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EVALUATION OF SUSTAINED RELEASE FROM FLUORIDE-LOADED CARBON NANOTUBES

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ABSTRACT: The developments in nanotechnology have led to significant advances in many areas of science and technology including medical sciences and dentistry. Carbon nanotubes (CNTs) have some unique properties such as their tube-shaped structure which allows for drug delivery and makes them very suitable agents for use with biomaterials in various fields of medicine. The aim of this research was to evaluate the fluoride delivery potential of fluoride-loaded carbon nanotubes in two different artificial saliva solutions of pH 7.4 and pH 5.5. After buckypaper samples were prepared from CNTs, the dispersant agent Triton X, and distilled water, the samples were loaded with NaF. According to our results, the release of fluoride from the fluoride-loaded carbon nanotubes was achieved although the release occurred faster than was expected. The fluoride release was significantly higher in the artificial saliva with pH 5.5 which is a critical pH for enamel than with pH 7.4. We concluded that further research should be performed to regulate the fluoride release and to increase the potential for a slower fluoride release from the fluoride-loaded nanotubules through developing an amplified interaction between fluoride and the carbon nanotubes.

Keywords: Carbon nanotubes; Drug delivery; Fluoride-loaded carbon nanotubes; Sustained release.

1. INTRODUCTION

1.1. Fluoride and dentistry: Dental caries is an infectious and multifactorial disease in which lesions are formed by the acidogenic activities of microorganisms in dental plaque, resulting in the loss of dental hard tissues. Traditionally, dental caries are treated with restorative methods.[1,2,3]

Fluoride's role in the reduction of dental caries incidence was discovered in the 1940s when the dental caries incidence found to be high in the areas that were using drinking water with a low level of fluoride; and low in the areas that were using drinking water with a high level of fluoride.[4] However further research showed that an excessive systemic fluoride intake could adversely effect the reproductive system in mammals and cause sportiness abortions, be a risk factor for hypertension, and negatively effect thyroid stimulating hormone (TSH) levels.[5-9]

Still, the topical remineralizing effect of fluoride on the teeth enamel might be beneficial. In fully developed enamel, the principal mineral is hydroxyapatite. Fluoride combines with this mineral forming the stronger structure of fluorapatite; which demineralizes less in an acidic oral environment.[4,10]

1.2. Nanotechnology and dentistry: Nanotechnology involves the control and manipulation of particles that are smaller than 100 nanometers. Engineering of

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nanosized particles allows the researchers to work in tiny areas.[12] Carbon is an element that has the ability to form a variety of different structures. At the atomic scale, carbon nanotubes (CNTs) are constituted by hexagonal graphite leaves that are wrapped in a single or multiwall manner. Due to their cylindrical symmetries, carbon bonds and single dimensional structure, they have unique thermal, electrical, and mechanical properties. Their electrical capacities are 1,000 times better than copper. They can remain stable until 2,800ºC degrees under a vacuum. Their tensile strength can vary between 50–150 GPa. As far as we know, their elasticity modulus and strength are above those of the strongest steel. All these properties have increased the attention given to biomaterials that are strengthened with carbon nanotubes.[18]

Nanotechnology research in dentistry started in the early 1990's with the usage of nanoparticles. In dentistry, nanotechnology could be used in (i) dental restorative materials; (ii) bone defect replacement therapies; (iii) protein, gene and drug delivery; and (iv) cancer therapies.[14]

The aim of the present research was to combine nanotechnology with dentistry by evaluating the slow fluoride release potential of fluoride-loaded CNTs.

2. MATERIALS AND METHODS

In this research, after loading the CNTs with sodium fluoride (NaF), the slow fluoride release from the nanotubes was studied.

2.1. Preparation of the CNTs: To provide the hydrophilic NaF entrance into the cylindrical shaped and hydrophobic CNTs, 9 differently formulated suspensions with CNTs, Triton-X, and distilled water were prepared in line with the research of Randhawa et al. (Table 1).[15] Briefly, the exact amount of CNT was weighed before a dispersant agent Triton-X was added onto the CNTs, and the dispersion was then diluted with distilled water.

