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Comparison of the Fluoride Ion Release from nanofluorapatite-modified orthodontic cement under different pH Conditions - an *in Vitro* Study

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Abstract

Purpose Construction of the orthodontic bracket promotes food accumulation, which is the cause of plaque formation. Modern trends in the design of adhesive orthodontic cements focus on the ability to release cariostatic fluoride ions. One of the methods is to incorporate the material with fluorapatite nanoparticles. The aim of the study was to determine the fluoride release capacity of orthodontic cement doped with nanosized fluorapatite in selected media and solution pH over a 12-week period.

Methods A commercial light-curing two-component orthodontic cement GC Fuji Ortho®LC was modified by the addition of nanofluorapatite at 2% or 5% by weight. Each of the three groups (5 samples in 9 different media of varying ionic composition and pH). Fluoride determination was performed at 18 time intervals.

Results

The largest amount of fluoride is released in the first hour of incubation of all samples. Incorporating cement with 5% w/w nFAp significantly increases the amount of ions released. Low pH and artificial saliva rich in calcium cations promote significantly lower fluoride detection, which is associated with the formation of CaF2 conglomerates. The erosion of the surface layer was confirmed by the SEM image to be responsible for the ability to release the largest amounts of fluoride ions in an acidic environment.

Conclusion

The selection of experimental media for studying the fluoride release capacity of biomaterials is important in terms of the results achieved. The nanofluorapatite content correlates with the amount of fluorine released. Some limitations of the current research require further studies.

Keywords: fluoride release; fluoride recharge; orthodontic cement, glass-ionomer cement; nanosized particle

1. **Introduction**

Fixed appliances are one of the most common ways to treat malocclusion today. Mechanical properties of orthodontic appliance components affect the treatment of dental malpositions [29]. In fixed appliances, which are attached directly to the surface of the teeth, bite forces are transmitted to the teeth by means of orthodontic brackets bonded to the tooth surface.

Such mastication, as well as the orthodontic appliance itself, creates dynamic stresses that, during cyclic changes, can weaken the bonding strength of the bracket and its premature detachment during the treatment period [2].

Construction of the orthodontic bracket promotes food accumulation, which is the cause of plaque formation. With the growth of this plaque, there is a general increase in the activity of carious bacteria on the surface of the tooth in places where plaque adheres. As a consequence of the production of acids by the plaque-forming bacteria, a demineralization process occurs, which starts caries, otherwise known as white spot lesions WSL [5],[34].

In other words, treatment with fixed orthodontic appliances is directly related to the risk of decalcification of the enamel surface adjacent to the components of the appliance.

That's why one of the main currents of research on treatment with braces is still the search for solutions in terms of minimizing the risk of caries formation. Currently, promising in preventing the problem may be the use of fluoride-containing materials, and modifying them in such a way as to ensure greater release of fluoride ions, which will help prevent pathological changes.

Fluoride is an element that has wide clinical applications in dentistry. It inhibits demineralization of hard dental tissues so that hydroxyl ion is exchanged for fluoride ion, which is more resistant to acidic environments than hydroxyapatite, and facilitates remineralization of tooth tissues, particulary enamel and dentin, particulary [3],[18],[24].

Another property is that fluoride, by combining with magnesium present in the active enolase, which is produced by the bacterium in the carious process, destabilizes this enzyme, thus having a strong antimicrobial effect [13],[16]. These properties are crucial in preventing the development of caries.

In the study, glass ionomer cements, which release fluoride ions, were used as a control group. These cements are based on the reaction of powdered alkaline glasses with weak polymeric acids. Bonding takes place in concentrated aqueous solutions and the final structure contains a large amount of unreacted glass, which acts as a filler to strengthen the bonded cement [31]. There are three basic components of glass ionomer cement, precisely , alkali glass (ion leachable), water-soluble polymeric acid, water.

The orthodontic adhesive GC Fuji Ortho®LC used in the examination, which is based on a glass-ionomer, has, in addition to releasing fluoride ions, high hydrophilic properties, excellent sealing with good radiopacity and biocompatibility. Although GJ does release fluoride ions, the current aim is to increase the amount of fluoride released, while keeping the physical properties of the material unchanged, e.g. adhesion and no negative impact on the material's biocompatibility. It is also important that the material is durable and has a prophylactic effect on the dental tissues.

The use of nanosized fluorapatite in the modification of glassionomer is promising. Nanosized fluorapatite particles are a material that is biocompatible, meaning that it does not cause negative immunological or toxic reactions in the body, it also helps to restore and protect tooth enamel, and has an antibacterial effect (Fig 1).

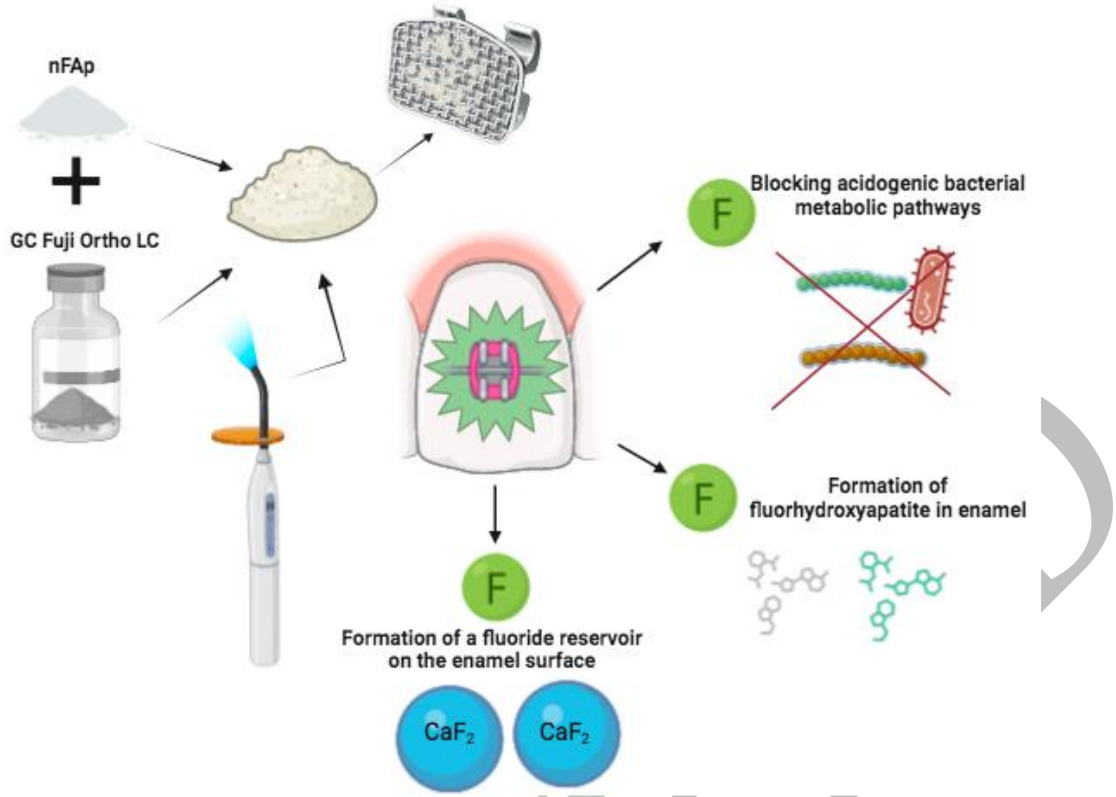

Fig. 1 Modification of the orthodontic cement with nFAp

According to studies by Wiglusz R.J. et al. [14], the long-term fluoride ion release capacity of nanosized fluorapatite has been demonstrated. In addition, this release is influenced by various factors, such as pH, among others. It therefore seems appropriate to modify fluoride-containing materials with fluorapatite nanoparticles, which will significantly improve the efficacy of tooth decay prevention, improve the efficacy of commercially sold orthodontic adhesives, through their improved bonding strength to dentin, and mechanical properties such as hardness, tensile and compressive strengths [4],[26].
Our research showed that the amount of fluoride released is 5-10 times higher in orthodontic adhesive doped with 5% nanosized fluorapatite in a deionized water medium compared to orthodontic adhesive without modification. In an in vitro study, the short-term release of fluoride from orthodontic adhesive samples was assessed. The next step will be a long-term study of the material in a system: natural tooth-adhesive material-orthodontic bracket.
The main objective of the present study was to determine the amount of fluoride ions released from orthodontic adhesive samples enriched with nanosized fluorapatite crystals in selected media of varying pH. The following media were used in the experiment: 0.9% NaCl solution, artificial saliva (AS) and deionized water.
In order to mimic the different environments of the human oral cavity, an artificial saliva from pH 4,5 to 7,5 was created for the study based on a recipe from the Department of Pediatric and Conservative Dentistry. The preliminary character of this study manifests itself in the short-term evaluation of fluoride release and is only in vitro in character. The next step will be the study of long term release and preparing materials in a real tooth.

2. Materials and Methods

A commercial light-curing two-component orthodontic cement GC Fuji Ortho®LC (including powder+liquid) was used as the reference group. The cement was modified by the addition of nanofluorapatite at 2% or 5% by weight of the cement powder, Nanosized fluorapatite was synthesized at the Institute of Low Temperature and Structure Research, Polish Academy of Sciences in Wrocław.

GC Fuji Ortho® LC

GC Fuji Ortho®LC – LIQUID (Light-cured glass ionomer for bonding of orthodontic appliances): Components: Polyacrylic acid 20-22 %, 2-Hydroxyethyl methacrylate 35-40%, Proprietary Ingredient 5-15 %, 2,2,4, Trimethyl hexamethylene dicarbonate 5-7 %, Triethylene glycol dimethacrylate 4-6%. GC Fuji Ortho®LC – Powder: Alumino-fluoro-silicate glass (amorphous)100%.

Synthesis of fluorapatite

Nanocrystalline fluorapatite powders were synthesized by co-precipitation method. Starting materials were calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$ ≥99% Acros Organics, Geel, Belgium), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$ ≥98% Avantor Performance Materials, Gliwice, Poland), ammonium fluoride (NH_4F, 98% Alpha Aesar, Haverhill, Ma, USA) and ammonia solution (NH_4OH ≥98% Avantor Performance Materials, Gliwice, Poland) for pH adjustment. In deionized water the stoichiometric amounts of starting materials were dissolved. Subsequently, the solutions were mixed and a synthesis was carried out on a magnetic stirring plate (Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) at 100 °C for 1.5 h. Aqueous ammonia was used to maintain the reaction at a pH ~ 10. The resulting precipitate was washed and centrifuged to a neutral pH, but not less than three times. Lastly, to form crystallized nanoparticles, the materials were dried for 24 hours at 70 °C and then heat-treated at 450 °C for 6 hours.

