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Cytotoxic Effect of Nano Polyvinylidene Fluoride on Human Gingival Fibroblast

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Abstract

Aim: This study examined the cytotoxic effects of Nano Polyvinylidene-Fluoride (PVDF) on Human Gingival Fibroblasts (HGF) cells in comparison to sodium fluoride (NaF) varnish.

Method: The MTT-Assay was performed to evaluate the cytotoxicity of the tested materials on HGFs. To assess the toxic effects, cell viability after the application of NaF varnish (2.26%) and PVDF (1.26%, 2.26%, and 3.26%) at each concentration.

Results: The findings indicated that all concentrations of PVDF exhibited lower cytotoxicity compared to NaF varnish. Within the PVDF groups, the 3.26% concentration demonstrated the least cytotoxicity, succeeded by 2.26% and 1.26% respectively. On the other hand, NaF varnish exhibited the greatest toxicity among the evaluated substances.

Conclusion: This innovative study demonstrates that Nano-PVDF, particularly at a concentration of 3.26%, is a promising material exhibiting enhanced biocompatibility and less cytotoxicity relative to traditional NaF varnish. All data represented that Nano-PVDF may function as a safer option in dental treatments, with exhibiting little adverse effects on oral cells.

keywords: Nano-PVDF, Cytotoxicity, MTT-Assay, Sodium fluoride varnish, Human gingival fibroblast, Biocompatibility.

Introduction

Dental caries continues to be a major public health concern, necessitating the development of effective preventive measures. NaF varnish is widely used due to its demonstrated efficiency in caries prevention and enamel remineralization [(1)] . However, the biocompatibility of NaF varnish, particularly its cytotoxic effects on HGFs, which is considered as a topic of currently ongoing research. Studies suggested that NaF varnish is efficacious; however, its cytotoxicity toward HGFs may pose potential risks to oral health [(2)]. PVDF has been introduced in nano-form as a result of the emergence of nanotechnology. This material has the power to provide benefits in dental applications due to its enhanced biocompatibility and mechanical properties [(3)]. Nano PVDF is currently being studied as an alternative to conventional fluoride treatments, with an emphasis on its effects on HGFs.

The objective of this study is to assess the potential of Nano-PVDF as a safer and potential alternative to fluoride-based treatments by comparing the cytotoxicity of three different PVDF concentrations (1.26%, 2.26%, and 3.26%) with that of NaF varnish (2.26%). This research is crucial for developing dental interventions that optimize caries prevention while minimizing the adverse effects on gingival tissues.

Materials And Methodologies

This study was approved by the Research Ethics Committee of the college of dentistry, Mustansiriyah University (Approval No. MUPRV005)

Preparation of Nano PVDF mixture by different concentrations

Preparation of 1.26%:

1.Exactly 0.315 g of nano PVDF (Nanochemazone, Canada) was dissolved in 25 ml of Triethyl citrate (Sigma-Aldrich, Germany) with a stir for 2 hours at 80 °C.

2. Methyl callouses (30%, 0.0945 g) were added to the above mixture with a contentious stir for 2 hours at the same temperature until a clear solution was obtained.

3. This process was repeated by getting (0.565 g PVDF, 0.169 g methyl callouses) and (0.815 g, 0.244 g methyl callouses) to prepare 2.26 and 3.26% respectively [(4) (5)] .

Sodium Fluoride Varnish

NaF varnish used in this study was manufactured by FluoroDose, USA, with concentration of 2.26%, the varnish was approved by ADA.

Cell viability assay (MTT assay)

The cytotoxic effects of varying concentrations of nano PVDF varnish and NaF varnish on Human Dermal Fibroblasts (HDF) were evaluated using the MTT assay. The HDFs were cultured in RPMI-1640 medium. Subsequently, the cells were inoculated into 96-well plates and permitted to adhere for the night. Nano PVDF solutions and NaF varnish were prepared at 0.5 µL/mL concentration. The culture medium was replaced with media that contained varying concentrations of the medications after cell adhesion. The cells were cultured in a 5% of CO2 incubator at 37°C for 24, 48, and 72 hours. Immediately following each incubation period, 20 µL of MTT solution was added to each well and allowed to incubate for an additional 4 hours. Afterward, the medium was withdrawn, and the formazan crystals were dissolved by adding approximately 100 µL of solubilization solution to each well. A micro plate reader was employed to measure absorbance at a wavelength of 570 nm. The percentage of control cells was used to evaluate cell viability using the following formula: Viability of cells (%) = (Treated cells absorbance / Control cells absorbance) [6] .