Table 1. Solutions prepared with carbon nanotubes (CNT) and Triton X in different concentrations

Component	Formulated suspension								
	F0	F1	F2	F3	F4	F5	F6	F7	F8
Triton X (mL)	2 mL	4 mL	6 mL	8 mL	2 mL	2 mL	2 mL	2 mL	2 mL
CNT (mg)	40 mg	20 mg	40 mg	40 mg	50 mg	60 mg	30 mg	20 mg	10 mg
Distilled water (mL)	20 mL	20 mL	10 mL	10 mL	20 mL	20 mL	20 mL	20 mL	20 ml
CNT %	200%	200%	200%	200%	250%	300%	150%	100%	50%

All the formulations were sonicated (10×5 pulse and 40 amplitude) for 10 minutes with the ultrasonic mixing device VCX 130; (Sonics®, Switzerland). According to the results, the most homogenous suspension was the one with the formulation of 2 mL Triton X, 40 mg CNT, and 20 mL distilled water (F0 formulation in Table 1) in

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line with the research of Randhawa et al.[15] In order to provide the standardization, this formulation was used for all the experiments.

The dispersion was filtered with a 0.45 μm membrane filter using a filtering kit (Merck Millipore, Germany). Membrane filters were dried in a drying oven. After that, in order to separate the CNTs from the filters easily, acetone was added to the filters and they were dried again in the drying oven in line with the research from Gou.[16]

Another suspension with 4 mg/mL NaF concentration was prepared using ethyl alcohol. The fluoride solution in ethyl alcohol was dropped on membrane filters gradually, with an injector so that the exact amount of the NaF suspension used could be measured. This process was repeated, until there were visible white dots on the membrane filters, indicating that the CNTs were full with NaF so that the additional fluoride particles stayed outside the CNTs and on the top of the membrane filters. The macro whiteness visibility appeared after 4.0 mL of NaF solution in ethyl alcohol; therefore, this amount was used for the samples to achieve standardization. Following the NaF loading process, the membrane filters were cut into 1×1 cm pieces. The CNTs were peeled from the filters resulting in the formation of 1×1 cm fluoride-loaded buckypapers. (A buckypaper is a macroscopic aggregate of carbon nanotubes which owes its name to the buckmininsterfullerene, the 60 carbon fullerene, an allotrope of carbon with a similar bonding that is sometimes referred to as a "buckyball" in honour of R Buckminster Fuller). Eight samples were constituted to allow for testing in triplicate for each of the two pH values (3 parallel samples or replicates and one control for each pH value).

2.2. Preparation of artificial saliva: The slow fluoride release potential of the CNTs was evaluated using artificial saliva. Artificial saliva was prepared with 40 g of sodium chloride, 0.95 g of monobasic potassium phosphate, 11.9 g of disodium hydrogen phosphate, and 5 L of distilled water in line with the research of Marques et al.[17]

In order to evaluate whether the pH of the saliva affected the slow release potential, the experiments were performed in saliva with a neutral pH 7.4 and pH 5.5, which is known as the critical pH for enamel. After preparing the artificial saliva, hydrochloric acid was used to achieve the desired pH concentration. The pH measurements were conducted with a pH meter (Isolab Labergerate GmbH, Germany®).

2.3. Release experiments: Release experiments were performed with the United States Pharmacopoeia (USP) Apparatus 2 (paddle), in compliance with the standard dissolution testing methodologies (VanKel-Varian-Agilent VK 7000®, Agilent, CA, USA) at 37ºC, for the 2 groups of artificial salivae in the two different pHs of 7.4 and 5.5. Each group had 3 parallels besides the control parallel which was pure artificial salivae, in other words, the fluoride-free medium. Two mL of sample were collected in the 1st, 3rd, 5th, 10th, 15th, 30th, 45th, 60th, 90th, 120th, 150th, 180th, 240th, and 360th minutes for both groups. After collecting each samples, 2 mL of artificial saliva has added to the device to hold the volume of saliva steady.

2.4. Fluoride electrode measurements: The amount of fluoride in the samples was measured using an ion selective electrode (Consort C863®, Belgium). For the measurements, the electrode was calibrated on every experimental day. To provide

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the essential information relating the reading in mV with the fluoride concentration, total ionic strength adjustment buffer (TISAB) solutions with 100 mg/L, 10 mg/L, 1 mg/L, 0.1 mg/L, and 0.05 mg/L concentrations were measured with the device and graphed. Each sample had 3 minutes of measuring time. The electrode has cleaned and dried before each measurement. The fluoride concentration has calculated as in equation 1:

$$y = -53.486x + 72.177 \quad \text{.....Equation 1}$$

2.5. Statistical analysis: The statistical analysis of the data obtained in the research was conducted using the INSTAT statistical analysis programme. The data was evaluated with ANOVA, comparisons between the groups with post-hoc and Tukey Kramer. When the p-value was below 0.05, the data were recorded as being statistically significant.