Test sample preparation

The test material consisted of cylindrical samples (ϕ=6mm and h-=2.5mm high) divided into 3 measurement groups: reference samples (GC Fuji Ortho®LC cement), GI cement with 2% w/w nFAp and cement with 5% w/w nFAp, prepared at INTiBS PAN, was mixed with the cement, and then each sample was polymerised with an Elipar II LED polymerisation lamp (3M ESPE, St Paul, MN, USA) emitting light in the wavelength range of 400–515 nm with a maximum intensity of 800 $mW^{-2}cm$, for a period of 20 s.

Fluoride ions release measurements

To determine the exact amount of fluoride ion released, an ORION Model 9609 ion-selective electrode (Thermo Fisher Scientific Co., Waltham, MA) was used in conjunction with a pH/ion meter equipped with a CPI-551 Elmetron microcomputer. Before each further measurement, the system was calibrated three times and repeated to determine the mean value. The samples were then immersed in 5 ml of the test solution and left in a closed container at 37 °C without stirring for the appropriate time until the fluoride released in the material is determined (measurements are performed after 1, 3, 24 , 48, 72, 96, 168, 336, 504, 672, 840, 1008, 1176, 1344, 1512, 1680, 1848 and 2016 hours). Five samples of each material were prepared for each environment (total n=135).

Statistical analysis

Statistical analysis was performed using MS Excel Professional 2016 (Microsoft Co, USA) and Statistica v.13.3 (Tibco Software Inc., Palo Alto, CA, USA). All experiments were done five times and descriptive data were presented as a mean and a standard deviation (±SD). Distribution of the data was tested with the Shapiro–Wilk normality test, and the homogeneity of variances were analyzed by Leven's test. One-way analysis of variance ANOVA was used for multiple comparison procedures, and the post-hoc Tukey test was used for intergroup comparisons. Pearson's correlation coefficients (r) were calculated to evaluate associations between the time of incubation and release of fluoride ions from study materials. Because Pearson's correlation analysis assumes a linear correlation, whereas the ion release vs. time dependence should resemble a logarithmic function, data for this analysis were logarithmically transformed. Values with $p \leq 0.05$ were considered to be statistically significant.

SEM measurements

Scanning electron microscope (SEM) micrographs were made on an FEI Nova NanoSEM 230 microscope (Hillsboro, OR, USA).

3. Results

In the Table 1 has been shown the results of the *in vitro* release of fluoride ions from the Fuji ORTHO®LC glass ionomer GC (GI) into nine different solutions with varying solution compositions and pH levels at selected time intervals. ANOVA analysis of the dependent samples revealed statistically significant differences in F^- ions released at specific time intervals under incubation conditions ($p<0.0001$ for all). For all groups, the highest release of fluoride ions from the unmodified cement - occurred in the 1st and 3rd hours of the experiment (Table 1). However, the most fluoride ions were released in deionized water and also artificial saliva at low pH 4,5 without calcium ions. Over time, a reduction in the amount of fluoride ion release was observed in all study groups. on 84th days of the experiment, the amount was the lowest.
Glass ionomer GC Fuji Ortho®LC (GI) showed the highest cumulative release of fluoride ions into the physiological saline solution 293.144±30.485 [μg/mm^2], followed by the deionized water solution 274.812 ± 27.665 [μg/mm^2], and the artificial saliva solution (AS) at pH 7,5 (202.481± 41.056 [μg/mm^2]) (Table 2, Fig. 2).
In contrast, the lowest emission was observed in the artificial saliva solution at pH 5,5 with added calcium 75.044 ± 12.669 [μg/mm^2] (Table 2). After the first hour, the pure orthodontic cement released the most fluoride in the artificial saliva medium at pH 5,5 without calcium addition 7.726 ±1.435 [μg/mm^2], followed by that in 0.9% NaCl (7.473 ± 1.974 [μg/mm^2] and artificial saliva at pH 7 without added calcium 7.392 ± 0.921 [μg/mm^2], and least to artificial saliva solution at pH 4,5 with added calcium 4.544 ± 0.158 [μg/mm^2] (Table 1).
The average increase of emission related to fluoride ions was highest in the artificial saliva medium at pH 7,5 without addition of calcium ions, the artificial saliva medium at pH 4,5 without calcium addition, and in the artificial saliva solution at pH 7,0 without calcium addition, amounting to an average value 0.9±0.14 [μg/mm^2]. There is demonstrated statistically

significant correlations between selected time intervals and release of fluoride ions from the Fuji ORTHO®LC glass ionomer GC (GI) in all studied solutions (*p<0.0001*) (Tale. 2).

Table 1. In nine distinct solution environments characterized by varying pH values and solution compositions, the release of fluoride ions from glass ionomer GC Fuji Ortho®LC (GI) exhibits differential patterns. For each solution, five samples were made. Mean ± and standard deviation (±SD) were used to present descriptive data

Time (hours)	Deionized H_2O (1) [μg/mm]	0.9%NaCl (2) [μg/mm^2]	AS pH 4,5 (3) [μg/mm^2]	AS pH 5,5 (4) [μg/mm^2]	AS pH 6,0 (5) [μg/mm^2]	AS pH 7,0 (6) [μg/mm^2]	AS pH 7,5 (7) [μg/mm^2]	AS + Ca^{2+} pH 4,5 (8) [μg/mm^2]	AS + Ca^{2+} pH 5,5 (9) [μg/mm^2]	***p-value*
1	7,168 ±1,296	7,473 ±1,974	7,006 ±0,891	7,726 ±1,435	6,333 ±2,161	7,392 ±0,921	6,935 ±1,007	4,544 ±0,158	6,277 ±0,395	*0.017**
3	3,699 ±0,423	2,824 ±0,373	6,939 ±1,094	4,572 ±0,565	4,447 ±0,869	5,774 ±1,383	7,092 ±0,835	2,435 ±0,139	3,188 ±0,546	*<0.0001**
24	1,039 ±0,116	0,695 ±0,041	0,548 ±0,175	0,763 ±0,087	0,596 ±0,020	1,070 ±0,111	0,713 ±0,129	0,376 ±0,049	0,496 ±0,067	*<0.0001**
48	0,643 ±0,091	0,540 ±0,069	0,445 ±0,082	0,365 ±0,042	0,361 ±0,047	0,512 ±0,054	0,424 ±0,081	0,290 ±0,015	0,282 ±0,022	*<0.0001**
72	0,502 ±0,061	0,536 ±0,029	0,308 ±0,078	0,297 ±0,012	0,278 ±0,015	0,325 ±0,020	0,318 ±0,047	0,250 ±0,025	0,191 ±0,052	*<0.0001**
96	0,430 ±0,040	0,498 ±0,020	0,202 ±0,014	0,194 ±0,033	0,274 ±0,203	0,221 ±0,052	0,248 ±0,021	0,192 ±0,011	0,173 ±0,016	*<0.0001**
168	0,292 ±0,041	0,287 ±0,032	0,212 ±0,030	0,163 ±0,011	0,169 ±0,015	0,063 ±0,011	0,202 ±0,038	0,149 ±0,003	0,106 ±0,014	*<0.0001**
336	0,124 ±0,006	0,121 ±0,005	0,088 ±0,011	0,069 ±0,004	0,064 ±0,001	0,105 ±0,009	0,084 ±0,015	0,069 ±0,007	0,044 ±0,008	*<0.0001**
504	0,123 ±0,005	0,121 ±0,004	0,094 ±0,012	0,066 ±0,002	0,068 ±0,008	0,078 ±0,011	0,076 ±0,019	0,048 ±0,019	0,034 ±0,009	*<0.0001**
672	0,116 ±0,008	0,120 ±0,003	0,093 ±0,017	0,067 ±0,003	0,068 ±0,003	0,097 ±0,021	0,098 ±0,022	0,027 ±0,021	0,023 ±0,005	*<0.0001**
840	0,141 ±0,017	0,126 ±0,006	0,084 ±0,017	0,065 ±0,004	0,069 ±0,012	0,071 ±0,004	0,083 ±0,020	0,018 ±0,014	0,016 ±0,006	*<0.0001**
1008	0,119 ±0,006	0,121 ±0,004	0,076 ±0,011	0,057 ±0,006	0,059 ±0,009	0,064 ±0,011	0,077 ±0,018	0,011 ±0,005	0,012 ±0,002	*<0.0001**
1176	0,105 ±0,010	0,118 ±0,003	0,056 ±0,010	0,044 ±0,003	0,040 ±0,006	0,052 ±0,007	0,060 ±0,012	0,009 ±0,004	0,009 ±0,002	*<0.0001**
1344	0,083 ±0,009	0,109 ±0,002	0,049 ±0,009	0,039 ±0,004	0,035 ±0,005	0,046 ±0,004	0,059 ±0,013	0,010 ±0,004	0,008 ±0,001	*<0.0001**
1512	0,071 ±0,008	0,098 ±0,024	0,042 ±0,007	0,028 ±0,004	0,033 ±0,003	0,037 ±0,008	0,052 ±0,013	0,027 ±0,012	0,008 ±0,001	*<0.0001**
1680	0,065 ±0,006	0,103 ±0,021	0,035 ±0,005	0,032 ±0,002	0,029 ±0,002	0,040 ±0,004	0,053 ±0,010	0,006 ±0,002	0,004 ±0,000	*<0.0001**
1848	0,052 ±0,008	0,085 ±0,025	0,038 ±0,012	0,036 ±0,006	0,036 ±0,002	0,051 ±0,010	0,057 ±0,014	0,004 ±0,001	0,003 ±0,000	*<0.0001**
2016	0,064 ±0,004	0,103 ±0,027	0,024 ±0,004	0,022 ±0,002	0,028 ±0,003	0,015 ±0,001	0,058 ±0,012	0,004 ±0,001	0,004 ±0,000	*<0.0001**
Mean ± SD	0,824 ±0,119	0,782 ±0,147	0,907 ±0,137	0,811 ±0,123	0,721 ±0,188	0,889 ±0,146	0,927 ±0,129	0,470 ±0,027	0,604 ±0,063	*<0.0001**
***p-value	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	-
post-hoc Tukey test	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups;	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups;	p=0,001* for 1h vs 24-2016h; p=0,0001* for 3h vs 24-2016h	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups;	p=0,0001* for 1h *vs* all time subgroups; p=0,0001* for 3h *vs* all time subgroups;	-

p=0,0001* for 24h *vs* 168-2016h	p=0,0001* for 24h *vs* 168-2016h	p<0,05* for 24h *vs* 72-2016h; p<0,001* for 48h *vs* 168-2016h; p<0,05* for 72 *vs* 336-2016h; p<0,05* for 96 *vs* 336-2016h; p<0,05* for 168 h *vs* 672-2016h	p<0,05* for 24h *vs* 168-2016h

