Int J Physiol Pathophysiol Pharmacol 2026;18(2):29-38
www.ijppp.org /ISSN:1944-8171/IJPPP0170201

Original Article

Investigating the cytotoxic effect of polyethyleneimine nanodendrimer on fibroblast cells

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Received November 23, 2025; Accepted March 31, 2026; Epub April 15, 2026; Published April 30, 2026

Abstract: Introduction: Oral health is associated with an individual's overall health and daily function, as well as having an impact on mental health. As such, this study evaluated the cytotoxic and biocompatibility profile of fourth-generation polyethyleneimine (PEI) nanodendrimers using a human gingival fibroblast cell line [1]. The aim was to determine the concentration- and time-dependent cytotoxic effects of PEI nanodendrimers to assess their suitability for potential dental applications. Materials and methods: This laboratory study investigated the potential cytotoxicity and biocompatibility of fourth-generation PEI nanodendrimers. PEI nanodendrimers were synthesized, and their cytotoxic effects were tested on human gingival fibroblast cells (2PI2HGF) at varying concentrations (1-128 µg/mL) over 48 and 72 hours. Viability of the cells was determined by MTT assay, and growth curves were constructed to determine the rate of cell proliferation in response to the addition of the PEI dendrimer. Results: Cytotoxicity was shown to increase with increasing concentrations of PEI dendrimers. For example, after 48 and 72 hours of exposure to a range of concentrations (1-128 µg/mL), viability levels dropped from 99.18% to 67.7% and 97.3% to 65.1%, respectively. It should also be noted that the extent of reduced cell viability was influenced by both the duration of exposure and the dendrimer concentration. Conclusion: These results indicate that while PEI dendrimers show significant potential for use in dentistry, they also demonstrate considerable cytotoxicity and subsequent concern over their biocompatibility. Future research should therefore focus on improving the safety and efficacy of dendrimer materials in the context of developing therapies for oral health. These findings contribute to the growing body of evidence on nanomaterials in dentistry and highlight the need to balance antimicrobial efficacy with acceptable cytotoxicity for clinical translation.

Keywords: Polyethyleneimine dendrimers, cytotoxicity, human gingival fibroblast cells, nanotechnology, oral health

Introduction

The quality of an individual's life is significantly affected by their oral health. Speaking, eating, and social interactions are all essential functions that are affected by poor oral health. Due to the significance of oral health in maintaining an individual's overall well-being, and the emphasis placed upon prevention rather than treatment, caring for your mouth and stopping the growth of pathogens is paramount [2-4]. Dental plaque is a significant factor in the development of caries and periodontal disease, so reducing dental plaque should be the primary focus for prevention. Cultural ignorance regarding oral health and the high expense of treatments have increased interest in globally adopting preventive measures and the need for continued research in this area [5].

Dental caries (tooth decay) is one of the main conditions affecting oral health. This happens when bacteria metabolize sugar in the mouth to produce acids that weaken tooth enamel. With continued acid production and subsequent demineralization (the loss of minerals such as

https://doi.org/10.62347/GQXA3269

calcium and phosphate from enamel), there is a greater chance that the cycle of demineralization will lead to dental caries [6, 7]. The development of bacterial biofilm associated with dental caries begins with the initial reversible attachment of microorganisms to the tooth surface (enamel), followed by irreversible attachment and the development of an extracellular polymeric substance (EPS) matrix. The bacteria form microcolonies that create a multilayer three-dimensional biofilm structure. The ecological plaque hypothesis suggests that changes in the microenvironment around the plaque biofilm can lead to the development of dental diseases, including dental caries and periodontal disease [8, 9]. Therefore, preventive strategies that target biofilm formation, including mechanical and chemical approaches, are central to maintaining oral health.

One of the many chemical means of preventing the development of biofilms is using a mouthwash product; therefore, this type of product has become one of the significant areas of emphasis in the area of preventive dentistry. There are many different types of chemical products included in the formulation of mouthwashes, which provide for greater safety and effectiveness of the anti-plaque properties that they produce. Nanoparticle compounds are also a new class of compounds to be in cluded in this formulation, and they have demonstrated a significant ability to improve the overall state of oral health [10]. Nanoparticle compounds are also a new class of agents in mouthwash formulations and have demonstrated significant potential to enhance antimicrobial activity and biofilm control in the oral cavity.