Statistical analysis

The Statistical Package for the Social Sciences, version 28, was used to analyse the data. Here is the data presented:

1- Descriptive Analysis: Calculation of mean and standard deviation for quantitative variables.

2- Inferential analysis:

A- Shapiro-Wilk test: utilized for assessing the normality of distribution in quantitative variables.

B-Levene's test: test the equality of variances among groups.

C-One Way ANOVA test: to test the hypothesis for a quantitative dependent

variable by an independent variable followed by post hoc test (Bonferroni).

A level of p–value less than 0.05 was considered as significant.

Results

Cell vitality values were assessed at three distinct time intervals: 24 hours, 48 hours, and 72 hours. Table 1 presents a summary of descriptive statistics for each group, including the minimum, maximum, mean, and standard deviation (SD) of the cell vitality values.

Group 1 (26% nano PVDF) showed a decrease in cell vitality over the time, with mean values dropping from 23.6 at 24 hours to 8.1 at 72 hours. Group 2 (26% nano PVDF) also showed a decrease in cell vitality, with mean values decreasing from 33.4 at 24 hours to 8.0 at 72 hours. Similarly, group 3 (26% nano PVDF) exhibited a similar trend, with cell vitality, with values falling from 35.0 at 24 hours to 9.8 at 72 hours.

The positive control group (NaF varnish) maintained relatively stable cell vitality values over time, with a slight decrease from 16.3 at 24 hours to 7.7 at 72 hours. In contrast, the negative control group consistently exhibited a cell vitality value of 100 across all time points, indicating no observed toxicity (Figure .1).

The normality distribution test (Shapiro-Wilk) results showed that Cell Vitality values at all-time points were normally distributed ($p > 0.005$) Table 2.

The comparison of cell vitality between groups at different time points was conducted using a One-Way ANOVA test. The results are presented in Figure 2 and in Tables 3, 4, and 5 for 24-hour, 48-hour, and 72-hour time points, respectively.

The data showed a significant results from the One-Way ANOVA test, on the other hand, pairwise post-hoc comparisons using the Bonferroni correction were performed to determine specific differences between groups at the 24-hour, 48-hour, and 72-hour time point's (tables 6,7 and 8).

Discussion

The cytotoxicity of dental materials is essential for evaluating their safety, particularly concerning oral tissues like HGF. In this comparison, nano PVDF exhibited reduced cytotoxicity compared to NaF varnish, suggesting its viability as a safer option for prolonged clinical use. Previous cytotoxicity evaluations using the MTT-assay have previously indicated that NaF, although efficacious in remineralization, might cause cellular damage at elevated concentrations, leading to impaired fibroblast activity and potential apoptosis [(7)] . The hazardous profile of NaF can be ascribed to its effects on critical physiological processes, including oxidative stress and calcium metabolism, which affect fibroblast viability and wound healing. This corresponds with data demonstrating that fluoride exposure elevated production of inflammatory markers, such as FGF-2 and TGF-β, signifying tissue stress and remodelling activity [(8)] . Conversely, the inert and biocompatible characteristics of PVDF make it less likely to induce negative cellular reactions, thereby facilitating its use for enamel protection while preserving gingival health [(9)] . Moreover, these findings underscore necessity of investigating alternatives to fluoride-based therapies, particularly in formulations aimed at sensitive tissues such as gingival fibroblasts. As the cytotoxic potential of fluoride and other negative halogens becomes increasingly apparent with prolonged contact, PVDF emerges as a viable option for safer dental care. Additional research is advised to corroborate these findings over extended durations and evaluate PVDF's efficacy in other various therapeutic contexts.