*statistically significant; AS: artificial saliva; ***p-value* ANOVA for independent groups; ***p-value (ANOVA for dependent samples)

Table 2. The cumulative release of fluoride ions [µg/mm²] from glass ionomer GC Fuji Ortho®LC (GI) was characterized across nine environments with varying pH values and solution compositions. For each solution, five samples were prepared. Mean and standard deviation (±SD) were used to summarize descriptive data. Data for correlation analysis were logarithmically transformed

Time (hours)	Deionized H_2O (1) [µg/mm²]	0.9%NaCl (2) [µg/mm²]	AS pH 4,5 (3) [µg/mm²]	AS pH 5,5 (4) [µg/mm²]	AS pH 6,0 (5) [µg/mm²]	AS pH 7,0 (6) [µg/mm²]	AS pH 7,5 (7) [µg/mm²]	AS + Ca^{2+} pH 4,5 (8) [µg/mm²]	AS + Ca^{2+} pH 5,5 (9) [µg/mm²]
1	7,168 ±1,297	7,473 ±1,974	7,006 ±0,891	7,726 ±1,435	6,333 ±2,161	7,392 ±0,921	6,935 ±1,007	4,544 ±0,158	6,277 ±0,395
3	14,567 ±2,143	13,122 ±2,721	20,885 ±3,080	16,870 ±2,566	15,229 ±3,900	18,941 ±3,688	21,120 ±2,678	9,414 ±0,436	12,654 ±1,487
24	36,392 ±4,592	27,738 ±3,595	32,403 ±6,764	32,899 ±4,398	27,745 ±4,323	41,419 ±6,028	36,101 ±5,390	17,318 ±1,468	23,077 ±2,899
48	51,836 ±6,794	40,713 ±5,254	43,101 ±8,733	41,667 ±5,413	36,410 ±5,469	53,710 ±7,345	46,291 ±7,344	24,289 + 1,846	29,868 + 3,437
72	63,891 ±8,274	53,599 ±5,969	50,501 ±10,618	48,801 ±5,705	43,103 + 5,849	61,523 + 7,826	53,925 + 8,486	30,304 + 2,454	34,461 + 4,695
96	74,225 ±9,236	65,569 ±6,471	55,373 ±10,970	53,477 ±6,501	47,690 + 6,333	66,835 + 9,084	59,888 + 9,001	34,919 + 2,727	38,622 + 5,098
168	95,299 ±12,162	86,278 ±8,783	70,657 ±13,156	65,257 ±7,319	59,924 ±7,472	71,379 ±9,944	74,463 ±11,742	45,711 ±3,000	46,281 ±6,134
336	116,242 ±13,297	106,743 ±9,714	85,543 ±15,095	76,858 ±7,993	70,805 ±7,724	89,045 ±11,584	88,631 ±14,351	57,439 ±4,249	53,707 ±7,526
504	136,914 ±14,150	127,171 ±10,487	101,401 ±17,248	88,098 ±8,399	82,272 ±9,076	102,231 ±13,533	101,563 ±17,572	65,616 ±7,525	59,517 ±9,049
672	156,558 ±15,547	147,353 ±11,120	117,134 ±20,131	99,842 ±9,034	93,842 ±9,594	118,615 ±17,184	118,161 ±21,296	70,276 ±11,196	63,535 ±9,896
840	180,325 ±18,494	168,645 ±12,213	131,314 ±23,143	110,386 ±9,720	105,508 ±11,616	130,573 ±17,951	132,265 ±24,782	73,427 ±13,554	66,322 ±11,042
1008	200,473 ±19,646	189,041 ±12,936	144,246 ±25,124	119,986 ±10,846	115,463 ±13,171	141,419 ±19,883	145,203 ±27,974	75,282 ±14,555	68,432 ±11,488
1176	218,149 ±21,384	209,015 ±13,482	153,770 ±26,955	127,544 ±11,424	122,233 ±14,283	150,316 ±21,179	155,387 ±30,090	76,894 ±15,261	70,013 ±11,879
1344	232,231 ±22,954	227,483 ±13,933	162,157 ±28,547	134,159 ±12,116	128,199 ±15,266	158,210 ±22,018	165,329 ±32,338	78,699 ±16,010	71,524 ±12,135
1512	244,159 ±24,411	243,980 ±18,042	169,258 ±29,829	138,817 ±12,909	133,817 ±15,909	164,589 ±23,439	174,104 ±34,650	81,281 ±23,219	72,875 ±12,317
1680	255,233 ±25,456	261,338 ±21,615	175,302 ±30,809	144,466 ±13,324	138,794 ±16,378	171,313 ±24,172	183,075 ±36,442	82,322 ±23,593	73,646 ±12,394
1848	263,974 ±26,898	275,755 ±25,818	181,825 ±32,841	150,668 ±14,400	144,964 ±16,732	179,885 ±25,871	192,729 ±38,899	83,007 ±23,821	74,263 ±12,544

2016	274,812 ±27,665	293,144 ±30,485	185,887 ±33,638	154,406 ±14,837	149,743 ±17,299	182,501 ±26,088	202,481 ±41,056	83,750 ±24,086	75,044 ±12,669
Correlation (Pearson test)	r=0.793 *p<0.001**	r=0.828 *p<0.001**	r=0.801 *p<0.001**	r=0.790 *p<0.001**	r=0.795 *p<0.001**	r=0.768 *p<0.001**	r=0.798 *p<0.001**	r=0.729 *p<0.001**	r=0.712 *p<0.001**

*: statistically significant; r: correlation coefficient; AS: artificial saliva

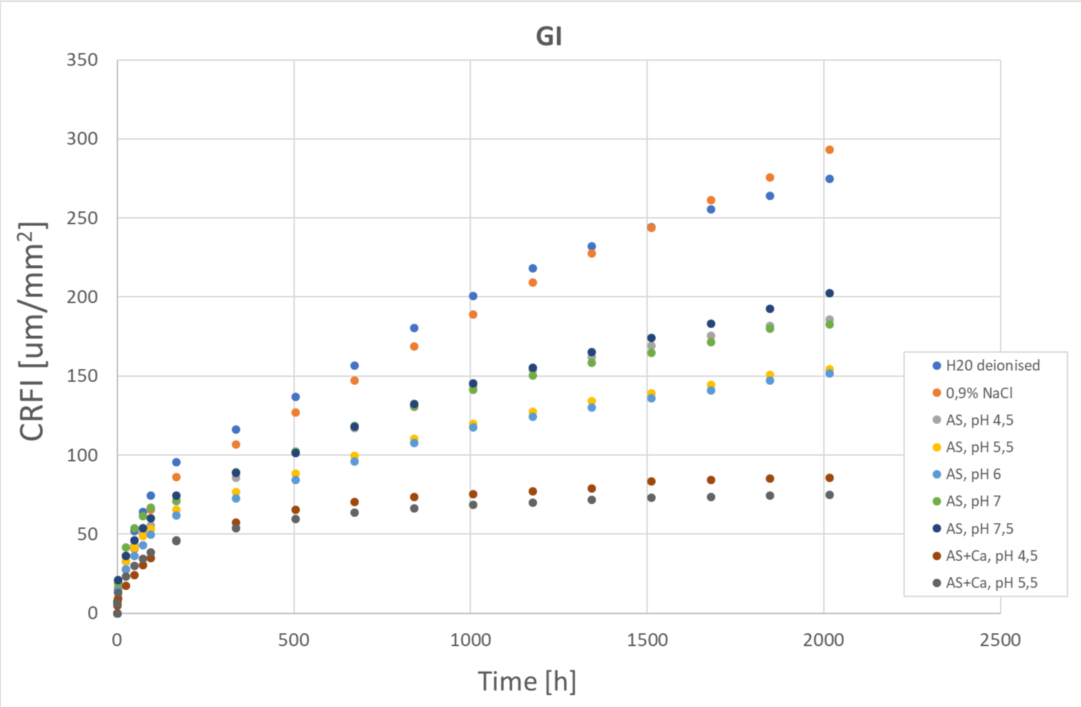

Fig. 2. The release of fluoride ions [µg/mm2] from GC Fuji Ortho®LC glass ionomer (GI) into nine different solutions was cumulatively measured. Mean measurements are represented by points along time intervals. AS refers to artificial saliva; CRFI – Cumulative release of F^- ions factor

Statistically significant differences in F^- ion release were observed over time periods of GC Fuji Ortho®LC (GI) plus 2% w/w nanosized fluorapatite (nFAp) in all tested incubation solutions ($p < 0.0001$) (Table 3).

The material groups analyze for above study showed the significantly highest levels of fluoride ion release after 1 hour and 3 hours of incubation, particularly in AS at pH 5,5 (10,067±1,937 µg/mm^2/h and 6,112±0,625 [µg/mm^2], respectively), AS pH at 4,5 (8,400±0,981 [µg/mm^2] and 6,084 ±0,813 [µg/mm^2], respectively), and AS at pH 6,0 (8,294±4,568 [µg/mm^2] and 5,858±0,746 [µg/mm^2] respectively).

The cumulated levels of fluoride ion release from the modified cement with 2% nFAp were highest during incubation in 0.9% NaCl (312,478±38,255 [µg/mm^2]) and deionized H_2O (303,857±32,465 [µg/mm^2]) solutions. Significantly lower values of cumulated F^- ions were observed in AS + Ca^{2+} at pH 4,5 (82,371 ± 14,991 [µg/mm^2]) and AS + Ca^{2+} at pH 5,5 (84,460±22.528 [µg/mm^2]) media (Table 4 and Fig. 3).

All tested solutions showed significant correlations between the time of incubation and the release of F^- ions ($p < 0.001$) (Table 4).

The addition of 2% w/w nFAp to the orthodontic cement powder resulted in an increase in the amount of fluoride released, with the highest amounts observed in the saline solution (data in Table 4 vs Table 2). The high amount of fluoride ions was also revealed in a solution of artificial saliva characterized by the absence of calcium ions (Table 4, Fig. 3).