Nanotechnology has transformed many different areas and specifically in medicine, it provides a lot of potential to improve the way drugs are delivered. The term nanoparticle refers to a particle measuring 100 nm or smaller. The most well-known nanocarrier is a dendrimer, which has similarities with dendritic cells due to its unique structure and various capabilities [11]. A dendrimer is made from a series of iterations where multiple polymer layers are added to a central core, creating a dendrimer with multiple generations. Dendrimers are known for their distinct properties, which emerge with the creation of generations. Dendrimers are widely recognised as being polyvalent, due to the many functional groups located on their surfaces. The polyvalence allows for targeted drug transport, in addition to the ability to accurately manipulate the size, shape, and surface properties of the dendrimer, therefore allowing for the effective design of new drugs [12, 13]. In dentistry, dendrimer-based systems have been explored for antimicrobial delivery, biofilm disruption, and improved retention in the oral cavity, but their cytotoxicity toward oral fibroblasts remains a critical concern.

However, despite the promising antimicrobial and drug-delivery applications of polyethyleneimine (PEI) nanodendrimers in dentistry, data on their concentration- and time-dependent cytotoxicity in human gingival fibroblasts remain limited. Therefore, this study aimed to characterize fourth-generation PEI nanodendrimers and evaluate their cytotoxic effects on a human gingival fibroblast cell line across a range of concentrations and exposure times. We hypothesized that: (1) The effects of PEI nanodendrimers on human gingival fibroblast cells are dependent on the administered concentration [11]; (2) Increasing exposure time increases cytotoxicity toward these cells [2]; (3) Structural features of PEI nanodendrimers, such as size, shape and surface functional groups, influence their cytotoxic potential [14]; (4) PEI nanodendrimers may induce cytotoxicity through mechanisms including membrane disruption and oxidative stress [15]; and (5) Chemical modification and functionalization of PEI nanodendrimers can yield formulations with a more favorable cytotoxicity profile and improved biocompatibility for dental applications [16]. In addressing these hypotheses, this study seeks to provide a better understanding of the potential use of PEI nanodendrimers in medicine and dentistry, particularly in the context of oral health.

Material and methods

Materials and equipment collection

This laboratory-based experimental study included several major components, including the selection of materials and equipment, synthesis of fourth-generation polyethyleneimine (PEI) nanodendrimers, acquisition and activation of human gingival fibroblast cells (2PI2-HGF), preparation of different dendrimer con-

centrations, assessment of cell viability at multiple post-exposure time points, plotting of growth curves over 9 days, and data collection and analysis [17, 18].

Cell line and dendrimer preparation

The cells used in this study were human gingival fibroblast cells (166C, 2PI2HGF), obtained from the National Gene Bank of Iran (Tehran, Iran). Fourth-generation PEI nanodendrimers synthesized as described above were used in all experiments. For the MTT assay, 5,000 cells were seeded per well, and for growth curve experiments, 7,500 cells were seeded per well.

Ethical approval

Before conducting the laboratory study, the Research Ethics Committee of Ardabil University of Medical Sciences approved the protocol (IR.ARUMS.REC.1401.167). The study did not involve research on humans or animals; therefore, no ethical issues were anticipated. Material safety data sheets (MSDS) were available for all hazardous substances, and all procedures complied with institutional safety and environmental regulations.