Conclusions

This study concluded that 3.26% nano PVDF exhibits the highest biocompatibility and the lowest cytotoxicity on HGF cells, making it a promising candidate for clinical applications in the treatment of dental caries. Continuing study is essential to validate these findings and explore the potential applications of this novel nanopolymer.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Acknowledgments

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Table 1: The cell vitality means and standard deviations of all groups in different phases.

Groups		24 Hour	48 Hour	72 Hour
1.26 PVDF	Min.	23.0	10.9	7.6
	Max.	24.1	14.1	8.3
	Mean (SD)	23.6 (0.5)	12.1 (1.7)	8.1 (0.4)
2.26 PVDF	Min.	30.0	13.8	7.2
	Max.	36.6	16.4	8.5
	Mean (SD)	33.4 (3.2)	15.5 (1.4)	8.0 (0.7)
3.26 PVDF	Min.	33.2	14.9	9.4
	Max.	37.7	22.9	10.2
	Mean (SD)	35.0 (2.3)	17.9 (4.3)	9.8 (0.4)
F.Varnish	Min.	14.8	12.7	7.3
	Max.	17.2	14.0	8.1
	Mean (SD)	16.3 (1.3)	13.5 (0.7)	7.7 (0.4)
Neg. Cont.	Min.	100	100	100
	Max.	100	100	100
	Mean (SD)	100 (0)	100 (0)	100 (0)

*****PVDF= Polyvinylidene fluoride
*****F.Varnish= Sodium fluoride varnish
*Neg.Cont= Deionized water
*****SD= Standard deviation
*****Min= Minimum
*****Max= Maximum

Table 2: Normality distribution test of the Cell Vitality at different time points

Phases	Groups	Shapiro Wilk		
		Statistic	df	P value
24 Hour	1.26 PVDF	0.9	3	0.5
	2.26 PVDF	0.9	3	0.9
	3.26 PVDF	0.8	3	0.3
	F.Varnish	0.8	3	0.1
	Neg. Cont.		3	
48 Hour	1.26 PVDF	0.8	3	0.2
	2.26 PVDF	0.7	3	0.08

	3.26 PVDF	0.8	3	0.1
	F.Varnish	0.7	3	0.057
	Neg. Cont.		3	
72 Hour	1.26 PVDF	0.7	3	0.06
	2.26 PVDF	0.8	3	0.1
	3.26 PVDF	0.9	3	0.8
	F.Varnish	0.9	3	0.97
	Neg. Cont.		3	
* p– value less than 0.05 was considered as significant.				

Table 3: The comparison of the cell vitality (mean ± SD) between all the study groups after 24 hour.

24 Hour					
Groups	N	Mean (SD)	Statistics	df	*P-Value
1.26 PVDF	3	23.6 (0.5)	914.4	4	**< 0.001**
2.26 PVDF	3	33.4 (3.2)			
3.26 PVDF	3	35.0 (2.3)			
F.Varnish	3	16.3 (1.3)			
Cont. Neg.	3	100 (0)			

Table 4: The comparison of the cell vitality (mean ± SD) between all the study groups after 48 hours.

48 Hour					
Groups	N	Mean (SD)	Statistics	df	*P-Value
1.26 PVDF	3	12.1 (1.7)	894.0	4	**< 0.001**
2.26 PVDF	3	15.5 (1.4)			
3.26 PVDF	3	17.9 (4.3)			

F.Varnish	3	13.5 (0.7)			
Cont. Neg.	3	100 (0)			

Table 5: The comparison of the cell vitality (mean ± SD) between all the study groups after 72 hour.

72 Hour					
Groups	N	Mean (SD)	Statistics	df	*P-Value
1.26 PVDF	3	8.1 (0.4)	23290.7	4	**< 0.001**
2.26 PVDF	3	8.0 (0.7)			
3.26 PVDF	3	9.8 (0.4)			
F.Varnish	3	7.7 (0.4)			
Cont. Neg.	3	100 (0)			

Table 6: Pairwise post-hoc comparisons of cell vitality values between groups at 24 hour's time point.

