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ON THE POSSIBILITY OF INCORPORATING ANTIMICROBIAL COMPONENTS INTO GLASS-IONOMER CEMENTS

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A b s t r a c t: Apart from their release of fluorine ions, GICs can potentially be used as templates for the release of other active antimicrobial ingredients. The aim of this study was to investigate the possibility of incorporating an antimicrobial compound into a glass-ionomer cement. For the realization of the aim set we used the comercially available ChemFlex glass-ionomer cement, a material widely used in restorative dentistry. Three groups of the commercial ChemFlex glass-ionomer were prepared, with no antimicrobial compound added, of 5 samples each – to serve as a control group; and another three groups of the same cement of 5 specimens each were also prepared, but each with a different concentration of benzalkonium chloride added to it – 1%, 2% and 3%, respectively – a total of 15 samples. The concentrations of released ions – fluoride and chloride – were measured at predetermined time instants. In order to determine the amount of ions (Cl^- and F^-) released into the medium (deionized water) in which the samples had been placed, we conducted measurements with ion-selective electrodes for chloride and fluoride at different time instants. We carried out an analysis of the release of chloride in order to see whether and in what concentrations such release of chlorine, a constituent of benzalkonium chloride, occurs, and thus to conclude about the possibility of the antimicrobial effect of the glass-ionomer cement. The common feature of all three percentages of benzalkonium chloride was that there were differences between the concentrations of chloride released by the samples with and without the antimicrobial compound added.

Key words: glass-ionomer cements, benzalkonium chloride, ion-selective electrodes, fluoride ion, chloride ion, antimicrobial compounds.

Introduction

Modern science identifies three main factors involved in the development of dental caries: pathogenic micro-organisms, incorrect nutrition and xerostomy, and several protective factors: saliva, fissures sealing, use of antimicrobial agents, fluorides and correct nutrition. These, on the one hand pathogenic, and on the other hand protective, factors are permanently in a state of balance which varies during the day. Dental caries will or will not develop depending on the direction of the change in this balance [36].

Although several pathogenic factors were emphasized, there is no dental caries without micro-organisms. This is a generally accepted notion based on a number of scientifically proven facts. Certain micro-organisms of the oral cavity flora can cause experimental dental caries *in vitro;* experimental animals raised in sterile conditions do not develop caries despite a cariogenic diet; certain bacteria, which can be proven histologically and microbiologically in the cariosic enamel, the dentine and the dentine tubes, can be isolated from the cariosic lesion [12].

From a preventive viewpoint there are an enormous number of products, both commercial and professional, which have in their structure one or several different antimicrobial components. As main examples we could cite fluorine, chlorhexidine, cetylpyridinium chloride, benzalkonium chloride, triclosan, listerine, and xylitol.

Fluorine has been used since 1940 because of its acknowledged anticaries effects, which mainly refer to the process of demineralisetion/remineralisation. However, substances containing fluorine manifest antimicrobial characterristics as well in the reduction of levels of salivary cariogenic microorganisms, and affect bacteria in a number of ways, which depends mainly on the concentration of fluorine [2, 35].

The most frequently used antimicrobial substance, for professional use mostly, is chlorhexidine. There is a quantity of research that has investigated the effect of chlorhexidine in various forms, various percental presences, and in combinations with other compounds, with the goal of determining the best method of application for reduction of oral cariogenic flora – in saliva and plaque [1, 10, 14, 20, 22].

Cetylpirydinium chloride (CPC), as an active component of oral antiseptics, has a broad antimicrobial spectrum with a strong bactericidal effect on gram^{+} pathogenics and a fungicidal effect. Its effectiveness against gram^{-} pathogenics and mycobacteria is questionable. The application of CPC in a concentration of 0.05% in rinses results with a direct reduction of bacteria counts [12, 27]. In comparison to chlorhexidine, CPC has fewer residual effects, but as a result its effect against plaque and gingivitis is weaker.

The official US pharmacopoeia accepts benzalkonium chloride as an auxiliary antimicrobial agent [6]. It is the major antimicrobial agent in numerous toothpastes and mouthrinses [11].