Dendrimer synthesis

Fourth-generation PEI nanodendrimers were synthesized divergently from an ethylenediamine core. Briefly, 4.4 g of acrylonitrile was added to an aqueous solution of ethylenediamine (10.33 mL per gram). The exothermic reaction increased the temperature to 40°C, after which the mixture was heated to 85°C for two h to ensure complete reaction of the reagents. Excess acrylonitrile was removed by vacuum extraction at 50°C and 18 mbar, yielding a solid half-generation polyethyleneimine dendrimer. Hydrogenation of this intermediate to the first-generation dendrimer was carried out using Raney nickel in 100 mL of 2 M sodium hydroxide and 100 mL of distilled water under 30 atm at 75°C for three h. After cooling, the mixture was filtered, and the solvents were removed by rotary evaporation under reduced pressure, yielding the first-generation dendrimer. Higher generations up to the fourth, with 16 terminal amino groups, were obtained by repeating these steps with excess acrylonitrile. FTIR analyzed structural and functional characteristics of the dendrimers, and TEM and SEM evaluated their size and morphology.

Cell culture conditions

The effects of fourth-generation PEI nanodendrimers on fibroblast cell lines were evaluated using the MTT assay. The 2PI2HGF fibroblast cell line was sourced from the National Gene Bank of Iran (Tehran, Iran). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; [Gibco, Thermo Fisher Scientific, Waltham, MA, USA]) supplemented with 10% heat-inactivated fetal bovine serum (FBS; [Gibco, Thermo Fisher Scientific, Waltham, MA, USA]), two mM L-glutamine, penicillin (50 U/mL) and streptomycin (50 µg/mL) (all from [Company, City, Country]). Cultures were maintained at 37°C in a humidified incubator with 5% CO_2 and 85% relative humidity, and the medium was changed every three days [19].

Cell counting and preparation for dendrimer exposure

For cell counting, 10 µL of the cell suspension was loaded into a Neubauer counting chamber and examined under a light microscope. Approximately 5,000 cells were seeded into each well of a 96-well plate containing 100 µL of DMEM. After 24 h, once the cells had adhered, the medium was removed and the wells were washed three times with sterile PBS (0.01 M). Subsequently, 200 µL of medium containing different concentrations of fourth-generation PEI nanodendrimers (1, 4, 16, 32, 64, and 128 µg/mL) was added to each well, and the plates were incubated for 48 or 72 h.

MTT assay for cell viability

After incubation with nanodendrimers, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, shaken at 120 rpm for 5 min, and incubated at 37°C for four h. During this period, mitochondrial succinate dehydrogenase in viable cells reduced the yellow MTT to purple formazan crystals. The medium was then removed, and 200 µL of DMSO was added to dissolve the crystals. Absorbance at 570 nm was measured using a microplate reader, and cell viability was calculated as a percentage of the negative control (cells cultured without nanodendrimers) [20, 21].

Doubling time and growth curve evaluation

To assess the effect of fourth-generation PEI nanodendrimers on fibroblast doubling time,

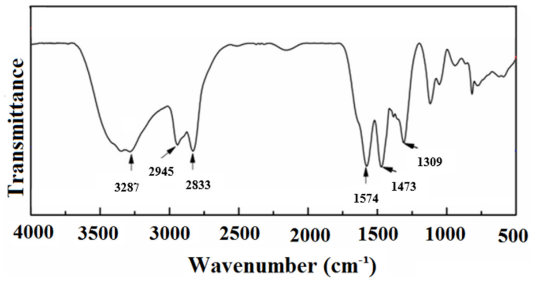

Figure 1. FT-IR spectrum of the fourth-generation polyethyleneimine nanodendrimer.

different dendrimer concentrations (1, 4, 16, 32, 64, and 128 µg/mL) were added to 12-well plates containing 7,500 fibroblast cells per well, one day after seeding. Cells were exposed for 48 h, after which the medium was replaced with fresh, dendrimer-free medium, and incubation continued under standard culture conditions until day 9. Daily cell counts were performed using a Neubauer chamber and light microscopy, and growth curves were generated. Doubling time (Td) was calculated using the Peterson equation.

Statistical analysis

Statistical analyses were performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). After checking variance homogeneity and normality, independent t-tests and one-way ANOVA followed by Tukey's post hoc test were applied. A p-value <0.05 was considered statistically significant.

