ORIGINAL ARTICLE

Does Adding Fluorescein to Toothpastes Increase their Cytotoxic Effect on the Oral Cells?

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Abstract

Background: The role of fluorescein in detecting dental plaque has been introduced recently. However, the effect of adding fluorescein to toothpastes and its cytotoxic effects on gingival cells are still unknown. The aim of the present study was to evaluate the cytotoxic effects of fluorescein on human gingival fibroblasts using MTT assay.
Methods and Materials: In this in-vitro study, 0.1% fluorescein was added to three different toothpastes including Sensodyne ProNamel, Signal Complete 8, and Darougar 1. Next, different concentrations of each toothpaste (3%, 25% and 50%) were prepared. The same concentrations of each toothpaste without fluorescein were served as controls. Human gingival fibroblast cells in contact with various concentrations of toothpastes were incubated for 2, 5 and 10 minutes. The mean cell viability was evaluated by MTT assay.
Results: The mean cell viability of toothpastes with and without fluorescein was reported to be 40.88% ± 31.62% and 47.18% ± 31.82%, respectively. No significant difference was found between the cell viability of these groups (P=0.73). There was a significant difference in cell viability between the three different concentrations of each toothpaste (P<0.0001). A significant difference in cell viability was also found between the three different types of toothpastes (P<0.0001). Pronamel and Darougar1 toothpastes had the highest and lowest cell viability, respectively. As the exposure time increased, the mean cell viability decreased. However, the difference was not statistically significant.
Conclusion: It seems that the addition of fluorescein to toothpastes did not increase their cytotoxic effects on gingival fibroblasts.

Keywords: Fluorescein, Toothpaste, Cytotoxicity

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INTRODUCTION

Different methods have been proposed for complete plaque removal around orthodontic appliances or fixed prosthetics [1-5]. One of the recent developments in this field has been the introduction of fluorescein to dentistry [6,7]. Fluorescein is an artificial organic material, which is usually used as a fluorescent marker. The fluorescein absorbs the light in the UV spectrum and emits a visible yellow-green light in the 400-500nm wavelength. Its fluorescence depends on the environmental PH and its optimal fluorescence happens at 5.5 PH [8]. In medical applications, fluorescein is used in ophthalmology as an angiography material for detecting the obstruction of retinal arteries, foreign objects, and the assessment of corneal damage [9,10]. Because of the low PH of carious dental plaques, the fluorescence of this marker can be easily detected. The main advantage of fluorescein as a disclosing agent is that it only marks the dental plaque and does not color the tongue, gingiva or dental fillings. Moreover, it has an acceptable taste and shows a better contrast between teeth and gingiva for digital assessments. But it is not noticeable at daylight [11,12]. Fluorescein has been shown to help patients with oral healthcare problems reduce dental plaque and lower the incidence of caries and periodontal diseases [13-15]. The fluorescein studied in these studies has been in the form of mouthwash. To the best of our knowledge, no study, to date, has investigated the effect of adding fluorescein to toothpastes as a disclosing agent.

Most people brush for an average of 2 minutes to eliminate most of the dental plaque [16]. Therefore, oral mucosal cells are usually exposed to toothpaste and its

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ingredients for several minutes. Some of the toothpastes have been shown to have cytotoxic effects on cultured epithelial cells [17]. Also, individual ingredients of toothbrushes, such as SLS (sodium lauryl sulfate) have been shown to have cytotoxic effects on the oral cavity. Their application has been limited in toothpastes especially for children and patients with aphthous lesions [18].

The effect of adding fluorescein to toothpastes and their cytotoxic effects on the gingival cells are still unknown. The aim of the present study was to evaluate the cytotoxic effects of fluorescein at different concentrations and periods of time on human gingival fibroblast cells using MTT assay method.

METHODS

In this *in-vitro* study, fluorescein was added to different concentrations of some routinely-used toothpastes to evaluate the cytotoxic effects of the mixture.

Toothpaste Preparation

0.1% fluorescein was added to three different toothpastes including: Sensodyne ProNamel (GlaxoSmithKline, Rio de Janeiro, Brazil), Signal Complete 8 (Signal, India), and Darougar 1 (Darougar, Tehran, Iran). Next, the toothpastes were diluted with DMEM (Dulbecco's Modified Eagles Medium) solution and three different concentrations of each toothpaste (3%, 25% and 50%) were prepared. Also, the same concentrations (3%, 25% and 50%) of each toothpaste without fluorescein were served as controls.