3. RESULTS

The amounts of fluoride released from the fluoride-loaded buckypapers in the artificial saliva group 1 with pH 5.5 for each time period were first observed as readings in mV. Group 1 included 3 parallels and the control sample. The results gained from the fluoride electrode in mV were used via the equation to calculate the amount of fluoride released in mg/mL (Table 2).The average amount of fluoride released for each parallel for each time period for group 1 are shown in Table 3. Significantly higher levels of fluoride were released from the fluoride-loaded buckypapers compared to the control ($p<0.05$) (Figure 1).

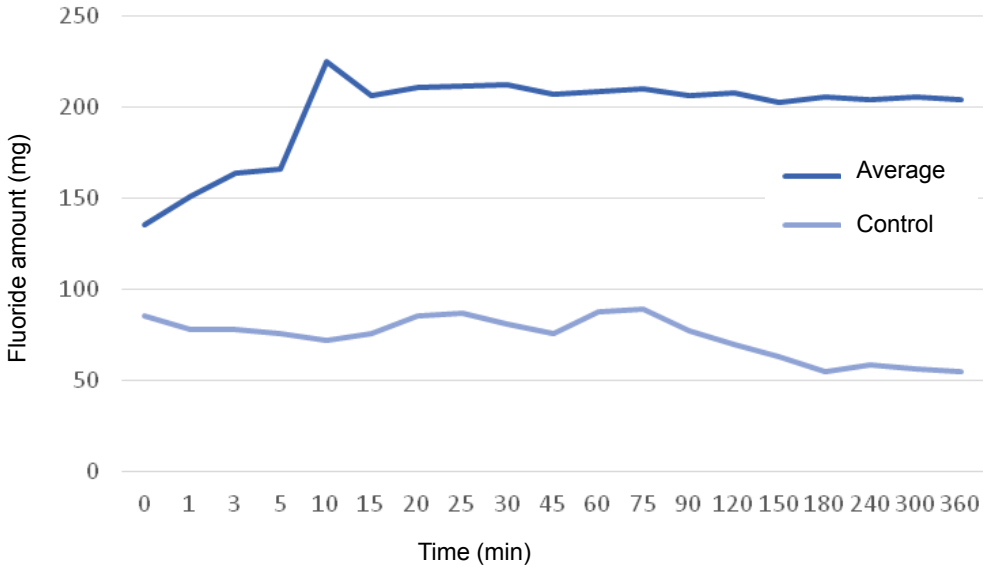

Figure 1. Fluoride release graph for group 1 with pH 5.5.

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Table 2. Calculated fluoride amount in the environment for group 1

Time (min)	Fluoride amount (mg)			
	Control	1st parallel	2.nd parallel	3rd parallel
0	85.75006	56.74923	183.1589	166.1572
1	77.95849	115.4832	186.6227	111.6937
3	78.26853	138.616	187.3232	104.409
5	75.82228	146.5367	184.5366	99.81841
10	72.29551	272.4699	177.0854	99.07318
15	75.52193	235.4263	176.4231	94.71721
20	85.41038	239.8785	181,7915	94.36298
25	86.77724	244.4149	179.0872	92.26522
30	80.79371	246.2534	177.7501	90.89269
45	76.12383	235.4263	179.0872	88.53971
60	87.81672	236.3101	181.1116	87.2226
75	89.57693	238.0876	181.7915	83.7007
90	77.64967	243.5008	169.2994	84.33029
120	69.75851	238.0876	177.7501	84.64686
150	63.16877	228.474	177.0854	84.64686
180	54.75778	233.6687	176.4231	84.96462
240	58.34807	229.3317	178.4174	85.28358
300	56.74923	234.5458	176.4231	84.33029
360	54.75778	226.7683	181.1116	83.7007

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Table 3. Calculated fluoride amount in the environment of the control for group 1 and the average and standard deviation values of the calculated fluoride amount of the parallels of group 1