Results

Characterization of fourth-generation polyethyleneimine dendrimers

The vertical loading characteristics on DC motors using the fourth-generation polyethyleneimine dendrimers were established by FT-IR analysis, as shown in **Figure 1**. The FT-IR spectrum of this material contains defined peak positions between 4000 and 400 cm^{-1} at 2945, 2833, and 1473 cm^{-1} relating to stretching and bending modes of the CH2 aliphatic functional group. The peak at 3287 cm^{-1} indicates the presence of H-N functional groups within the dendrimer, confirming the presence of amines within the structure of the dendrimer.

The morphology and dimensions of the fourth-generation polyethyleneimine dendrimers were confirmed by the analysis of the dendrimers via Transmission Electron Microscope (TEM). **Figure 2** illustrates that the TEM images of the dendrimers display a near-spherical, smooth-surfaced shape. TEM images display that the average size of the dendrimers is approximately 15 nm. A complementary analysis of the dendrimers using a Field Emission Scanning Electron Microscope (FE-SEM) also shows that the dendrimers are spherical in morphology and exhibit a greater average size of approximately 25 nm (**Figure 3**).

Cytotoxicity assessment

The MTT test determined cytotoxicity for polyethylenimine dendrimers of the fourth generation using multiple concentration points for a 48-hour and a 72-hour time period. **Figure 4** demonstrates that as the concentration increases and the time of exposure lengthens, the viability of the cells is reduced compared to the negative control. More than 40% fewer cells were viable after 48 hours in the 128 µg/mL concentration (67.7%) than at the lowest concentration of 1 µg/mL (99.18%). This represents a gradual decline in viability from 97.3% at 48 hours to 65.1% after a full 72-hour exposure at the same concentration. In contrast, the positive control (cisplatin) caused cell viability to drop to approximately 20% (20.18%) and 14% (14.22%) after 48 hours and 72 hours, respectively, using a 30 µg/mL cisplatin solution.

Growth curve analysis

Figure 5 shows the effect of varying concentrations of fourth-generation polyethylenimine dendrimers on fibroblast cell growth curves and doubling times. These data support what was seen in **Figure 5**, with increased concentrations of dendrimers having a decrease in fibroblast cell proliferation compared to the negative control (untreated) group. For example, a negative control cell count of 7,500 at day zero produced a count of 1,263,500 by day nine. In comparison, the exact cell count at exposure to 128 µg/mL of dendrimer-treated cells was only counted at 908,500 on day nine, a difference of over 350,000 by day nine.

At day three, a comparison of different dendrimer concentrations revealed that higher

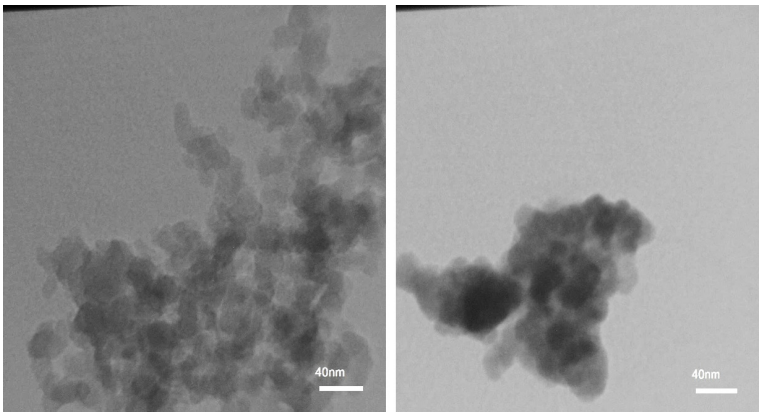

Figure 2. TEM image of the fourth-generation polyethyleneimine nanodendrimer.

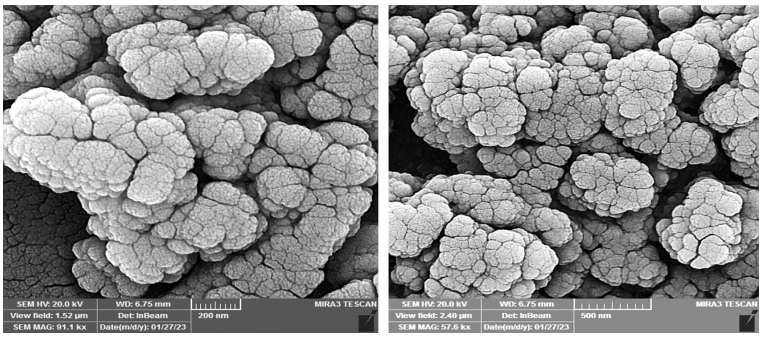

Figure 3. FE-SEM image of the fourth-generation polyethyleneimine nanodendrimer.