Post hoc Pairwise Comparisons (Bonferroni) – 24 Hour				
(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-Value
1.26 PVDF	2.26 PVDF	-9.7	1.5	**< 0.001**
	3.26 PVDF	-11.3	1.5	**< 0.001**
	F.Varnish	7.3	1.5	**0.008**
	Cont. Neg.	-76.3	1.5	**< 0.001**
2.26 PVDF	3.26 PVDF	-1.6	1.5	1.0
	F.Varnish	17.0	1.5	**< 0.001**
	Cont. Neg.	-66.5	1.5	**< 0.001**
3.26 PVDF	F.Varnish	18.6	1.5	**< 0.001**
	Cont. Neg.	-64.9	1.5	**< 0.001**
F.Varnish	Cont. Neg.	-83.6	1.5	**< 0.001**

Table 7: Pairwise post-hoc comparisons of cell vitality values between groups at 48-hour's time point.

Post hoc Pairwise Comparisons (Bonferroni) – 48 Hour				
(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-Value
1.26 PVDF	2.26 PVDF	-3.3	1.8	0.9
	3.26 PVDF	-5.7	1.8	0.09
	F.Varnish	-1.4	1.8	**1.0**
	Cont. Neg.	-87.8	1.8	**< 0.001**
2.26 PVDF	3.26 PVDF	-2.3	1.8	1.0
	F.Varnish	1.9	1.8	1.0
	Cont. Neg.	84.4	1.8	**< 0.001**
3.26 PVDF	F.Varnish	4.3	1.8	0.3
	Cont. Neg.	-82.0	1.8	**< 0.001**
F.Varnish	Cont. Neg.	-86.4	1.8	**< 0.001**

Table 8: Pairwise post-hoc comparisons of cell vitality values between groups at 72 hour's time point.

Post hoc Pairwise Comparisons (Bonferroni) – 72 Hour				
(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-Value
1.26 PVDF	2.26 PVDF	0.01	0.3	1.0
	3.26 PVDF	-1.7	0.3	**0.009**
	F.Varnish	0.3	0.3	1.0
	Cont. Neg.	-91.8	0.3	**< 0.001**
2.26 PVDF	3.26 PVDF	-1.7	0.3	**0.009**
	F.Varnish	0.3	0.3	1.0
	Cont. Neg.	-91.9	0.3	**< 0.001**
3.26 PVDF	F.Varnish	2.0	0.3	**0.003**
	Cont. Neg.	-90.1	0.3	**< 0.001**
F.Varnish	Cont. Neg.	-92.2	0.3	**< 0.001**