MTT Assay

Human gingival fibroblast cells (HGFs) were isolated from healthy patients referred to Mashhad Dental School (Iran) for the extraction of third molars. The cells were incubated at 37°C in 5% CO_2 and cultured in DMEM which contained 10% fetal bovine serum (FBS), 1 % penicillin/streptomycin (100 IU/ml penicillin and 100 µg/ml Streptomycin) with the 7.4 PH. The medium solution was sterilized using a 0.2 filter under the air hood. The treated cells with various concentrations of toothpastes (3%, 25% and 50%) were incubated for 2, 5 and 10 minutes. The cell viability was evaluated by MTT assay which is based on cell metabolic activity.

For long-term storage, cells were frozen so that they could be utilized when necessary. After removing the culture medium from the top of flasks, the flasks were washed three times using PBS buffering material, then the flasks were trypsinizated so that the cells could be detached from the bottom of flasks. Then DMEM medium was added and the solution was centrifuged with 1000 rpm in the falcon for 5 minutes. Again, the top medium was removed and 10% DMSO and 90% FCS solution was added and the cells were transferred to freeze-dried sterile vials. The vials were placed in -20° for one hour and then were moved to a -80°C freezer for 24 hours. The cells could be preserved for several days in -80° conditions but for longer periods they should be held in a nitrogen tank.

For defrosting, the vials were placed in a warm environment and their temperature were increased to 37°C and then 37° DMEM medium was added. They were pipetted several times so that the medium and cells were combined completely.

The cells were seeded in 96 well plates (5000 cell/well) and were exposed to different concentrations of toothpaste solution for 2, 5 and 10 minutes. Cell viability was assessed using MTT assay. The assessments were triplicated for every well. This assay has been performed using 10 microliter MTT (3-(4, 5- dimethylthiazol – 2)-2, 5 diphenyl tetrazolium) solution (5 mg/ml) which was added to each well in 96 well plates and incubated for three hours. After removing the solution, 100 microliters of DMSO solution were added to the remaining depositions (cells and MTT crystals). Plates were placed in Elisa reader (Stat FAX 303, USA) and light absorption of cells were measured at 570nm and 620nm wavelengths and their cell viability was measured.

One-way ANOVA and post-hoc Tukey were used for statistical analysis of cell viability. Also, regression analyses were performed to evaluate the effects of different toothpastes, concentrations, and exposure times. The statistical procedures were conducted using SPSS (Statistical package for social sciences, version 16.0; SPSS Inc., Chicago, Il) and the P-value < 0.05 was considered statistically significant.

RESULTS

The effect of adding fluorescein-added toothpastes on cell viability was investigated in this study. There were 27 groups including three toothpastes (Sensodyne Pronamel, Signal Complete 8, and Darougar 1), at three different concentrations (3, 25 and 50 percent) and three various exposure times (2, 5 and 10 minutes). Also, each group was subdivided to with and without fluorescein.

The mean cell viability of toothpastes with and without fluorescein was reported to be 40.88% ± 31.62% and 47.18% ± 31.82%, respectively. No significant difference was found between the cell viability of these groups (P=0.73).

Table 1 demonstrates that there was a significant difference in cell viability between the three different

Table 1. Mean gingival cell viability at three different concentrations of toothpastes

Concentration	Mean Cell viability (%)	SD	Maximum	Minimum	ANOVA Test
3%	65.99	24.51	102.4	18.9	P<0.0001
25%	36.18	32.18	108	6.1	
50%	29.92	25.98	106.1	5.1	
Total	44.03	31.78	108	5.1	

concentrations of each toothpaste (P<0.0001). Tukey test showed that cell viability of 3% concentration was significantly higher than both 25% and 50% (p<0.0001). As the concentration of the toothpaste increased, the cell viability decreased.

On the other hand, a significant difference in cell viability was also found between the three different types of toothpastes (P<0.0001). As is apparent in table 2, Sensodyne Pronamel and Darougar 1 toothpastes had the highest and lowest cell viability, respectively. Tukey test revealed that the difference was statistically significant between the Signal Complete 8 and Darougar 1 toothpaste (P=0.35).

Table 3 shows that as the exposure time increased, the mean cell viability decreased. However, the difference was not statistically significant (P=0.057).

Table 4 tabulates the results of regression analysis between different toothpastes, concentrations, exposure times, and the existence of fluorescein. There was a statistically significant difference between Sensodyne Pronamel and Darougar 1(P<0.0001). Also, Pronamel and Signal toothpaste had significant differences. Furthermore, the evaluation of different concentrations showed significant differences in cell vitality between 3% and 25%, and also 3% and 50% concentrations (P<0.0001). Similarly, different exposure times (2 min vs 10 min and 2 min vs 5 min) showed significant differences in their effect on cell vitality (P<0.0001). Finally, toothpastes with fluorescein showed 6% higher cell vitality compared to toothpastes without fluorescein and this difference was statistically significant (P<0.0001).