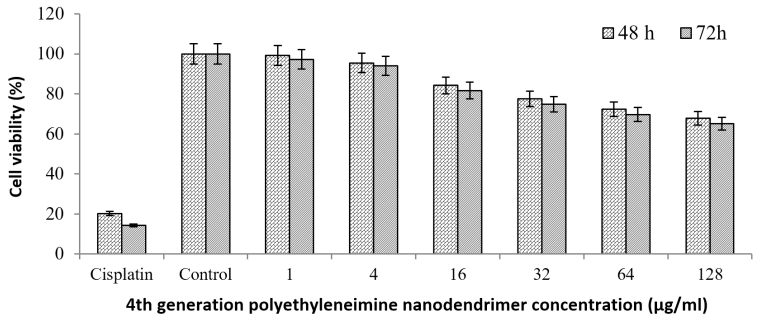

Figure 4. Evaluation of the cytotoxicity of the fourth-generation polyethyleneimine nanodendrimer at different concentrations (1 to 128 µg/ml) on fibroblast cells at contact times of 48 and 72 hours, with cisplatin at a concentration of 30 µg/ml as a positive control.

concentrations corresponded to fewer surviving cells. Specifically, at concentrations of 1 and 128 µg/mL, the cell counts were 117,400 and 76,200, respectively. By day nine, these numbers adjusted to 1,249,500 and 908,500.

Table 1 summarizes the impact of varying concentrations of fourth-generation polyethyleneimine dendrimers on the doubling time of fibroblast cells compared to the negative control. The average doubling time increased from 22.58 hours in the control group to 25.96 hours at a concentration of 128 µg/mL. Overall, while there was a trend toward increased doubling time with higher dendrimer concentrations, the increase was not statistically significant, indicating moderate cytotoxicity and showing that cells continued to proliferate, though at a reduced rate and with increased doubling time, particularly at higher dendrimer concentrations.

Statistical analysis

With an increase in the concentration of fourth-generation polyethyleneimine dendrimers, the viability of fibroblast cells relatively decreased over the 48 and 72-hour exposure times. One-way ANOVA analysis with a 95% confidence level showed that at some concentrations (1 µg/ml), there was no statistically significant difference ($P>0.05$) between the increase in the concentration of fourth-generation polyethyleneimine dendrimers and cellular toxicity compared to the negative control samples during the 48 and 72-hour exposure times. However, at some concentrations (4 to 128 µg/ml), there was a statistically significant difference ($P<0.05$) between the increase in the concentration of fourth-generation polyethyleneimine dendrimers and cellular toxicity compared to the negative control samples during the 48 and 72-hour exposure times (**Table 2**).

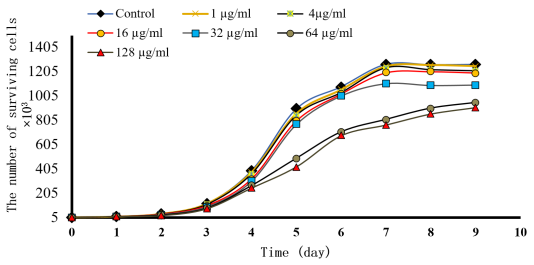

Figure 5. Cumulative growth curve of fibroblast cells in the presence and absence (negative control) of the fourth-generation polyethyleneimine nano dendrimer at different concentrations.

Comparing the cellular toxicity of fourth-generation polyethyleneimine dendrimers between the 48 and 72-hour exposure times, the statistical T-test analysis showed that there was a statistically significant difference ($P<0.05$) between the duration of treatment and the cellular toxicity of the dendrimers (**Table 3**).