Time (min)	Fluoride amount (mg)		
	Control (mg)	Average (mg)	Standard deviation of average (mg)
0	85.75006	135.3551	68.60340723
1	77.95849	151.3195	42.2955035
3	78.26853	163.3053	41.75510036
5	75.82228	165.8988	42.52522864
10	72.29551	225.1455	86.89057056
15	75.52193	206.4242	70.8499431
20	85.41038	211.2926	73.40611716
25	86.77724	212.2196	76.4901424
30	80.79371	212.4723	78.03043907
45	76.12383	207.7279	74.27687958
60	87.81672	209.1714	75.54049487
75	89.57693	210.4034	78.29813695
90	77.64967	206.8666	79.81840041
120	69.75851	208.3775	77.47762063
150	63.16877	203.2417	73.05502726
180	54.75778	205.4965	75.1669918
240	58.34807	204.3302	73.21455394
300	56.74923	205.9375	75.90745409
360	54.75778	204.3966	73.24514215

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The amounts of fluoride released from the fluoride-loaded buckypapers in the artificial saliva group 2 with pH 7.4 for each time period were first observed as readings in mV. Group 2 included 3 parallels and the control sample. The results gained from fluoride electrode in mV were used via the equation to calculate the amount of fluoride released in mg/mL (Table 4). The average amount of fluoride released for each parallel for each time period for group 2 are shown in Table 5. Significantly higher levels of fluoride were released from the fluoride-loaded buckypapers compared to the control ($p<0.05$) (Figure 2).

When the results for both groups were compared, it was found that the difference between the average of the group 1 parallels and the group 1 control sample, the difference between the average of the group 2 parallels and the group 2 control sample, and the difference between the average of the group 1 parallels and the group 2 control sample were all statistically significant at $p<0.001$. Even though there was a statistical difference between group 1 and group 2, the difference was moderate compared to the other results ($p<0.05$) (Table 6).

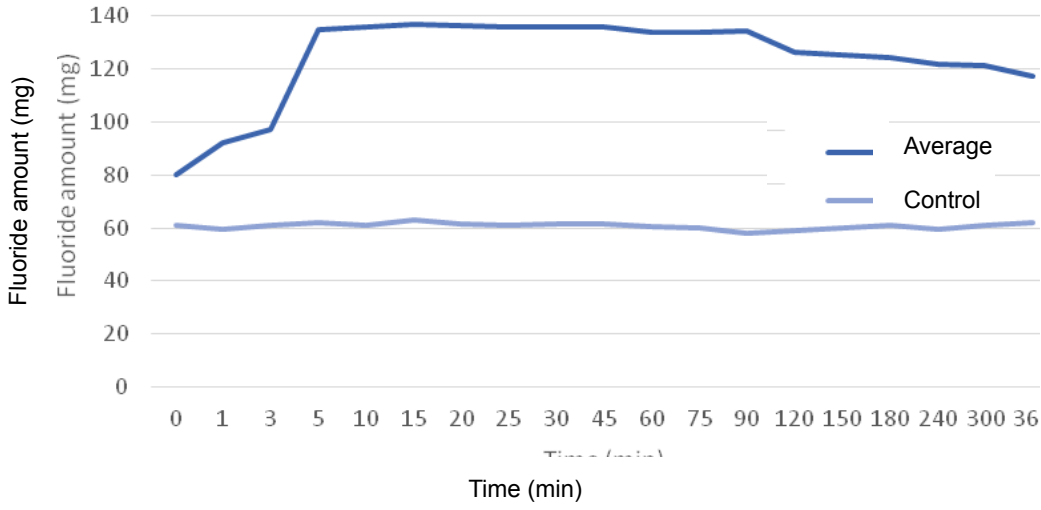

Figure 2. Fluoride release graph for group 2 with pH 7.4.

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Table 4. Calculated fluoride amount in the environment for group 2

Time (min)	Fluoride amount (mg)			
	Control	1st parallel	2nd parallel	3rd parallel
0	60.85188	81.51415	122.8442	78.71794
1	59.53871	82.58815	117.5995	75.68669
3	61.11796	87.02753	119.67	84.77879
5	62.19401	146.1538	122.8442	45.0324
10	60.85188	146.1538	125.5536	65.25181
15	63.01345	148.5953	124.4627	69.66525
30	61.38522	147.9811	123.3814	73.73099
45	60.85188	150.4532	121.2467	78.71794
60	61.65364	144.9482	121.7769	73.09038
90	61.38522	149.212	119.149	72.13989
120	60.58695	133.4621	118.6302	75.68669
180	59.79906	133.4621	115.0618	75.35718
240	58.00026	127.2079	115.5649	75.68669
300	59.02141	126.654	115.5649	76.01765
360	60.06054	119.149	115.0618	75.68669