The summary of the mean cell viability of the different groups are depicted in the Figure 1.

DISCUSSION

Fixed orthodontic appliances and dental prosthesis have long been known to cause accumulation of bacterial plaque

Table 2. Mean cell viability of the three different types of toothpastes.

Toothpaste	Mean Cell viability (%)	SD	Maximum	Minimum	ANOVA test
Pronamel	72.85	18.62	108	28.5	P<0.0001
Complete8	32.83	31.14	99.4	5.1	
Darougar	26.41	21.49	37.3	11	
Total	44.03	31.78	108	5.1	

Table 3. Mean cell viability at three different exposure times.

Exposure time (Minutes)	Mean Cell viability (%)	SD	Minimum	Maximum	ANOVA test
2	52.01	36.23	9	108	P<0.0001
5	42.4	29.43	8.1	96.2	
10	37.68	27.94	5.1	87	
Total	44.03	31.78	5.1	108	

Table 4. Regression analysis for percentage of vital cells based on toothpaste, concentration, exposure time and fluorescein

Variable	Type	B regression coefficient	Standard Error	t	P-value
Toothpaste	Sensodyne Pronamel	46.437	3.355	13.840	<0.0001
	Signal Complete 8	6.426	3.355	1.915	0.057
	Darougar 1	0[a]	-	-	-
Concentration	3%	36.062	3.355	10.748	<0.0001
	25%	6.251	3.355	1.836	0.064
	50%	0[a]	-	-	-
Exposure time	2 min	14.329	3.355	4.271	<0.0001
	5 min	4.723	3.355	1.408	0.161
	10 min	0[a]	-	-	-
Fluorescein	with	-6.304	2.739	-2.301	0.023
	without	0[a]	-	-	-