Comparing the cell growth on days 1 to 9 with the exposure to concentrations of 1 to 128 μg/ml of the dendrimer and the negative control samples, the statistical T-test analysis showed that there was no statistically significant difference ($P>0.05$) between these two variables (**Table 4**).

Discussion

The study objective was to examine the potential toxic effects of PEI dendrimers on fibroblasts while characterizing the structure and mechanisms of toxicity. Our findings provide insight into the potential use of PEI nanodendrimers in biomedical products, particularly dental applications, by linking their structural features to their cytotoxic behavior. In the following sections, we interpret these results in the context of structural characterization, cytotoxicity profiles, comparative data, and proposed mechanisms of toxicity.

Structural characterization of PEI dendrimers

Using Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM), the structure of the fourth-generation PEI dendrimer was characterized. Analysis using FTIR revealed the presence of many significant functional groups on the surface of the dendrimer that may contribute to the biological properties of PEI dendrimers. The peaks observed at 2945 cm^{-1}, 2833 cm^{-1}, and 1473 cm^{-1} represent the stretching and bending vibrations of aliphatic groups, while the bands at 3287 cm^{-1} and 1574 cm^{-1} belong to H-N groups. It is believed that these functional groups interact with cellular membranes, which in turn may increase the cytotoxicity of PEI dendrimers [22, 23].

The average diameter of the dendrimers found from examination of the images showed that the dendrimers are almost spherical. The arrangement of these dendrimers creates a very dense and compact structure, and therefore has a large surface area in comparison to the volume, allowing the dendrimers to interact with fibroblast cells effectively. The findings of this research match previous studies where PEI dendrimers were shown to possess the same morphology as those reported as modified nanoparticles. Therefore, we believe that our findings support current literature. The structure of the fourth-generation polyethyleneimine dendrimers is very irregular, dense, and branched, which is necessary for their functional properties [24, 25]. From a biological standpoint, this compact, highly branched architecture, together with the high density of surface amines, likely underlies both the efficient interaction with cell membranes and the cytotoxic effects observed in our fibroblast model.

Cytotoxicity assessment

The effects of cytotoxicity of PEI dendrimers were evaluated at different concentrations and exposure times to assess whether there was a correlation between the amount of dendrimers added to the media and how many of the number of fibroblast cells that were alive. The results of the cytotoxicity assessment showed a strong difference between the increasing amount of dendrimers and the amount of cell death caused by the dendrimers, as well as evidence that there was a significant loss in cell viability following the 24 hr and 72 hr exposure times to the highest concentration of 128 μg/mL at which only about 65% of the fibroblast cells were alive at 72 hr. This supports findings from Liu et al. (2016) that found that dendrimers have the ability to form pores or holes in the membranes of eukaryotic cells, which can have a cytotoxic effect [26]. In practical terms, this level of residual viability suggests that fourth-generation PEI nanodendrimers exhibit moderate

Table 1. Effect of the fourth-generation polyethyleneimine nanodendrimer at different concentrations on the doubling time of fibroblast cells and comparison with negative control samples

Day	0 µg/mL (Negative Control)	1 µg/mL	4 µg/mL	16 µg/mL	32 µg/mL	64 µg/mL	128 µg/mL
Day 0	7500	7500	7500	7500	7500	7500	7500
Day 1	246200	376000	36350	321000	301700	267421	246200
Day 2	36166	35400	34300	33550	30200	25950	20880
Day 3	12566	12500	12750	12775	12500	12500	12525
Day 4	118500	117400	112250	101750	92300	81700	76200
Day 5	897500	858750	844750	798300	769250	492000	420750
Day 6	1080250	1056000	1032500	1020500	1003900	709100	677154.5
Day 7	1264375	1247250	1236250	1195075	1105075	811407	765335
Day 8	1260500	1258150	1220000	1204000	1090500	903932	855750
Day 9	1263500	1249500	1211000	1193957	1094000	950018	908500
Doubling Time (hours)	32	32	31.4	22.2	23.8	26.8	32.6

Table 2. One-way ANOVA analysis between the factors of the fourth-generation polyethyleneimine nanodendrimer concentration and cell viability with negative control samples

Fourth-Generation Polyethyleneimine Dendrimer Concentration (µg/ml)	Exposure Time (48 hours)	Exposure Time (72 hours)
1	0.889	0.169
4	<0.001	<0.001
16	<0.001	<0.001
32	<0.001	<0.001
64	<0.001	<0.001
128	<0.001	<0.001

P<0.05 was considered statistically significant.