Table 3. In nine distinct solution environments characterized by varying pH values and solution compositions, the release of fluoride ions from glass ionomer GC Fuji Ortho®LC (GI) plus 2% w/w nanosized fluorapatite (nFAp) exhibits differential patterns. For each solution, five samples were made. Mean ± and standard deviation (±SD) were used to present descriptive data

Time (hours)	Deionized H_2O (1) [µg/mm]	0.9%NaCl (2) [µg/mm²]	AS pH 4,5 (3) [µg/mm²]	AS pH 5,5 (4) [µg/mm²]	AS pH 6,0 (5) [µg/mm²]	AS pH 7,0 (6) [µg/mm²]	AS pH 7,5 (7) [µg/mm²]	AS + Ca^{2+} pH 4,5 (8) [µg/mm²]	AS + Ca^{2+} pH 5,5 (9) [µg/mm²]	***p-value*
1	7,011 ±1,638	5,610 ±1,220	8,400 ±0,981	10,067 ±1,937	8,294 ±4,568	8,264 ±2,584	6,763 ±0,799	5,071 ±0,593	8,289 ±2,642	*<0,001**
3	3,858 ±0,696	3,940 ±0,302	6,084 ±0,813	6,112 ±0,625	5,858 ±0,746	5,297 ±1,035	5,869 ±0,569	2,688 ±0,223	3,430 ±0,369	*<0,0001**
24	1,172 ±0,106	0,781 ±0,075	0,869 ±0,033	0,921 ±0,065	0,766 ±0,087	0,786 ±0,244	0,746 ±0,166	0,457 ±0,059	0,451 ±0,115	*<0,0001**
48	0,699 ±0,077	0,686 ±0,096	0,409 ±0,051	0,476 ±0,018	0,418 ±0,080	0,435 ±0,085	0,385 ±0,088	0,313 ±0,043	0,306 ±0,085	*<0,0001**
72	0,561 ±0,050	0,606 ±0,108	0,327 ±0,014	0,330 ±0,021	0,317 ±0,029	0,289 ±0,032	0,290 ±0,014	0,272 ±0,018	0,240 ±0,063	*<0,0001**
96	0,500 ±0,039	0,566 ±0,062	0,193 ±0,013	0,229 ±0,046	0,219 ±0,013	0,222 ±0,031	0,213 ±0,022	0,225 ±0,016	0,170 ±0,048	*<0,0001**
168	0,296 ±0,037	0,311 ±0,024	0,204 ±0,012	0,184 ±0,018	0,169 ±0,010	0,066 ±0,011	0,173 ±0,012	0,158 ±0,014	0,129 ±0,031	*<0,0001**
336	0,144 ±0,019	0,135 ±0,011	0,088 ±0,007	0,078 ±0,008	0,073 ±0,003	0,093 +0,017	0,085 ±0,006	0,076 ±0,010	0,058 ±0,012	*<0,0001**
504	0,140 ±0,011	0,124 ±0,013	0,091 ±0,008	0,079 ±0,009	0,082 ±0,006	0,069 +0,009	0,074 ±0,011	0,041 ±0,020	0,037 ±0,008	*<0,0001**
672	0,123 ±0,009	0,123 ±0,013	0,082 ±0,008	0,086 ±0,003	0,075 ±0,007	0,072 ±0,008	0,088 ±0,014	0,022 ±0,015	0,024 ±0,007	*<0,0001**
840	0,150 ±0,017	0,143 ±0,0021	0,079 ±0,006	0,077 ±0,008	0,064 ±0,009	0,076 ±0,009	0,071 ±0,005	0,009 ±0,003	0,019 ±0,009	*<0,0001**
1008	0,139 ±0,013	0,131 ±0,017	0063 ±0,004	0,071 ±0,010	0,062 ±0,006	0,061 ±0,005	0,067 ±0,005	0,007 ±0,001	0,016 ±0,006	*<0,0001**
1176	0,118 ±0,006	0,123 ±0,023	0,052 ±0,006	0,063 ±0,005	0,049 ±0,006	0,050 ±0,007	0,047 ±0,002	0,007 ±0,003	0,009 ±0,004	*<0,0001**
1344	0,098 ±0,010	0,111 ±0,019	0,043 ±0,002	0,052 ±0,006	0,047 ±0,002	0,042 ±0,005	0,051 ±0,003	0,005 ±0,000	0,010 ±0,003	*<0,0001**
1512	0,078 ±0,008	0,103 ±0,011	0,039 ±0,002	0,043 ±0,003	0,040 ±0,003	0,037 ±0,004	0,048 ±0,009	0,003 ±0,000	0,007 ±0,002	*<0,0001**
1680	0,069 ±0,005	0,101 ±0,009	0,042 ±0,002	0,038 ±0,003	0,034 ±0,002	0,036 ±0,006	0,042 ±0,005	0,005 ±0,001	0,005 ±0,001	*<0,0001**
1848	0,060 ±0,009	0,082 ±0,007	0,044 ±0,003	0,047 ±0,003	0,044 ±0,002	0,042 ±0,006	0,051 ±0,004	0,003 ±0,000	0,003 ±0,000	*<0,0001**
2016	0,072 ±0,009	0,104 ±0,009	0,022 ±0,001	0,029 ±0,003	0,033 ±0,001	0,013 ±0,001	0,046 ±0,009	0,004 ±0,001	0,004 ±0,001	*<0,0001**
Mean ± SD	0,849 + 0,153	0,765 + 0,113	0,952 + 0,109	1,054 + 0,155	0,925 + 0,310	0,886 + 0,228	0,839 + 0,097	0,520 + 0,057	0,734 + 0,189	*<0,0001**
***p-value	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	-
post-hoc Tukey test	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs all	*p=0,0001** for 1 h vs all time subgroups; *p=0,0001** for 3 h vs	-

	all time subgroups; *p<0,05** for 24 h vs 336-2016h	all time subgroups; *p=0,041** for 24 h vs 1848 h	all time subgroups; *p<0,05** for 24 h vs 336-2016h	all time subgroups	all time subgroups	all time subgroups	all time subgroups; *p<0,05** for 24 h vs 168-2016h	time subgroups; *p<0,05** for 24 h vs 336-2016h	all time subgroups

*statistically significant; AS: artificial saliva

Table 4. The cumulative release of fluoride ions [µg/mm²] from glass ionomer GC Fuji Ortho®LC (GI) plus 2% w/w nanosized fluorapatite (nFAp) was characterized across nine environments with varying pH values and solution compositions. For each solution, five samples were prepared. Mean and standard deviation (±SD) were used to summarize descriptive data. Data for correlation analysis were logarithmically transformed

Time (hours)	Deionized H_2O (1) [µg/mm²]	0.9%NaCl (2) [µg/mm²]	AS pH 4,5 (3) [µg/mm²]	AS pH 5,5 (4) [µg/mm²]	AS pH 6,0 (5) [µg/mm²]	AS pH 7,0 (6) [µg/mm²]	AS pH 7,5 (7) [µg/mm²]	AS + Ca^{2+} pH 4,5 (8) [µg/mm²]	AS + Ca^{2+} pH 5,5 (9) [µg/mm²]
1	7,011 ±1,638	5,610 ±1,220	8,400 ±0,981	10,067 ±1,937	8,294 ±4,568	8,264 ±2,584	6,763 ±0,799	5,071 ±0,593	8,289 ±2,642
3	14,729 ±3,030	13,490 ±1,825	20,569 ±2,608	22,292 ±3,188	20,012 ±6,062	18,859 ±4,655	18,502 ±1,939	10,448 ±1,040	15,150 ±3,382
24	39,357 ±5,267	29,894 + 3,421	38,833 + 3,301	41,641 + 4,573	36,114 + 7,901	35,368 + 9,799	34,169 + 5,441	20,047 ±2,285	24,632 ±5,798
48	56,136 ±7,115	46,360 ±5,746	48,664 ±4,542	53,069 ±5,029	46,157 ±9,829	45,827 ±11,851	43,421 ±7,564	27,575 ±3,334	31,988 ±7,858
72	69,603 ±8,317	60,905 ±8,357	56,522 ±4,885	61,007 ±5,534	53,767 + 10,542	52,764 + 12,636	50,390 + 7,907	34,117 + 3,776	37,757 ±9,387
96	81,609 ±9,271	74,506 ±9,856	61,164 ±5,213	66,506 ±6,645	59,028 ±10,865	58,095 ±13,401	55,514 ±8,436	39,532 ±4,183	41,856 ±10,555
168	102,940 ±11,944	96,899 ±11,588	75,869 ±6,128	79,794 ±7,983	71,240 ±11,649	62,874 ±14,207	68,008 + 9,339	50,924 ±5,196	51,150 ±12,840
336	127,208 ±15,301	119,665 ±13,592	90,734 ±7,464	92,902 ±9,363	83,602 ±12,204	78,653 ±17,217	82,420 ±10,360	63,755 ±6,903	60,992 ±15,012
504	150,770 ±17,199	140,572 ±15,928	106,147 ±8,875	106,225 ±11,026	97,429 ±13,352	90,328 ±18,764	94,887 ±12,213	70,807 ±10,334	67,223 ±16,366
672	171,521 ±18,816	161,285 ±18,269	120,024 ±10,331	120,814 ±11,669	110,184 ±14,550	102,553 ±20,272	109,825 ±14,612	74,628 ±12,994	71,413 ±17,695
840	196,786 ±21,774	185,330 ±21,809	133,362 ±11,398	133,892 ±13,082	121,016 ±16,193	115,335 ±21,879	121,900 v15,590	76,242 ±13,614	74,769 ±19,232
1008	220,208 ±24,024	207,341 ±24,776	143,980 ±12,125	145,935 ±14,860	131,499 ±17,253	125,617 ±22,855	133,257 ±16,434	77,517 ±13,947	77,539 ±20,390
1176	240,066 ±25,136	228,055 ±28,677	152,775 ±13,278	156,561 ±15,852	139,816 ±18,296	134,026 ±24,043	141,297 ±16,786	78,777 ±14,455	79,147 ±21,105
1344	256,636 ±26,877	246,764 ±31,882	160,095 ±13,757	165,327 ±16,990	147,757 ±18,656	141,202 ±24,897	149,998 ±17,309	79,667 ±14,560	80,891 ±21,658
1512	269,854 ±28,305	264,081 ±33,821	166,813 ±14,260	172,577 ±17,581	154,565 ±19,168	147,433 + 25,706	158,161 ±18,880	80,310 ±14,664	82,076 ±22,075
1680	281,559 ±29,309	281,144 ±35,388	174,028 ±14,675	179,050 ±18,144	160,396 ±19,643	153,503 ±26,723	165,339 ±19,747	81,157 ±14,799	83,033 ±22,292
1848	291,748 ±30,838	294,933 ±36,597	181,456 ±15,314	186,968 ±18,736	167,932 ±19,991	160,648 ±27,797	173,996 ±20,458	81,679 ±14,875	83,640 ±22,393
2016	303,857 ±32,465	312,478 ±38,255	185,252 ±15,552	191,876 ±19,375	173,632 ±20,170	162,898 ±27,929	181,814 ±22,053	82,371 ±14,991	84,460 ±22,528
Correlation (Pearson test)	r=0,787 *p<0,0001**	r=0,799 *p<0,0001**	r=0,789 *p<0,0001**	r=0,798 *p<0,0001**	r=0,793 *p<0,0001**	r=0,797 *p<0,0001**	r=0,795 *p<0,0001**	r=0,696 *p<0,0001**	r=0,735 *p<0,0001**