Triclosan and listerine had been used in antiseptic treatment of skin for years. As oral antiseptics they have been used since the late eighties [2]. Numerous analyses have been made on the efficacy of listerine for inhibition of both cariogenic and overall viable oral flora, both from the plaque and the saliva [8, 10, 11, 15, 18].

The glass ionomer cements distinguish themselves as the most acceptable restorative materials possessing the positive characteristics of fluorine in the processes of remineralisation and antimicrobial action. Glass ionomer cements have been used in restorative dentistry for thirty years. They appeared as a result of the early studies of Alan Wilson and Brian Kent in the Laboratory of the Government Chemist in London by the last century's late sixties [24]. Commercial dental cements of this type were launched in 1975, though they then had quite inferior characteristics compared with materials being used today [23, 24]. The original glass ionomer cements are composed of a water solution of poly(acrylic)acid in a concentration of about 45% which enters into a reaction with a powder composed of calcium fluoraluminosilicate glass. This glass is of the same generic type that was used in old dental silicate cements [4, 21, 23, 24, 30].

Glass ionomer cements are used most often for restorations of non-retentive and cervical cavities, and of cavities in milk teeth. The use in these cases is determined by their characteristic of releasing fluorine [3, 5, 13, 33, 34] and of participating in the mechanism of inhibition at the development of secondary caries [4, 21, 23, 24]. At the same time they also act on the surrounding bacteria by reducing the cariogenic microorganisms [32, 16].

In addition to the release of fluoride ions, GICs can potentially be used as templates for the release of other active antimicrobial components.

In order to improve the antimicrobial characteristics of both conventional and resin-modified GICs, antimicrobial compounds such as chlorhexidine have been added.

Chlorhexidine has been described as a golden standard for antimicrobial application [19]. The incorporation of chlorhexidine dihydrochloride and chlorhexidine diacetate in glass ionomer cements has the ability to increase the antimicrobial effects of the glass ionomers without seriously disrupting their physical characteristics [17, 28].

Several studies describe the measurement of the quantity of CHX released from GICs as a function of time. Ribeiro and Ericson [28] acknowledge only two concentrations (13.3 and 6.65%) incorporated into a commercial luting cement (Aquacem) [25].

Chlorhexidine has been added in concentrations from 0.5% to 13% of the weight of the GICs. The whole measurable chlorhexidine was released within 22½ hours, although this was less then 10% of the total mass incorporated in the samples. The percental increase of CHA incorporated in the powder also yields an increased release into the neighbourhood. The largest part of CHA stays tied in the cement [25].

Unfortunately, in the literature there is very little data referring to the incorporation of other antimicrobial components in GICs. Although some of them have a confirmed effect in the reduction of cariogenic salivary flora when used in mouthrinses or toothpastes, [2, 6, 8, 9, 10, 11, 15, 18, 22, 26, 27] results regarding their incorporation in glass ionomer cements are still scarce.

The experiment through which a comparative analysis has been made of the antimicrobial effects of various antimicrobial agents added to conventional glass ionomer cement – Fuji IX, is interesting. Besides chlorhexidine hydrochloride, cetylpyridinium chloride, cetrimide and benzalkonium chloride were added in concentrations of 0, 1, 2, and 4% of the weight. The antimicrobial samples of GICs showed significant inhibition which declined in various time periods during the research. The addition of antimicrobial agents to Fuji IX creates a GIC material with a significant *in vitro* antimicrobial activity which depends on the concentration and type of the antimicrobial agent, and is mainly correlated to its release from the surface layer of the samples [7].

Considering that microorganisms are the main cause of the occurrence of dental caries, that glass ionomer cements are materials of choice mainly in pediatric dentistry, and that they can potentially be used as a medium for slow release of active antimicrobial components, we have defined the null hypothesis – that no differences appear before and after incorporation of antimicrobial compounds into glass ionomer cements, all the characteristics of glass ionomer cements remain preserved and satisfactory.

We tested the null hypothesis by:

– Incorporating various concentrations of an antimicrobial compound into a commercial glass ionomer cement;

– Determining the effects of incorporated antimicrobial compound on some of the physical characteristics of the commercial glass-ionomer cement through measurement of a working time

– Measuring the quantity of released antimicrobial component with different concentrations, from the commercial glass ionomer cement through measurement of the level of released ions (chloride) in various time periods;

– Determining the level of release of fluoride ions from commercial glass ionomer cements in different time periods.