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Table 5. Calculated fluoride amount in the environment of the control for group 2 and the average and standard deviation values of the calculated fluoride amount of the parallels of group 2

Time (min)	Fluoride amount (mg)		
	Control (mg)	Average (mg)	Standard deviation of average (mg)
0	60.85188	80.11605	24.7087
1	59.53871	92 .16781	22.52726
3	61.11796	97.36312	19.57738
5	62.19401	134.7287	52.96738
10	60.85188	136.1526	42.16089
15	63.01345	136.8309	40.53893
30	61.38522	135.9846	37.91266
45	60.85188	136.1514	36.15694
60	61.65364	133.6644	36.75591
90	61.38522	134.4769	38.92659
120	60.58695	126.3443	30.0913
180	59.79906	124.542	29.7628
240	58.00026	121.6625	27.08499
300	59.02141	121.3792	26.67769
360	60.06054	117.3745	24.05894

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Table 6. Statistical comparison of the results from both groups

Compared samples	Average difference	q value	p value
Group 1 and group 1 control	149.51	9.372	p<0.001
Group 1 and group 2	87.022	5.455	p<0.05
Group 1 and group 2 control	144.21	9.040	p<0.001
Group 1 control and group 2	–62.491	3.917	p>0.05 ns
Group 2 and group 2 control	57. 183	12.816	p<0.001

4. DISCUSSION

Dental caries is one of the most common chronic diseases that affects people worldwide. Every person is at risk for dental caries from birth. The primary cause of pain and tooth loss in the oral cavity is dental caries.[18] It has been stated that dental caries could be controlled with a reduction of sugar consumption and an increase of fluoride usage.[19] On the other hand some research indicates that a high systemic fluoride intake can be toxic.[5-9]

The appearance of the nanotechnology concept in 1959 has led to attention being given to the potential application of this field in medicine and dentistry.[20] The nanotechnology concept includes the use of devices and systems which are at the molecular level. Recently research has been conducted on nano-sized particles and their application for drug delivery, gene transfers, diagnostic purposes, and tissue engineering. Carbon nanotubes are one type of these nanosized particles and they have specific characteristics with various application potentials.[13,21]

CNTs, due to their unique physicochemical properties, have become a popular tool in cancer diagnosis and therapy. They are considered as one of the most promising nanomaterials with the capability of both detecting cancerous cells and delivering drugs or small therapeutic molecules to these cells. Various researches have been conducted to show the drug delivery potential of CNTs. Chen et al. reported an anti-cancer effect had been observed with the effect being exactly as it was designed and it having a high potency towards specific cancer cell lines.[22] Ji et al. reported that, after the injection of an anti-cancer agent to mice abdominally or subcutaneously, the nanotubes reached to targeted tissue through the lymphatic system, and the release of the agent from the nanotubes in the desired area was observed.[23] Heister et al. evaluated the effect of anti-cancer drug delivery with CNTs on colon cancer and stated that the anti-cancer effect could be achieved by a slow drug release from CNTs.[24]

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Nanotechnology in drug delivery has received a significant amount of attention with the goal of developing drugs with long-term higher efficacy. Due to their sub-micrometer size and high surface area to volume ratio, a variety of nanomaterials such as liposomes, dendrimers, nanoparticles, etc. show key differences in comparison to bulk materials.[25,26] Among the nanomaterials, CNTs are of special interest in the area of drug delivery by virtue of their high surface area and the tendency to surface modification either by adsorption or by covalent attachment. The cores of CNTs can be filled with drug molecules or drugs can be adsorbed onto CNT surfaces.[27,28] The amount of drug delivered and the release mechanism of the drug depend on the attraction of the drug molecule to the walls of the CNTs and where the drug loaded.[29]

The present research was conducted to examine the hypothesis that the slow release of fluoride might be possible from fluoride-loaded carbon nanotubes.