*statistically significant; r: correlation coefficient; AS: artificial saliva

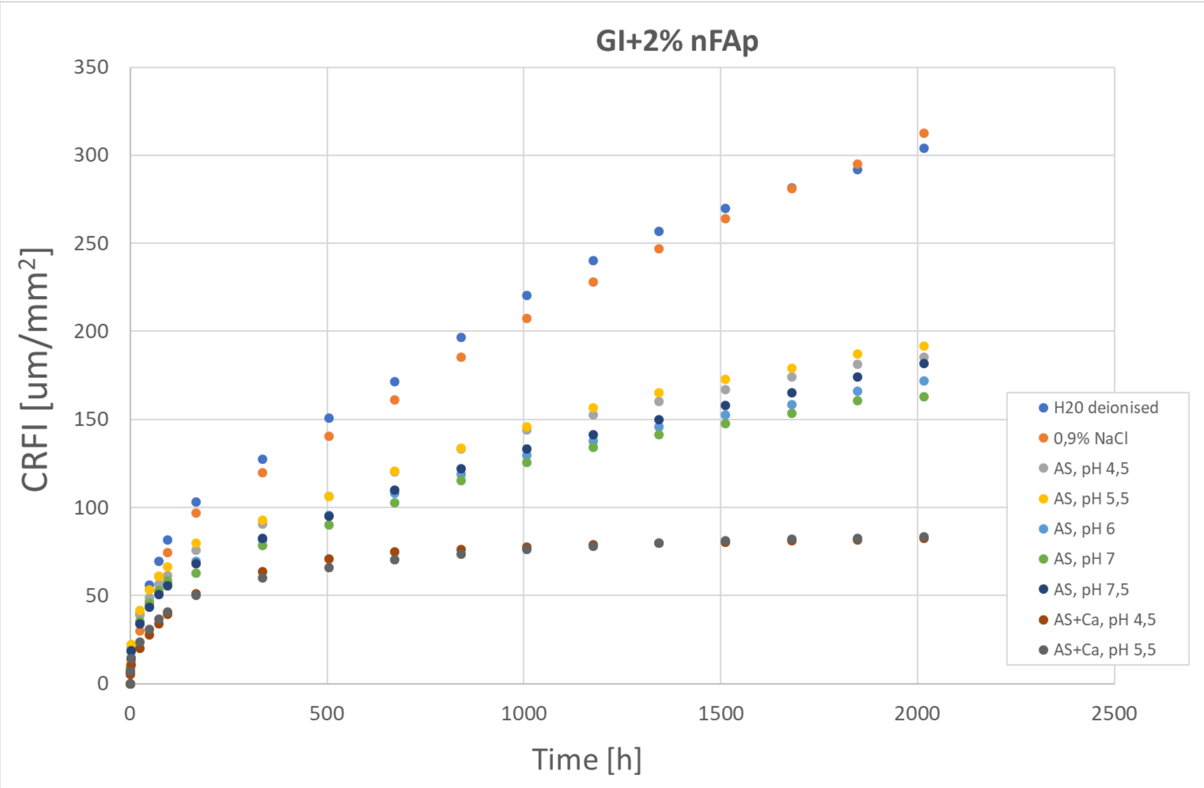

Fig. 3. The release of fluoride ions (µg/mm2) from GC Fuji Ortho®LC glass ionomer (GI)) plus 2% w/w nanosized fluorapatite (nFAp) into nine different solutions was cumulatively measured. Mean measurements are represented by points along time intervals. AS refers to artificial saliva; CRFI – Cumulative release of F^- ions factor

The ANOVA analysis of the dependent samples showed significant differences in the release of F^- ions at specific time intervals during incubation conditions in the case of GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) (*p <0,0001* for all) (Table 5).
For such orthodontic cement the highest fluoride release in all solutions also occurred in the 1st and 3rd hours of the study.
According to the experimental media, most fluoride ions were released in the artificial saliva solution without calcium ions at pH 7,0 (10,792±1,128 [$\mu g/mm^2$]) and at pH 4,5 (10,042±2,068 [$\mu g/mm^2$]). A systematic decrease of the released fluoride was observed in subsequent time intervals, The lowest amount of the released fluoride for all artificial saliva solutions and also for saline and deionized water was observed after 1848 hours (77 days) and 2016 hours (84 days) of the experiment, respectively.
GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite showed the highest cumulative release of fluoride ions into the deionized water solution (346,108 ± 36,516 $\mu g/mm^2$) followed by the physiological saline solution 305,108 ± 25,390 [$\mu g/mm^2$] and the artificial saliva solution at pH 7,5 (208,454 ± 16,861[$\mu g/mm^2$] (Table 6, Fig, 4), On the other hand, the lowest release was observed in the artificial saliva solution containing calcium ions at pH 4,5 (75,822 ± 11,033 [$\mu g/mm^2$] (Table 6).
After the first hour, the orthodontic cement incorporated with 5% w/w nFAp released the highest amount of fluoride ions in the artificial saliva solution without calcium addition at pH

5,5, then pH 7,0 and pH 4,5, (11,136 ± 2,193 [µg/mm^2]; 10,792 ± 1,128 [µg/mm^2]; 10,042± 2,068 [µg/mm^2], respectively).

The average increase of emission associated with fluoride ions was highest in the artificial saliva medium without addition of Ca^{2+} ions at pH 5,5, followed by the artificial saliva medium without addition of Ca^{2+} ions at pH 7,0, and in the deionized water (Table 5).

Table 5. In nine distinct solution environments characterized by varying pH values and solution compositions, the release of fluoride ions from glass ionomer GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) exhibits differential patterns. For each solution, five samples were made. Mean and standard deviation (±SD) were used to present descriptive data

Time (hours)	Deionized H_2O (1) [µg/mm]	0.9%NaCl (2) [µg/mm^2]	AS pH 4,5 (3) [µg/mm^2]	AS pH 5,5 (4) [µg/mm^2]	AS pH 6,0 (5) [µg/mm^2]	AS pH 7,0 (6) [µg/mm^2]	AS pH 7,5 (7) [µg/mm^2]	AS + Ca^{2+} pH 4,5 (8) [µg/mm^2]	AS + Ca^{2+} pH 5,5 (9) [µg/mm^2]	***p-value*
1	9,692 ±2,731	7,005 ±1,837	10,042 ±2,068	11,136 ±2,193	7,018 ±2,667	10,792 ±1,128	6,837 ±0,571	5,077 ±0,395	8,336 ±0,604	*<0,0001**
3	4,609 ±0,830	3,399 ±0,240	6,496 ±0,472	6,724 ±0,846	6,517 ±0,960	6,516 ±1,327	7,524 ±1,386	2,733 ±0,280	3,515 ±0,464	*<0,0001**
24	1,324 ±0,147	0,750 ±0,082	0,888 ±0,044	0,908 ±0,066	0,708 ±0,125	0,898 ±0,115	0,779 ±0,063	0,415 ±0,057	0,480 ±0,054	*<0,0001**
48	0,794 ±0,068	0,637 ±0,071	0,446 ±0,048	0,469 ±0,026	0,414 ±0,082	0,511 ±0,049	0,453 ±0,034	0,312 ±0,024	0,301 + 0,018	*<0,0001**
72	0,696 ±0,075	0,578 ±0,054	0,308 ±0,021	0,329 ±0,011	0,266 ±0,025	0,325 ±0,051	0,323 ±0,023	0,264 ±0,015	0,250 + 0,021	*<0,0001**
96	0,603 ±0,103	0,562 ±0,041	0,206 ±0,020	0,237 ±0,027	0,370 ±0,022	0,215 ±0,030	0,238 ±0,006	0,223 ±0,056	0,168 ±0,015	*<0,0001**
168	0,366 ±0,051	0,296 ±0,026	0,225 ±0,025	0,173 ±0,002	0,168 ±0,012	0,085 ±0,021	0,187 ±0,006	0,124 ±0,007	0,117 ±0,016	*<0,0001**
336	0,166 ±0,008	0,125 ±0,003	0,095 ±0,011	0,079 ±0,006	0,077 ±0,013	0,125 ±0,023	0,093 ±0,006	0,078 ±0,005	0,070 ±0,016	*<0,0001**
504	0,142 ±0,009	0,124 ±0,006	0,096 ±0,007	0,079 ±0,006	0,078 ±0,012	0,093 ±0,015	0,082 ±0,004	0,036 ±0,017	0,035 ±0,006	*<0,0001**
672	0,138 ±0,013	0,122 ±0,005	0,093 ±0,009	0,087 ±0,005	0,079 ±0,010	0,093 ±0,017	0,104 ±0,004	0,013 ±0,003	0,019 ±0,006	*<0,0001**
840	0,171 ±0,013	0,130 ±0,005	0,084 ±0,006	0,078 ±0,007	0,078 ±0,010	0,082 ±0,005	0,093 ±0,014	0,007 ±0,001	0,015 ±0,004	*<0,0001**
1008	0,144 ±0,022	0,123 ±0,006	0,064 ±0,006	0,071 ±0,003	0,059 ±0,008	0,072 ±0,005	0,071 ±0,006	0,004 ±0,000	0,010 ±0,001	*<0,0001**
1176	0,123 ±0,006	0,122 ±0,008	0,061 ±0,008	0,066 ±0,002	0,046 ±0,003	0,062 ±0,009	0,060 ±0,002	0,005 ±0,001	0,009 ±0,003	*<0,0001**
1344	0,113 ±0,006	0,116 ±0,004	0,046 ±0,005	0,048 ±0,004	0,042 ±0,003	0,046 ±0,004	0,058 ±0,001	0,005 ±0,000	0,009 ±0,003	*<0,0001**
1512	0,093 ±0,014	0,104 ±0,012	0,043 ±0,005	0,037 ±0,004	0,041 ±0,003	0,042 ±0,005	0,053 ±0,006	0,005 ±0,000	0,006 ±0,004	*<0,0001**
1680	0,084 ±0,007	0,098 ±0,016	0,041 ±0,005	0,038 ±0,003	0,042 ±0,003	0,038 ±0,004	0,055 ±0,005	0,005 ±0,001	0,005 ±0,002	*<0,0001**
1848	0,066 ±0,004	0,082 ±0,012	0,045 ±0,003	0,048 ±0,004	0,043 ±0,003	0,052 ±0,008	0,060 ±0,003	0,002 ±0,001	0,004 ±0,000	*<0,0001**
2016	0,080 ±0,008	0,108 ±0,009	0,024 ±0,001	0,027 ±0,004	0,035 ±0,002	0,014 ±0,000	0,052 ±0,005	0,003 ±0,000	0,005 ±0,000	*<0,0001**
Mean ±SD	1,078 ±0,229	0,804 ±0,135	1,072 ±0,154	1,146 ±0,179	0,893 ±0,232	1,114 ±0,157	0,951 ±0,119	0,517 ±0,048	0,742 ±0,069	*<0,0001**
***p-value	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	*<0,0001**	-
post-hoc Tukey test	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;	p=0,0001* for 1 h *vs* all time subgroups;