Material and method

The commercially available glass-ionomer cement that was used in the analyses was ChemFlex, a material having been widely applied in restorative dentistry. The composition of this glass-ionomer cement is shown in Table 1.

Table 1 – Табела 1

Material used in research
Материјал употребуван во испитувањата

Material	Classification	Composition	Manufacturer
ChemFlex	*Conventional glass-ionomer cement*	Strontium, aluminium, fluoride, silicate, tartaric acid, pigments, polyakrylic acid	DENTSPLY DeTrey, Konstanz, Germany

The antimicrobial compound used was Benzalkonium Chloride. The compound was procured from the factory in a powdery crystal-like aggregate state.

Five samples of the commercial ChemFlex glass-ionomer with no antimicrobial compound added were prepared – to serve as a control group; and another three groups of 5 specimens of the same cement were also prepared, but each with a different concentration of benzalkonium chloride added to it – 1%, 2% and 3%, respectively – a total of 15 samples.

Samples with no antimicrobial agent incorporated were prepared by mixing a certain amount of powder and liquid on glass mixing plates by means of a metal trowel (according to the manufacturers' instructions). The freshly mixed paste was then put into 6 mm high metal moulds of 4 mm diameter. The moulds were closed by metal plates on both sides and were then placed in special clamps. Then the specimens were placed into an incubator at 37^{o}C for one hour.

The benzalkonium chloride antimicrobial compound was first incorporated into the glass-ionomer cement's polyacrylic acid by mixing, and then the powder was gradually added, quantity by quantity, into the previously prepared acid and antimicrobial compound mixture, while care was taken to mix them together until complete saturation. The antimicrobial agent was added in strict portions of 1, 2 and 3% of the weight of the cement, the concentrations of 1, 2 and 3% being equivalent to 0.0022 g, 0.0044 g and 0.0066 g, respectively. The necessary weights of Benzalkonium Chloride were measured using an analytical scale having an inaccuracy less than 0.0001 g.

Determination of the working time was done using a Gilmore needle. The Gilmore needle used to determine the working time had a weight of 28 g. The following procedure was used. After the powder and the liquid without and with the established concentrations of antimicrobial compound have been mixed, and after the mixture has been placed in rubber moulds, a Gilmore needle is placed on the surface of the cement every 15 seconds. When placed on the cement's surface, the needle makes an impression. The surface is inspected for any impressions and the time is measured as long as the needle's tip makes impresssions. The first time the needle fails to make an impression on the material's surface stops the measurement of the time, and thus determines the cement's working time.

The quantities of released chloride and fluoride ions were determined at various time instants as follows: immediately, after 30 minutes, 45 minutes, and after 1, 2, 3, 4 and 24 hours.

The quantity of released F^- was determined by using an ion-selective electrode for fluoride. The instrument used for the determination of fluoride was a DIGITAL pH/mV and Temperature meter MODEL 7065, Electronic Instruments Limited, Kent, UK. The electrode was previously calibrated by standards with molarity within the range of the concentrations of fluoride that were to be measured (0.1, 1.0, 10.0, 100.0 and 1000.0 ppm). For the determination of the quantity of chloride an ion-selective chloride specific electrode and an ORION 4 star pH.ISE Benchtop instrument, Thermo Electron Corporation USA, were used. Following the calibration of the electrodes calibration curves were prepared. Bearing in mind that chlorine is the main ingredient of benzalkonium chloride and is at the same time the only relatively easily measurable element with this technique, it was considered that the quantity of released chlorine was equivalent to the quantity of released antimicrobial component. The release of fluoride and chloride ions was conducted in deionized water. Ten special glass vials were used, each containing 5 ml of deionisated water. The glass-ionomer cement samples having the aforementioned concentration of benzalkonium chloride were placed in five of these vials, whereas the other five vials were used for the placement of samples with no antimicrobial component. Following this, the electrodes were immersed into the vials sequentially – to determine the chloride ions and then the fluoride ions. The results thus obtained were expressed in millivolts and entered into tables for each of the time intervals measured, and were subsequently, through separate formulas, converted into concentrations of antimicrobial agent expressed in ppm in order to determine the real concentration of the ions in the water.