For biomedical applications of CNTs such as drug delivery, solubility is a critical essential for providing adequate absorption and biocompatibility as well as for the reduction of toxicity. It has been stated that in the preparation of a homogenous solution with CNTs, surface active materials can be used such as sodium dodecyl sulfate, dodecyltrimethylammonium bromide, octyl phenol ethoxylate (Triton X), Tween 80, and Tween 20. Among these materials, Triton X has been found to have the highest solvent capacity. Hence, Triton X was used in this research as the solvent for the CNTs.[15,30,31]

Despite its high solvent capacity, there are serious concerns about the cytotoxicity of Triton X. Kim et al. compared the biocompatibility of various solvents for CNTs and found that Triton X had the highest cytotoxicity with the difference being statistically significant.[32]

Randhawa et al. compared the functional capacity of solutions with various concentrations that had been prepared with CNTs, Triton X, and distilled water. They studied the effect on the functionalization of CNTs of various chemical treatments and dispersion using a surfactant via ultrasonication. The authors stated that the distribution of CNTs with the surfactant, Triton X-100, via ultrasonication helped in their unbundling.[15]

In the present research, in line with Randhawa et al.'s study, 11 different formulations of CNT, Triton X, and distilled water solutions were ultrasonicated in order to achieve homogenous solutions. Among the solutions; the one with the most homogenous macro appearance was chosen and the rest of the experiment was carried out with this formulation of 2 mL of Triton X, 40 mg CNTs, and 20 mL distilled water.[15]

Following the preparation of a homogenous solution from CNTs, in order to increase the manipulative capacity of the nanotubes, raising the functionality level becomes important. By filtering these solutions, very thin layers of CNTs can be obtained that are called "buckypaper." Buckypaper production by vacuum filtration was first reported by Liu.[33] This form of CNTs could also be used in nanocomposite production.[29] Buckypapers have some very important qualities such as mechanical and chemical stability, and thermal and electrical conductivity.[35] Various researchers have used buckypapers to improve the mechanical abilities of nanocomposites.[16,36]

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Liu et al. reported that they used methanol to ease the separation of buckypapers from membrane filters.[35] In this research, acetone was used and dried in the drying oven. This allowed the buckypapers to be easily peeled from the membrane filters.

We didn't encounter any other research in the literature aiming at demonstrating a slow fluoride release from fluoride-loaded CNTs.

Utilizing the advantage of the CNTs tube-like shape, NaF solution was added on to the buckypapers. This process was carried on until a white stain appeared. Our hypothesis is that until the appearance of the white stain, NaF has been entering into the tube-shaped CNTs and that after the appearance of the white stain there is no more entry of the NaF into the tubes and instead the NaF has started to accumulate on the buckypaper layer. The saturation level of drug accumulation was reached when the drug began to accumulate on the surface of the CNTs.

To imitate oral conditions, the slow release experiments carried out in artificial saliva. Artificial saliva was prepared according to the research of Marques et. al.[17] Two different artificial salivae were made; one with a neutral pH 7.4 and the other with pH 5.5, which is a critical pH for enamel. Previous research evaluating the fluoride release potential of glass ionomer cement showed that with a decrease in the pH of the saliva, the fluoride release increased. Our results, showing a statistically significant higher fluoride release occurred in the artificial saliva with 5.5 pH, in comparison to the saliva with a neutral pH of 7.4, are consistent with the literature.[37,38]

In order to determine the fluoride solution concentration to be used, preliminary tests were conducted with fluoride solutions with concentrations of 8 mg/mL, 4 mg/mL, 2 mg/mL, and 1 mg/mL. It was hypothesized that, following the functionalization of the CNTs with Triton X, covalent bonds would be established between the fluoride and the inner walls of the CNTs. When the inner walls become saturated, fluoride will begin to accumulate on the surface of buckypapers which will be visible as white spots on the black buckypapers. According to the results from the preliminary tests, 4 mL of a solution of 4 mg fluoride/mL provided the optimal conditions and this concentration was chosen for the rest of the research. The fluoride electrode (Consort C863®, Belgium) was used according to the manufacturer's instructions.

The results showed that there was a statistically significant difference in the fluoride release between the control samples that are free of fluoride (group 1-control and group 2-control) and the fluoridated samples (group 1 and group 2). These results indicated that there was fluoride release from the fluoridated buckypapers.

According to time-fluoride release graph, the fluoride release from the samples of group 1 and group 2 almost stopped after 10 minutes. This finding indicates that fluoride release occurred quickly and we interpreted this as indicating that covalent bonds between the CNTs and the fluoride had not been able to be established as expected. It may be possible for stronger bonds to be formed by changing some of the parameters such as pressure or temperature. More research will be needed to provide this further information.

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CONCLUSIONS

As in many fields of science, nanotechnology has a great potential for creating changes in dentistry. Further research in preventive dentistry within the nanotechnology concept would be likely to be helpful in the development of more effective preventive strategies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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