 h. According to the test results, they reported that NeoMTA Plus, MTA Angelus and the tricalcium silicate cement were biocompatible on hDPSCs.[34]

Tomas Catala et al.[35] researched the effects of the NeoMTA Plus, MTA Angelus and MTA Repair HP on dental pulp stem cells by using MTT test on days 1, 3 and 7. As a result they reported that there was a high level of cell proliferation and binding in all three materials stated.[35]

In these studies about NeoMTA Plus, the only cell viability was examined using MTT test. In this study, the cell viability as well as the dead cell rates (early apoptotic, late apoptotic and necrotic cell rates) were evaluated differently from other studies using flow cytometry analysis.

The results of this study showed that the all groups exhibited the similar cell viability and demonstrated low percentage of early apoptotic, late apoptotic and necrotic cells. At all time intervals, the cell viability and dead cell ratios didn't show statistical significant difference between all experimental groups. When the datas presented in the tables at the end of the study are evaluated, some 'SD' values can be seen as higher than the 'Mean' values due to the nonparametric distribution of the datas.

The main composition of the MTA material is tricalcium silicate, tricalcium oxide, tricalcium aluminate, silicate oxide and bizmuth oxide. The most important difference of NeoMTA Plus is that it does not contain bismuth oxide and contains a important amount of tantalum pentoxide. NeoMTA Plus is bismuth free and contains a significant amount of tantalum pentoxide. Tantalum has been used for orthopedic plates, membranes and plates due to its inertness.

The results of this study showed that tantalum did not affect the biocompatibility of these cements. This result is consistent with the results of the studies of Tanomaru et al. and Tomas Catala et al. The effect of NeoMTA Plus on cell viability in these two studies was found to be similar to our study when compared with MTA and Biodentine.[34,35]

Tantalum oxide has been reported to have adequate physicochemical properties such as sufficient radiopacity and calcium hydroxide production and also does not discolorize on dental tissues.[36]

Liu et al. showed that iRootBP Plus a tricalcium silicate-based cement which contains Ta_2O_5 promoted proliferation of hDPCs.[37] The present study confirmed that NeoMTA Plus (tricalcium silicate based material associated with tantalum oxide) showed biocompatibility and similar cell viability compared with Biodentine, ProRoot MTA and DMEM.

This study has some limitations due to in vitro conditions not being able to fully reflect the mouth. Therefore, there is a need for support by in vivo experiments.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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