All chemical analyses were conducted at the School of Chemical and Life Science, University of Greenwich, Chatham Maritime, Chatham, Kent, UK.

Results

Determination of the working time

This was done using a Gilmore needle. One representative sample was taken from each of the investigated groups with different concentrations of benzalkonium chloride. The working time of the glass-ionomer cement with no antimicrobial agent added had been previously measured. The results of the analyses are shown in tables.

Table 2 – Табела 2

Working time
Време на рабо̄та

Without	5’
1%	5’
2%	5’10’’
3%	5’10’’

Determination of sample weights

The weights of the samples were determined with an analytical scale before and after 24 hours, i.e. after the investigation, separately for each concentration. The average values of the samples with, (A-E), and the samples without, (F-J), the antimicrobial agent are shown.

The results are shown in the tables.

Table 3 – Табела 3

Sample weights – 1% benzalkonium chloride – average values
Тежина на ӣримероци – 1% benzalkonium chloride – средни вредносӣи

	A - E	F - J
0’	0.13828	0.1455
24h	0.14414	0.14956

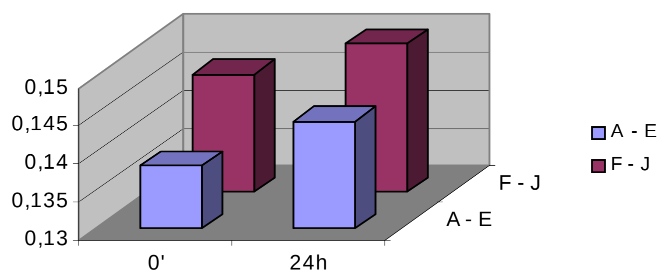

Diagram 1 – Sample weights – 1% benzalkonium chloride – average values
Графикон 1 – Тежина на ӣримероци – 1% benzalkonium chloride – средни вредносӣи

Table 4 – Табела

Sample weights – 2% benzalkonium chloride – average values
Тежина на примероци – 2% benzalkonium chloride – средни вредности

	A - E	F - J
0’	0.13696	0.14812
24h	0.14482	0.15222

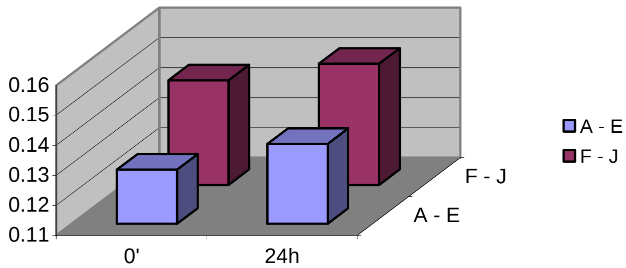

Diagram 2 – Sample weights – 2% benzalkonium chloride – average values
Графикон 2 – Тежина на примероци – 2% benzalkonium chloride – средни вредности

Table 5 – Табела 5

Sample weights – 3% benzalkonium chloride – average values
Тежина на примероци – 3% benzalkonium chloride – средни вредности

	A - E	F - J
0’	0.12802	0.14486
24h	0.13662	0.15038

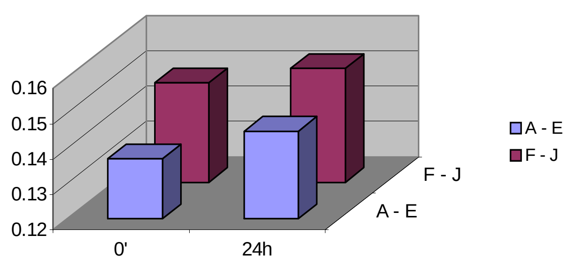

Diagram 3 – Sample weights – 3% benzalkonium chloride – average values
Графикон 3 – Тежина на примероци – 3% benzalkonium chloride – средни вредности

Determination of the levels of released ions

The measured values had been originally displayed in millivolts and were subsequently converted, using separate formulas for each of the elements, into concentrations expressed in parts per million. For the results obtained in millivolts, the following parameters were determined: mean, maximum, minimum and standard deviation. Then formulas derived from the previously obtained calibration curves for chlorine and fluorine were used to determine the ion concentrations in ppm. The results are shown in summary tables separately for each of the ions analyzed and each of the concentrations used over the time intervals of interest. The tables show both the values obtained with and without antimicrobial agent added.