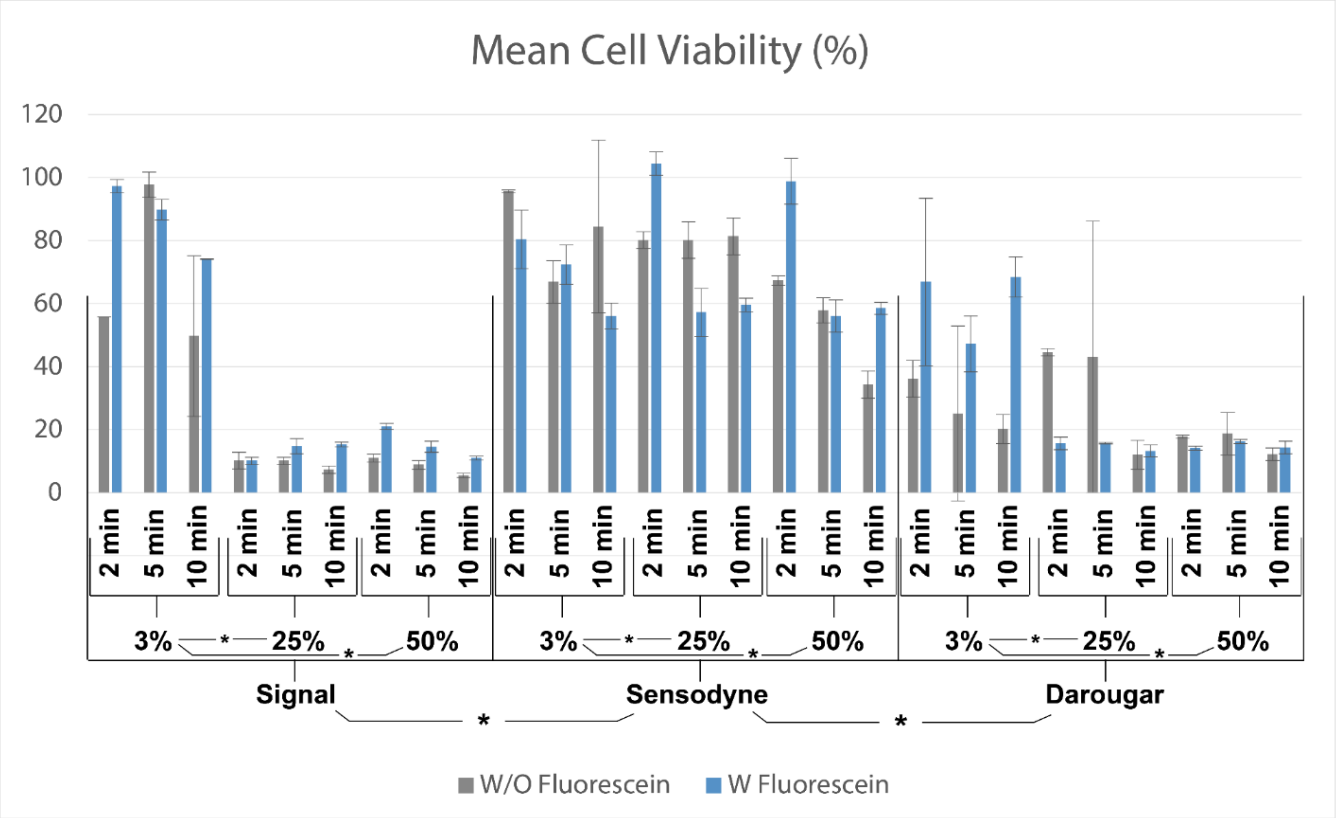

Figure 1. Mean difference of the cell viability in the evaluated groups

** The difference between groups are statistically significant*

and thus increase the development of dental caries and periodontal diseases [19-22]. Disclosing agents can help patients better identify the plaque residue and consequently better eliminate remnant plaque [23,24]. Fluorescin has recently been used as a disclosing agent in the form of mouthwash [14,21]. However, adding disclosing agents to toothpastes is more convenient and can demonstrate the residues of dental plaque simultaneously with brushing [25]. To the best of our knowledge, there are no studies that investigate the effect of adding fluorescein to the toothpaste, and our study is the first. However, before the application of fluoresceine-added toothpastes in the market, it is essential that their cytotoxic effects on gingival cells be investigated.

Fluorescein has been shown to have several advantages over the other disclosing agents. It only marks the dental plaque and does not color tongue, gingiva or dental fillings, has an acceptable taste, shows a better contrast between teeth and gingiva for digital assessments, and is not noticeable at daylight [11, 13, 14].

In present study, we investigated the cytotoxic effects of adding fluorescein to three different toothpastes at different concentrations and exposure times. We found that there was not a considerable difference in cell viability when they were exposed to fluorescein-added toothpastes compared to the toothpaste alone. This result shows that the 0.1% concentration of fluorescein can be added to toothpastes without considerable increased cytotoxicity. This result is in line with Yankell et al. [26] who found that until 3000 mg/kg systemic dose of fluorescein, no acute toxic effects could be detected in rats. The amount of fluorescein used in a toothpaste tube would be around 75 mg and only a trace amounts of this material would be used during brushing which is a lot lower than the Yankell et al's safe dosage. Similarly, fluorescein has been proved to be a safe marker in different cellular and clinical assessments like angiography, ophthalmological tests, the assessment of skin, and the detection of cancer cells [27-30].

Different brands of toothpaste have been shown to have different cytotoxic effects. Hence, they could influence the results of our study. In the reviews of Ghapanchi et al. [31] and Souza et al. [32] different brands of toothpastes have been shown to have different cytotoxic effects. Cells exposed to Sensodyne toothpaste had the highest cell viability compared to other toothpaste brands. These results are similar to the findings of our study in which Sensodyne had the highest cell viability. Furthermore, Cvikl et al. [17] found that SLS (sodium lauryl sulfate) and fluoride amine limits the cell growth and lowers the cell viability. In our study, both Darougar and Complete8 toothpastes had SLS which could explain the lower amount of cell viability that was found after exposure to these toothpastes. Nevertheless, no significant difference in cell viability was found between fluorescein containing toothpastes and controls. Therefore, while the specific brand of toothpaste effects the viability of cells, the

inclusion of fluorescein does not influence the cytotoxicity of these toothpastes.

In present study, increased concentrations of the toothpastes had also adverse effects on the cell viability of gingival fibroblast cells. Considering this toxic effect, Vennet et al. [18] suggested that toothpastes should not remain for a long time inside the oral cavity and should be thoroughly washed after brushing.

The cell viability decreased with longer exposure time to toothpastes in our study. However, the effect of exposure time was not statistically significant. Nevertheless, because of the effects of other confounding variables, the effects of time might be unclear. Since most of the dental plaque is usually eliminated after 2 minutes of brushing, the results of 2 minutes exposure to toothpastes seem to be most relevant to the clinical settings. The cell viability at 2 minutes of exposure for most groups was over 50% in our study.

The results of the present *in-vitro* study should be interpreted cautiously. Further clinical studies are strictly recommended to confirm the results of this study.

CONCLUSION

Based on the results of this study, it can be argued that the addition of fluorescein to toothpastes does not increase the cytotoxic effect of toothpastes on gingival fibroblasts. Therefore, fluorescein could be added to toothpastes to increase the ability of patients to remove the dental plaque.

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