	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups	p=0,0001* for 3 h *vs* all time subgroups; p=0,001* for 24 h *vs* 168-2016 h; p<0,05* for 48 h *vs* 504-2016 h	p=0,0001* for 3 h *vs* all time subgroups; p<0,05* for 24 h *vs* 504-2016h	p=0,0001* for 3 h *vs* all time subgroups

*statistically significant; AS: artificial saliva

Table 6. The cumulative release of fluoride ions [µg/mm²] from glass ionomer GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) was characterized across nine environments with varying pH values and solution compositions. For each solution, five samples were prepared. Mean and standard deviation (±SD) were used to summarize descriptive data, Data for correlation analysis were logarithmically transformed

Time (hours)	Deionized H_2O (1) [µg/mm²]	0.9%NaCl (2) [µg/mm²]	AS pH 4,5 (3) [µg/mm²]	AS pH 5,5 (4) [µg/mm²]	AS pH 6,0 (5) [µg/mm²]	AS pH 7,0 (6) [µg/mm²]	AS pH 7,5 (7) [µg/mm²]	AS + Ca^{2+} pH 4,5 (8) [µg/mm²]	AS + Ca^{2+} pH 5,5 (9) [µg/mm²]
1	9,692 ±2,731	7,005 ±1,837	10,042 ±2,068	11,136 ±2,193	7,018 ±2,667	10,792 ±1,128	6,837 ±0,571	5,077 ±0,395	8,336 ±0,604
3	18,911 ±4,393	13,804 ±2,319	23,035 ±3,013	24,584 ±3,886	20,053 ±4,587	23,824 ±3,783	21,887 ±3,344	10,545 ±0,957	15,367 ±1,533
24	46,721 ±7,493	29,571 ±4,052	41,697 ±3,939	43,661 ±5,274	34,921 ±7,230	42,686 ±6,200	38,248 ±4,676	19,267 ±2,165	25,454 ±2,684
48	65,781 ±9,139	44,861 ±5,758	52,402 ±5,105	54,932 ±5,900	44,876 ±9,217	54,956 ±7,400	49,127 ±5,507	26,765 ±2,764	32,689 ±3,131
72	82,497 ±10,962	58,738 ±7,075	59,799 ±5,617	62,843 ±6,169	51,264 ±9,833	62,775 ±8,645	56,901 ±6,066	33,124 ±3,132	38,701 ±3,654
96	96,978 ±13,458	72,229 ±8,067	64,748 ±6,099	68,548 ±6,836	60,148 ±15,286	67,953 ±9,383	62,632 ±6,223	38,477 ±4,492	42,750 ±4,015
168	123,394 ±17,158	93,562 ±9,943	80,998 ±7,938	81,055 ±7,016	72,290 ±16,178	74,127 ±10,945	76,142 ±6,686	47,439 ±5,030	51,202 ±5,221
336	151,303 ±18,525	114,571 ±10,559	97,042 ±9,924	94,366 ±8,042	85,304 ±18,386	95,129 ±14,960	91,781 ±7,773	60,663 ±6,029	63,022 ±7,928
504	175,280 ±20,133	135,458 ±11,627	113,306 ±11,140	107,783 ±9,143	98,557 ±20,536	110,794 ±17,503	105,721 ±8,482	66,726 ±8,992	69,017 ±9,060
672	198,556 ±22,404	156,119 ±12,633	129,044 ±12,775	122,490 ±10,027	111,917 ±22,373	126,494 ±20,484	123,317 ±9,258	68,980 ±9,576	72,323 ±10,093
840	227,370 ±24,640	177,994 ±13,603	143,264 ±13,876	135,691 ±11,355	125,070 ±24,140	140,344 ±21,350	139,100 ±11,650	70,262 ±9,892	75,005 ±10,919
1008	251,660 ±28,453	198,749 ±14,642	154,026 ±14,992	147,645 ±12,004	135,038 ±25,634	152,517 ±22,340	151,138 ±12,767	71,061 ±10,014	76,786 ±11,214
1176	272,458 ±29,480	219,339 ±16,004	164,320 ±16,352	158,737 ±12,488	142,909 ±26,208	162,985 ±23,956	161,349 ±13,122	71,967 ±10,321	78,333 ±11,850
1344	291,524 ±30,556	238,931 ±16,786	172,105 ±17,324	166,856 ±13,286	149,971 ±26,829	170,768 ±24,755	171,140 ±13,441	72,946 ±10,437	79,866 ±12,448
1512	307,243 ±32,991	256,469 ±18,813	179,435 ±18,331	173,216 ±14,112	156,863 ±27,367	177,948 ±25,686	180,163 ±14,498	73,806 ±10,558	80,980 ±13,124
1680	321,406 ±34,280	273,081 ±21,528	186,359 ±19,263	179,735 ±14,688	163,936 ±27,973	184,498 ±26,442	189,544 ±15,396	74,740 ±10,814	81,871 ±13,501
1848	332,642 ±35,089	286,884 ±23,710	193,972 ±19,871	187,850 ±15,503	171,303 ±28,495	193,240 ±27,822	199,649 ±16,015	75,204 ±10,985	82,609 ±13,651
2016	346,108 ±36,516	305,108 ±25,390	198,145 ±20,185	192,401 ±16,250	177,240 ±28,900	195,630 ±27,945	208,454 ±16,861	75,822 ±11,033	83,462 ±13,799
Correlation (Pearson test)	r=0,792 *p<0,0001**	r=0,815 *p<0,0001**	r=0,800 *p<0,0001**	r=0,801 *p<0,0001**	r=0,783 *p<0,0001**	r=0,805 *p<0,0001**	r=0,792 *p<0,0001**	r=0,689 *p=0,002**	r=0,723 *p=0,001**

*statistically significant; r: correlation coefficient; AS: artificial saliva

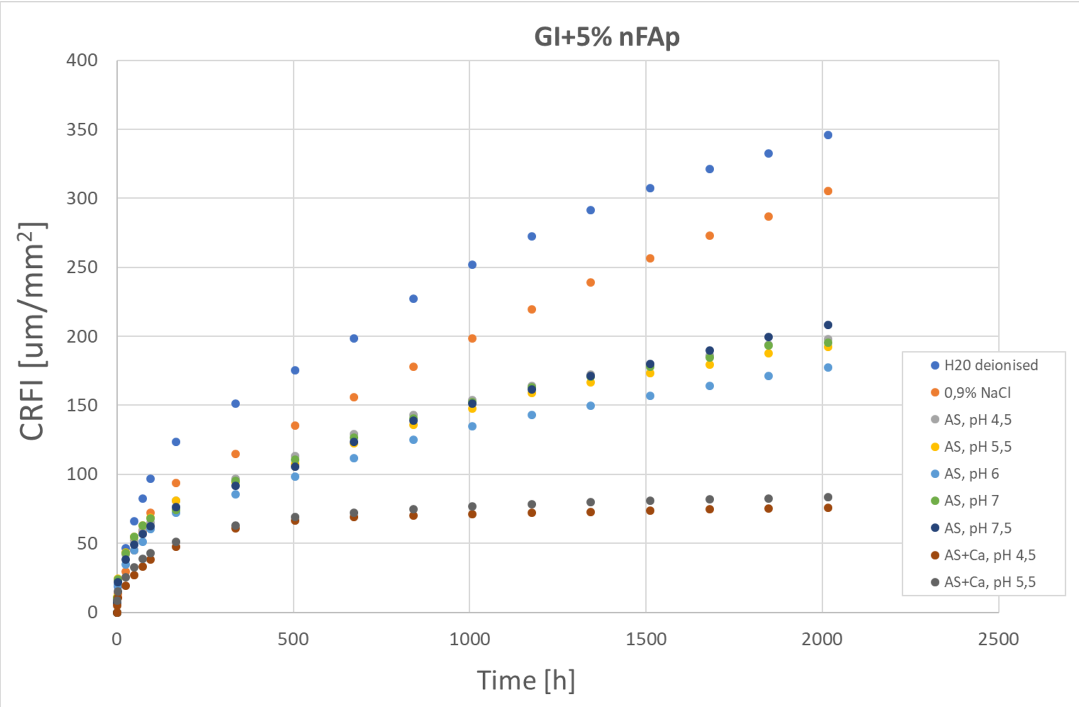

Fig. 4. The release of fluoride ions [μg/mm^2] from GC Fuji Ortho®LC glass ionomer (GI) plus 5% w/w nanosized fluorapatite (nFAp) into nine different solutions was cumulatively measured. Mean measurements are represented by points along time intervals, AS refers to artificial saliva

Comparative analysis of orthodontic adhesive samples enriched with nanosized fluorapatite crystals showed the highest cumulated amount of fluoride ion release from the GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) into deionized water solution, artificial saliva without calcium addition at pH 4,5 and pH 7,0 ($p<0,0001$ for all) (Table 7, Fig. 5). The GC Fuji ORTHO®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) revealed the lowest cumulative release of fluoride ions into artificial saliva solution added calcium at pH 4,5 ($p<0,001$).
The cumulated lowest emission of fluoride ions was observed from glass ionomer GC Fuji Ortho®LC (GI) than from the GC Fuji Ortho®LC (GI) plus 2% w/w nanosized fluorapatite (nFAp) and the GC Fuji Ortho®LC (GI) plus 5% w/w nanosized fluorapatite (nFAp) into deionized water, 0,9% NaCl, artificial saliva without calcium addition at pH 5.5, pH 6,0 and artificial saliva with calcium addition at pH 5.5 ($p<0,001$ for all) (Table 7, Fig. 5).