Table 3. T-test analysis between the two factors of 48 and 72-hour exposure times of fourth-generation polyethyleneimine dendrimers and cell viability

Fourth-Generation Polyethyleneimine Dendrimer Concentration (µg/ml)	Significance Level (p)	T Value
1	<0.001	9.87
4	<0.001	6.682
16	0.024	3.015
32	<0.001	6.505
64	0.007	4.077
128	<0.001	12.587

P<0.05 was considered statistically significant.

Table 4. T-test analysis between the negative control group and concentrations of 1 and 128 of the fourth-generation polyethyleneimine nanodendrimer and cell viability

Group/Concentration	T Value	Significance Level
Negative Control	2.206	0.136
1 µg/ml	2.20	0.115
128 µg/ml	2.207	0.113

cytotoxicity toward gingival fibroblasts at higher concentrations. For dental applications, this indicates that careful optimization of dose and exposure time would be required to remain within an acceptable safety margin.

It should also be noted that as the generation of dendrimers increases, the amount of amine groups also increases, thereby increasing the cytotoxicity associated with the higher generation of dendrimers. The number of amine functional groups is doubled at each generation level, causing a higher charge density and thus having an effect on the interaction of dendrimers with cellular membranes. When considering the interaction between a positively charged amine functional group with a negatively charged bacterial membrane, the positively charged amine group on the dendrimers can interact preferentially with and cause toxicity to bacteria rather than eukaryotic cells [27]. Due to the targeted delivery of antimicrobial agents, the finding applies to dental applications. In our study, although only one dendrimer generation was tested, the apparent concentration-dependent loss of fibroblast via-

bility is consistent with this concept and underscores the need to minimize surface charge density or shield amine groups when designing PEI-based formulations for oral use.

The study also revealed that the time of exposure to dendrimers has an impact on cellular viability, and the longer the duration the cells are in contact with PEI dendrimers, the less viable they are [28]. Understanding the duration of exposure and toxicity associated with the materials will help to establish a safety profile for these materials when used therapeutically. The statistically significant differences between 48- and 72-hour exposures in our data indicate that prolonged or repeated contact in the oral cavity could substantially increase the risk of fibroblast damage, which is particularly relevant for sustained-release or adherent dental products.

Comparative analysis with previous studies

Previous studies have shown that polyelectrolyte-containing dendrimers can exhibit considerable cytotoxic activity depending on their chemical structure and biological context [2, 29]. Bąchor et al. (2023) discovered that some isoxazole derivatives would significantly decrease the growth of biofilms without having an increased cytotoxic effect [29]. Furthermore, ElGammal et al. (2023) demonstrated that some recently developed antibiotics have less than ideal cytotoxic effects on cells when compared to some others and indicated that the cytotoxic profile of nanoparticles is heavily dependent on their chemical structures and how they are functionalized [2]. In contrast to these reports, our findings in gingival fibroblasts indicate that PEI nanodendrimers maintain a measurable but not extreme cytotoxicity within the tested concentration range, suggesting a potential therapeutic window if formulations are carefully tuned.

Our results comparing PEI dendrimers with silver nanoparticles revealed that silver nanoparticles can exhibit cytotoxicity above 20 ppm. In contrast, PEI dendrimers appear to exert their primary cytotoxic effect through membrane disruption, which helps to differentiate PEI dendrimers from other nanoparticles. Understanding the ability of PEI dendrimers to penetrate through bacterial membranes supports their practical use in treating bacterial infections, especially in situations where biofilms are prevalent, as would be the case in many dental environments. Together, these comparisons suggest that PEI nanodendrimers may offer a more controllable balance between antibacterial activity and host-cell toxicity than some metallic nanoparticles, provided that their concentration and exposure are carefully controlled in clinical formulations.