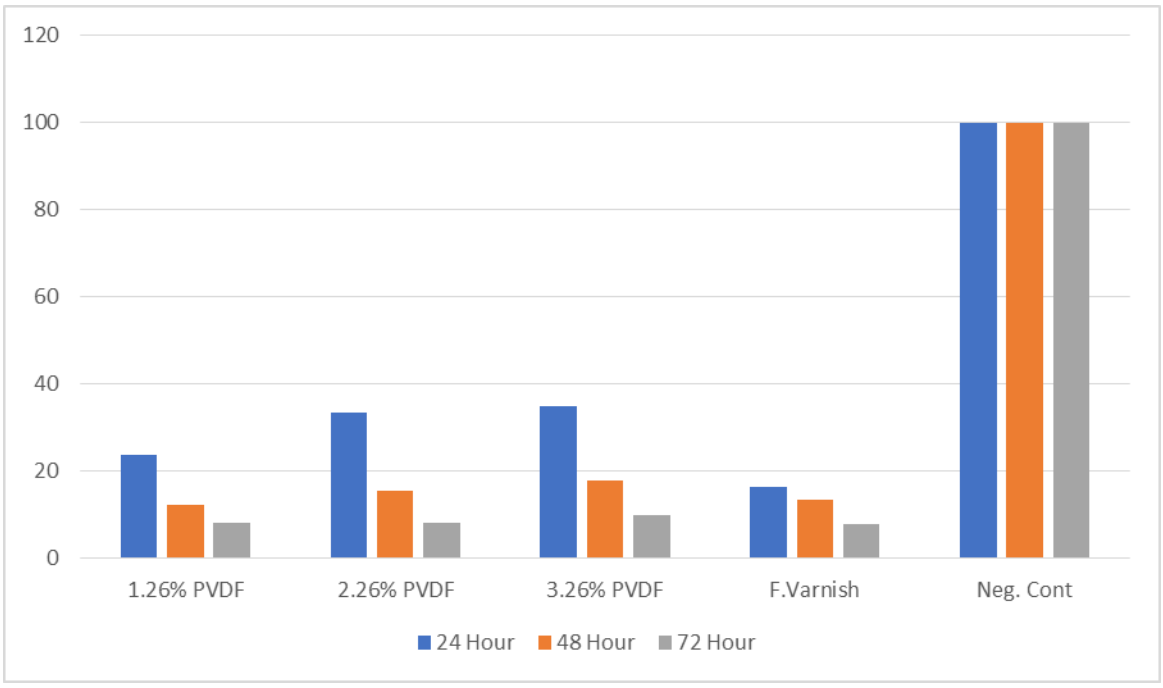

Figure 1: The cell vitality values of all study groups at different time points

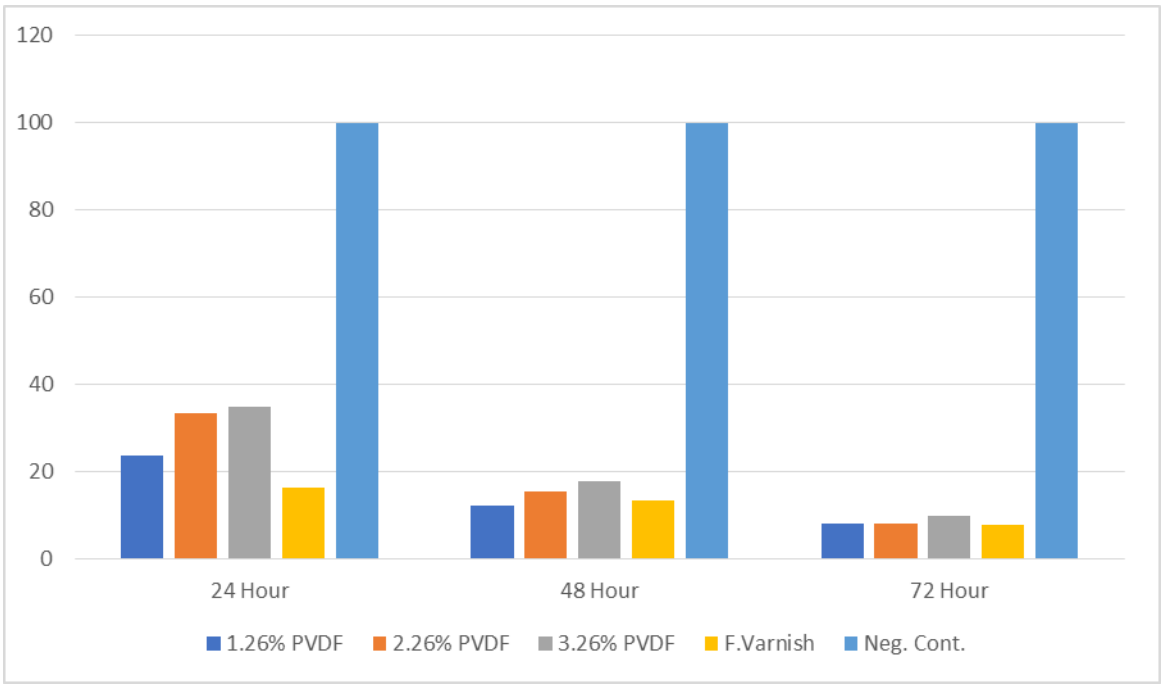

Figure 2: the cell vitality means of the study groups at different time points