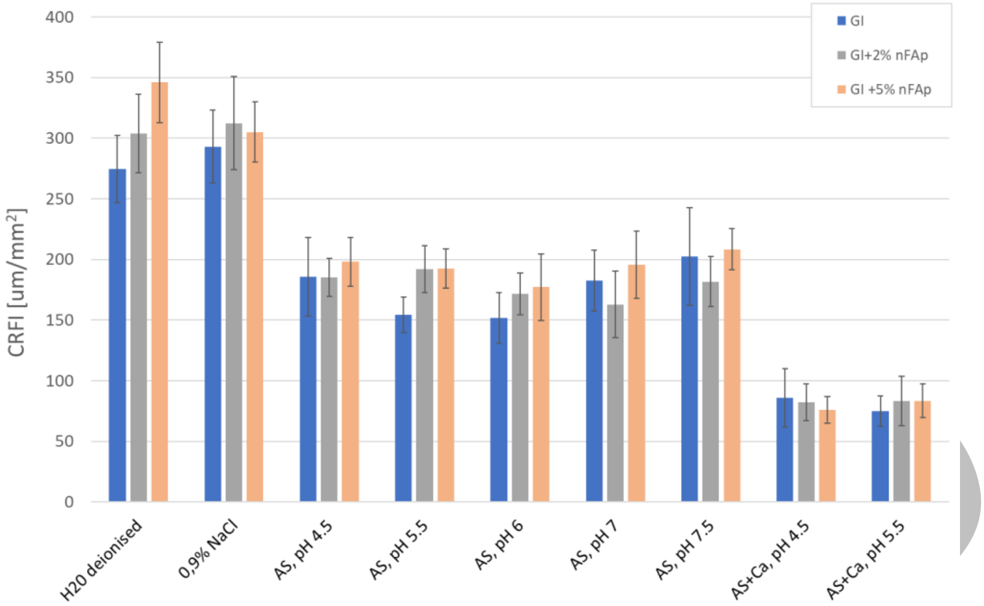

Fig. 5. Comparison of total cumulative release of fluoride ions [µg/mm^2] from glass ionomer GC Fuji Ortho®LC (GI), glass ionomer GC Fuji Ortho®LC (GI) plus 2%w/w nanosized fluorapatite (nFAp) and glass ionomer GC Fuji Ortho®LC (GI) plus 5%w/w nanosized fluorapatite (nFAp) into nine different solutions, ANOVA analysis showed statistically significant differences between analyzed materials for all solutions. AS: artificial saliva

Table 7. Comparison of total cumulative release of fluoride ions [µg/mm^2] from glass ionomer GC Fuji Ortho®LC (GI), glass ionomer GC Fuji Ortho®LC (GI) plus 2%w/w nanosized fluorapatite (nFAp) and glass ionomer GC Fuji Ortho®LC (GI) plus 5%w/w nanosized fluorapatite (nFAp) into nine different solutions

Material	Deionized H_2O [µg/mm^2]	0,9%NaCl [µg/mm^2]	AS pH 4,5 [µg/mm^2]	AS pH 5,5 [µg/mm^2]	AS pH 6,0 [µg/mm^2]	AS pH 7,0 [µg/mm^2]	AS pH 7,5 [µg/mm^2]	AS + Ca^{2+} pH 4,5 [µg/mm^2]	AS + Ca^{2+} pH 5,5 [µg/mm^2]
GI (1)	274,812 ±27,665	293,144 ±30,485	185,887 ±33,638	154,406 ±14,837	149,743 ±17,299	182,501 ±26,088	202,481 ±41,056	83,750 ±24,086	75,044 ±12,669
GI + 2% nFAp (2)	303,857 ±32,465	312,478 + 38,255	185,252 ±15,552	191,876 ±19,375	173,632 ±20,170	162,898 ±27,929	181,814 ±22,053	82,371 ±14,991	84,460 ±22,528
GI + 5% nFAp (3)	346,108 ±36,516	305,108 ±25,390	198,145 ±20,185	192,401 ±16,250	177,240 ±28,900	195,630 ±27,945	208,454 ±16,861	75,822 ±11,033	83,462 ±13,799
p-value (ANOVA for independent groups)	*< 0,0001**	*< 0,0001**	*< 0,0001**	*< 0,0001**	*< 0,0001**	*< 0,0001**	*< 0,0001**	*0,006**	*< 0,0001**
post-hoc Tukey test	p<0,0001* for 1 vs 2 1 vs 3 2 vs 3	p=0,0002* for 1 vs 2 p=0,004* for 1 vs 3 p=0,132 for 2 vs 3	p=0,877 for 1 vs 2 p=0,003* for 1 vs 3 p<0,0001* for 2 vs 3	p<0,0001* for 1 vs 2 1 vs 3 p=0,821 for 2 vs 3	p<0,0001* for 1 vs 2 1 vs 3 p=0,333 for 2 vs 3	p<0,0001* for 1 vs 2 2 vs 3 p=0,001* for 1 vs 3	p<0,0001* for 1 vs 2 2 vs 3 p=0,201 for 1 vs 3	p=0,639 for 1 vs 2 p=0,005* for 1 vs 3 p<0,001* for 2 vs 3	p<0,001* for 1 vs 2 p<0,0001* for 1 vs 3 p=0,719 for 2 vs 3

*statistically significant; GI: glass ionomer GC Fuji Ortho®LC; nFAp: nanosized fluorapatite; AS: artificial saliva

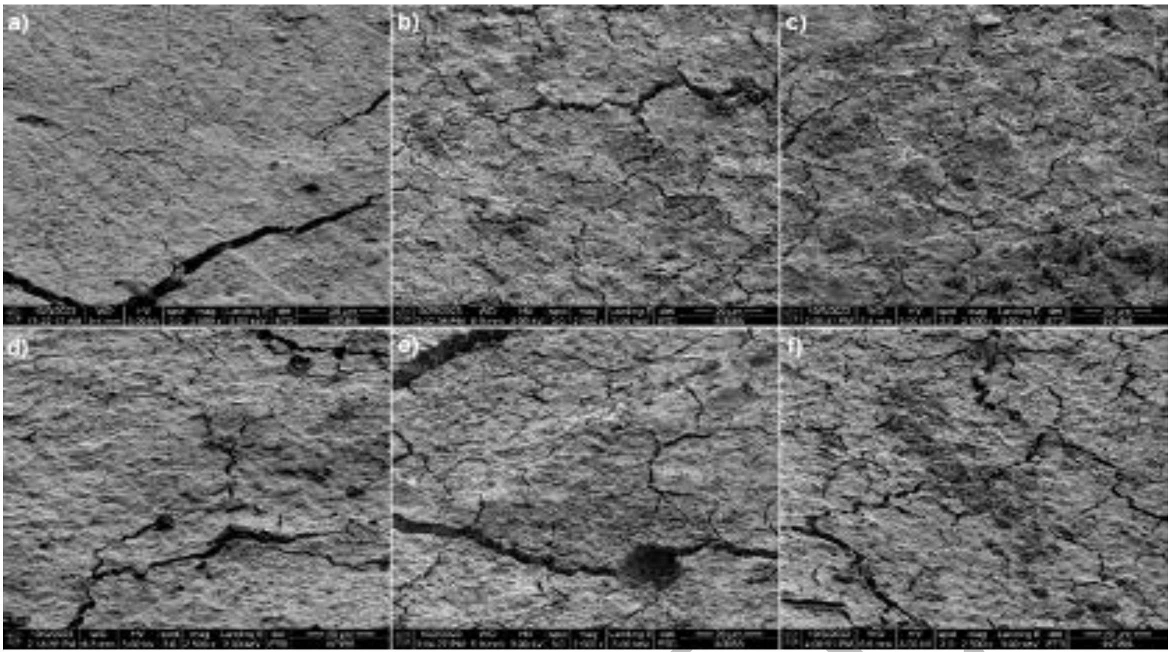

Fig. 6. SEM images of pure GC Ortho after release in deionized water (a), AS pH 4,5 (b) and AS + Ca^{2+} pH 4,5 (c), GC Ortho with 5% FAp in deionized water (d), AS pH 4,5 (e) and AS + Ca^{2+} pH 4,5 (f)

For samples where significant surface changes were observed, we presented the electron microscope images. The SEM images are presented in Fig. 6. In upper row GC Ortho after release in deionized water (a), AS pH 4,5 (b) and AS + Ca^{2+} pH 4,5 (c) is visible, while in lower row GC Ortho with 5% FAp after release in the same conditions is visible. One can see that for sample with addition of fluorapatite some craters (d, e) and islands of FAp (f) are clearly visible, Moreover surface of FAp doped samples is more eroded than for GC Ortho without additions.

4. **Discussion**

Nowadays, fixed orthodontic braces are very often used in the treatment of patients with malocclusion. The construction of the brackets of fixed appliances favors the deposition of bacterial plaque around them. Long-term retention of the plaque initially leads to demineralisation that manifests clinically as white spot lesions [7],[33].

The risk of developing white spot lesions (WSLs) is highest during the initial months of orthodontic treatment. This is due to the new brackets and archwires, which can trap more plaque than the teeth are accustomed to [34].

One of the main directions of current research in the field of orthodontic materials science is the development of modern orthodontic bonding cements with properties that reduce the metabolism of plaque bacteria and induce the formation of a protective antibacterial layer on the enamel surface [10]. Orthodontic cements have been improved by the addition of nanoparticles of various materials, such as silver. M. Chen et al, in their study showed the improved antimicrobial activity of orthodontic cement doped with particles of nano silver (NAg), N-acetylcysteine (NAC) and 2-methacryloxyethyl phosphorylcholine (MPC) [8].

In many dental materials, including orthodontics, manufacturers add fluoride compounds due to their positive, multidirectional protective effect [18], [20],[24],[28],[34]. Fluoride ions have a favourable impact on tooth structure and counteracts the formation of the carious process. Fluoride disrupts the transport of glucose into bacterial cells, which impedes the activity of enolase, an enzyme that plays a crucial role in the bacterial metabolism of glucose. By inhibiting metabolic pathways, it prevents the production of acid by bacteria and tooth hard tissues destruction [32].

In addition, an adequate amount of supplied fluoride compounds influences the formation of fluorapatite on the enamel surface. The fluorapatite is formed by replacing hydroxyl ions with fluoride ions in hydroxyapatite, This results in an enamel with better crystalline properties, better stability, greater resistance to acid solubility, which has a protective effect on adjacent dentine [9],[28], Moreover, fluorine in the appropriate amount provided by dental materials influences the formation of calcium fluoride, which prevents tooth hypersensitivity [1],[21]. The bioactivity of fluorapatite is enhanced by its nanoscale structure, which provides a larger surface area for chemical interactions with tooth tissues. This allows nFAp to integrate more effectively with the enamel and dentin of the tooth, promoting better adhesion and integration with the natural tooth structure. Nanofluoropatite possesses improved strength and durability compared to FAp, making it suitable for various dental applications. The nanoscale structure of FAp reduces stress concentration and improves overall material integrity, resulting in improved mechanical properties [14],[22]. Nanofluorapatite has been used as an additive in various dental and orthodontic materials as a source of fluoride ions [14],[18],[22],[24],[34]. The nFAp used in the current study, manufactured at the Institute of Low Temperature and Structure Research Polish Academy of Sciences, is a well-proven component of glass ionomer, composite and compomer materials used in restorative dentistry.