Mechanisms of cytotoxicity

PEI dendrimers have several ways of inducing toxicity via cellular dysfunction. The high density of amines results in strong electrostatic interactions between PEI and a cell's negatively charged membrane, leading to breach of the membrane and therefore, a more permeable state, resulting in lysis of the cell. As well, aggregates of PEI dendrimers may increase the degree of cytotoxicity by increasing the amount of PEI dendrimers localized at the cell membrane interface [26, 30]. The gradual, dose-dependent decline in fibroblast viability observed in our MTT and growth curve experiments is compatible with this membrane-driven mechanism, where higher local concentrations of PEI at the cell surface translate into greater functional impairment and cell loss.

Reactive oxygen species (ROS) generated from exposure to PEI dendrimers may also contribute to increased oxidative stress, thereby increasing the damage to the cell. The disturbance of the membrane and ROS generation represents another complication in the overall understanding of how PEI dendrimers interact with cells. A better understanding of the interplay between ROS and membrane disturbance would lead to a greater understanding of how these complex interactions occur.

Implications for dental applications

Thus, there are critical implications for the use of PEI dendrimers in dentistry. However, there are concerns regarding cytotoxicity and the limit of biocompatibility; thus, their use for targeted drug delivery and as an antimicrobial agent should not be overlooked. The fact that PEI dendrimers can penetrate the bacterial cell membrane indicates that they may be valuable as potential therapeutic agents for infection treatment, especially dental infections, where biofilm is often present. However, our fibroblast data indicate that such benefits will only be acceptable clinically if PEI nanodendrimers are

used at concentrations below those that significantly impair gingival cell viability or if their surfaces are modified to reduce direct cytotoxicity.

Based on the cytotoxicity observed in this study, we believe that further optimization work will be necessary to improve the biocompatibility of dendrimer formulations, while preserving their antibacterial potential. Future studies should investigate modifying the surface chemistry of PEI dendrimers in order to reduce cytotoxicity to fibroblasts and increase their clinical usage as therapeutics. Possible methods for reducing toxicity include biocompatible coatings and modifying the chemical properties of existing functional groups.

Future directions

To increase the therapeutic efficacy and minimize the cytotoxicity of PEI dendrimers, continued investigation into the optimal design of PEI dendrimer formulations should be conducted. Possible avenues of investigation would include alternate modifications of PEI dendrimer architecture and the incorporation of different functional groups into the dendrimer. Additionally, the combination of PEI dendrimers with other types of therapeutic compounds may provide greater therapeutic benefit without increasing toxicity.

In addition to optimizing therapeutic efficacy and decreasing cytotoxicity, future studies should also determine the long-term impact of PEI dendrimers on cellular function, evaluate the cumulative toxicity due to the use of PEI dendrimers, and conduct in vivo studies to evaluate the safety and efficacy of using PEI dendrimer formulations in the clinic. The determination of the pharmacokinetics and biodistribution of these nanoparticles will be a critical factor in the successful application of these types of nanoparticles within the biomedical field.

Conclusion

Overall, this study demonstrates that fourth-generation PEI nanodendrimers exhibit concentration- and time-dependent cytotoxicity toward human gingival fibroblasts. A combination of structural characterization and assessment of cytotoxicity, along with an understanding of how nanoparticles interact with biological systems, has helped to determine the viability of using PEI dendrimers in the field of dentistry. Although there are many possibilities for the use of PEI dendrimers in dental medicine, additional research will be necessary to determine the risk/benefit ratio of their use in the clinic. In addition to adding to the growing body of literature on the use of nanomaterials for health care-related applications, this study serves as an important stepping stone toward improving future development of dental therapies. Future studies should focus on optimizing the design of PEI dendrimers to achieve the most therapeutic impact while reducing the risk of cytotoxicity. Thus, ensuring the safe use of PEI dendrimers in dental practice. From a translational perspective, these results emphasize that any incorporation of PEI nanodendrimers into dental products must be accompanied by rigorous biocompatibility testing in relevant oral cell types.

Disclosure of conflict of interest

None.

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