The ability to release fluoride ions from dental materials is tested in a variety of media with different pH values. An important element of in vitro testing is to replicate conditions that exist in the oral cavity, Saliva present in the oral cavity is a physiological fluid produced by the salivary glands. It performs many important functions, such as antibacterial activity, pre-digestion of food and remineralization of enamel, providing stability to apatite crystals thanks to the content of fluorides, phosphates and calcium ions [6],[33]. Due to the interaction of all saliva components and many variable agents, it is impossible to create saliva that will have the same composition as natural one. The in vitro model of artificial saliva that we used in our study was motivated by several factors. First, natural saliva is not stable outside the oral cavity and through bacterial colonization. Moreover, its composition can be modified by many different factors including demographic, physiological, pathological and environmental [29]. Therefore, the need to obtain stable conditions explains the use of artificial saliva in our study, To produce 1 L of artificial saliva in our experiment we used: 0,908 g $CaCl_2 \cdot 2H_2O$; 0,78 g $NaH_2PO4 \cdot 2H_2O$; 0,4 g NaCl; 0,4 g KCl; 0,005 g $Na_2S \cdot 9H_2O$. During the 12-week study, we observed significant differences in the value of fluoride ions - released from Fuji ORTHO LC GC cement, GC cement with 2% wt, of nFAp and cement with 5% wt, of nFAp. Above artificial saliva solution was an imitation of the natural conditions of the oral cavity. Due to the variability of pH in the oral cavity, which causes the release of different values of fluoride ions, we decided to use 7 different solutions with different pH and composition in our research. In addition to artificial saliva, we used physiological saline also used in fluoride release studies, as well as deionized water. 0,9% physiological saline solution is isotonic in relation to blood plasma, while deionized water does not react with ions released from the tested solutions. Moreover, NaCl solution is electrolytically similar to human saliva.

Acidic and neutral environments cause different fluoride release [17]. Fluoride release reaction in both environments occurs in a two-step process - rapid release of large amounts of fluoride ('early wash out') and a steady low-level of release [27]. In neutral solutions after early washout the prolonged diffusion can be observed. On the other hand under acidic conditions, there is still an initial wash-out, but a greater amount of fluoride is released. According to the neutral pH, diffusion occurs and is dependent on the square root of time ($\sqrt{t}$). Under acidic pH conditions, a gradual process of erosion occurs. This process is directly dependent on time, Kinetic equation has been established to describe the release profiles [11]. The equation is describe (1).

$$F_c = \frac{F_1 t}{(t + t_{1/2})} + \beta\sqrt{t} + \alpha t \qquad (1)$$

where: F_c - concentration of fluoride ions in the fluid at time t; F_1 - denotes the quantity of fluoride released during the early wash out stage; t - time $t_{½}$ - half-time of fluoride release; β, α- parameter determining the fluoride release rate. Kinetic parameters: F_1, $t_{1/2}$,α, β depend on the composition of the material while refers to neutral pH conditions.

Taking into account the half-time of fluoride release, the highest amount was observed for the material containing 2% fluorapatite by weight in deionised water and 0,9% of physiological saline solution. As the pH increased to the limit of 7.0, the amount of fluoride ions released decreased in GC Ortho and GC Ortho + 2% FAp, in contrast to GC Ortho + 5% FAp (Table 8). The addition of calcium ions to the artificial saliva resulted in a decrease in the amount of fluoride as the pH increased. In other studies, the highest release of fluoride ions was also observed in neutral solutions of deionised water and physiological saline [19],[20]. The use of a more acidic pH determined the release of a greater amount of fluoride ions [17],[22]. Artificial saliva containing calcium determines the lower fluoride levels observed [19].

The relationship of orthodontic cement incorporation with nanofluorapatite to the dynamics of the fluoride release process were analysed. The evaluation of this dynamics in terms of the influence of the pH value and the fact of calcium ion addition was also performed. As the weight content of nFAp rises, the dynamics of the fluoride release process in the deionized water environment increases. According to the NaCl 0,9% medium, the addition of nFAp did not result in significant differences in the fluoride release rate. In turn, the consecutive addition of nFAp to the orthodontic cement incubated in artificial saliva with low pH values initially induced a slight increase in the dynamics of the F^- release process. In alkaline artificial saliva (pH 7,0; 7,5), the addition of nanofluorapatite to the glass ionomer orthodontic material increased the dynamics of F-ions release. As the percentage of nFAp in the GI material raised, there was a slight decrease in the dynamics of fluoride ion release in the artificial saliva with the lowest pH containing calcium ions.

Table 8. Comparison of fluoride release kinetics from orthodontic cements GC Fuji Ortho®LC, glass ionomer GC Fuji Ortho®LC plus 2%w/w nanosized fluorapatite (nFAp) and glass ionomer GC Fuji Ortho®LC plus 5%w/w nanosized fluorapatite (nFAp) into nine different solutions

	GI (GC Fuji Ortho®LC)				GI + 2% nFAp				GI + 5% nFAp			
	F_1	$t_{1/2}$	β	α	F_1	$t_{1/2}$	β	α	F_1	$t_{1/2}$	β	α
deionized H_2O	15,7	7,8	5,5	0,01	22,8	16,3	5,7	0,01	19,6	7,7	7,3	-2,3
0,9% NaCl	5,1	1,2	5,2	0,02	8,9	45,8	6,1	0,01	7,5	3,3	5,5	0,02
AS pH 4,5	26,6	4,6	2,9	0,02	25,6	3,9	3,7	-0,002	25,6	2,6	4,1	-0,004
AS pH 5,5	22,4	3,5	3,1	-0,003	31,4	3,4	3,4	0,004	34,1	3,1	3,3	0,006
AS pH 6,0	16,2	3,2	3,2	-0,004	25,6	3,2	3,3	4,6	23,7	3,7	3,4	1,3
AS pH 7,0	40,6	6,9	2,5	0,02	29,2	3,8	2,6	0,01	32,8	3,3	3,4	0,01
AS pH 7,5	34,1	5,2	2,3	0,03	31,7	5,2	2,3	0,02	34,7	6,9	2,7	0,03
AS+Ca^{2+} pH 4,5	5,7	2,8	3,2	-0,03	3,6	1,6	4,1	-0,05	5,6	2,2	3,6	-0,05
AS+Ca^{2+} pH 5,5	13	2,7	2,8	-0,03	11,7	1,2	3,4	-0,04	12,2	1,4	3,5	-0,04

According to Mirna Habuda-Stanić et al, negatively charged fluoride ions can have a strong affinity for positively charged calcium ions [12]. Therefore, a conglomerate may form, which results in the release of fewer fluoride ions into the environment.

These conclusions, which involve the formation of a fluoride-calcium conglomerate, are consistent with our research results. In the authors' research, the accumulated values in artificial saliva solutions pH 4,5 and pH 5,5 with Ca^{2+} ions were significantly higher than the values determined in artificial saliva solutions without Ca^{2+}ions pH 4,5 and pH 5,5 in all three tested materials.

In the experiment, a solution of physiological saline and deionized water was used to compare the values of fluoride anions released from samples under neutral conditions. Deionized water was used as a solution that eliminated potential interactions between fluoride ions and other ions. Due to mimicking the average natural temperature of the human body, all nine tested solutions were incubated at 37 °C.

Our study shows that the total cumulative values of GC cement samples with 5%w/w of nFAp were significantly higher than the control sample (in the case of eight out of nine research media). The highest level of total cumulative release from GC cement with 5%w/w of nFAp among all nine environments (1-9) was observed in deionized water solution (1) (346,108±36,516[μg/mm^2]) - after 2016 hours. Lin et al, also demonstrated that orthodontic cements doped with higher levels of nanofluoropapatite are characterised by an increase in the amount of fluoride released [22]. The lowest level of cumulative release from samples immersed in all nine solutions (1-9) was found in artificial saliva with pH 4,5 with the addition of Ca^{2+}ions (8) (15,077± 0,395 [μg/mm^2]) - after 1 hour. During the first 24 hours of the experiment, a significant increase in the amount of fluoride ions released was observed in all tested materials. This phenomenon corresponds to the so-called ion explosion effect, which has also been confirmed by research by other authors [23].

Due to different experimental conditions, comparative analysis of our own results and those obtained by other authors is problematic. It should be emphasized that the choice of the experimental medium, (in particular the type of artificial saliva and its biochemical composition) is of fundamental importance for the obtained results. The addition of calcium ions causes the reduction of fluoride anions through the calcium fluoride precipitation reaction. Our results are consistent with the results of other authors, which show that the release of fluoride ions is greater in deionized water than in human artificial saliva solution [14],[20],[25]. The erosion of the surface layer was confirmed by the SEM image to be responsible for the ability to release the largest amounts of fluoride ions in an acidic environment. This image is consistent with observations from our previous research [19].

Conversely, the highest release of fluoride was observed when the samples of both tested materials were incubated in a deionized water solution. Only the control samples maintained the smoothest surface. This is due to the fact that depressions on the sample surface were observed around the embedded nanofluorapatite crystals.

The use of nanofluorapatite as a source of fluoride ions is an interesting method of increasing the cariostatic potential of orthodontic cements. However, it should be noted that the experimental model which has been used does not fully reflect the clinical conditions. The thickness of the cement layer between the enamel surface and the base of the orthodontic bracket reaches only about 1,0 mm.

In addition, the influence of physicochemical factors in the oral cavity, especially those related to diet, has not been considered, The current research is only an introduction to further in-depth analyses.

Conclusions: Nanosized fluorapatite is a valuable source of fluoride and can be used as an important reservoir of this element in adhesive orthodontic cements. The selection of experimental media for studying the fluoride release capacity of biomaterials is important in terms of the achieved results. The surface texture associated with the presence of nFAp crystals has an effect on the amount of fluoride that is released. The nanofluorapatite content correlates with the amount of fluorine released. An increase in the weight of nFAp in studied material enhances the fluoride release. The highest dynamics of fluoride release was observed in saline, deionized water, as well as at the extreme pH levels of artificial saliva (4,5 and 7,5) without calcium addition. These studies, however, point toward two processes that occur during fluoride release: a fast elution process during the early periods, and a long-term diffusive process, However, the increased content of nanofluorapatite needs to be assessed in order to maintain optimal adhesive properties. Some limitations of the current research require further studies,

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