Table 6 – Табела 6

Determination of Cl^- in ChemFlex without and with the addition of 1% Benzalkonium chloride – concentration/ppm
Одредување на Cl^- во ChemFlex без и со додаток на 1% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				1% benzalkonium chloride			
	$\bar{x}$	Min	Max	σ	$\bar{x}$	Min	Max	σ
0’	0.15	0.15	0.15	0	0.24	0.18	0.29	0.04
15’	12.52	1.7	47.55	19.69	27.68	5.16	66.67	25.91
30’	14.65	2.38	54.96	22.64	36.68	5.16	102.94	39.72
45’	16.05	2.5	57.68	23.39	48.99	5.68	144.32	55.97
1h	18.84	3.86	60.53	23.68	60.37	8.36	192.8	75.78
2h	19.03	3.51	63.52	25.08	87.64	7.59	312.43	127.27
3h	19.28	4.05	63.52	24.96	99.6	9.21	361.12	147.77
4h	20.09	5.16	63.52	24.53	141.06	11.17	557.61	233.93
24h	21.32	5.68	63.52	24.04	147.65	14.22	557.61	231.24

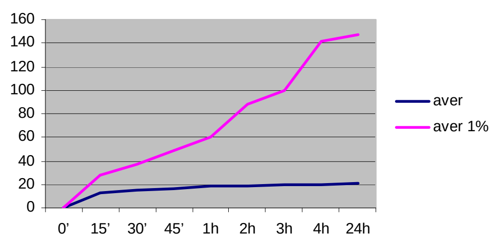

Diagram 4 – Average value of Cl^- in ChemFlex without and with the addition of 1% Benzalkonium chloride – concentration/ppm
Графикон 4 – Средна вредност на Cl^- во ChemFlex без и со додаток на 1% Benzalkonium chloride – концентрација/ppm

Table 7 – Табела 7

Determination of Cl^- in ChemFlex without and with the addition of 2% Benzalkonium chloride – concentration/ppm
Одредување на Cl^- во ChemFlex без и со додаток на 2% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				2% benzalkonium chloride			
	$\overline{x}$	Min	Max	σ	$\overline{x}$	Min	Max	σ
0’	0.17	0.16	0.18	0.01	0.42	0.3	0.79	0.21
15’	0.37	0.27	0.48	0.08	4.62	1.78	14.22	5.38
30’	0.54	0.44	0.68	0.1	9.22	2.76	30.79	12.09
45’	0.73	0.56	0.82	0.1	14.64	3.86	52.37	21.14
1h	0.94	0.87	1	0.06	18.01	4.92	63.52	25.51
2h	1.26	1.05	1.87	0.35	23.94	6.57	84.86	34.13
3h	1.56	1.21	2.5	0.54	31.87	8.78	113.37	45.65
4h	1.89	1.4	3.04	0.65	37.37	9.67	131.04	52.58
24h	2.36	1.78	3.34	0.66	66.79	14.93	245.44	100.26

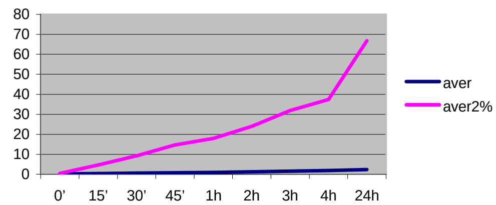

Diagram 5 – Average value of Cl^- in ChemFlex without and with the addition of 2% Benzalkonium chloride – concentration/ppm
Графикон 5 – Средна вредност на Cl^- во ChemFlex без и со додаток на 2% Benzalkonium chloride – концентрација/ppm

Table 8 – Табела 8

Determination of Cl^- in ChemFlex without and with the addition of 3% Benzalkonium chloride – concentration/ppm
Одредување на Cl^- во ChemFlex без и со додаток на 3% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				3% benzalkonium chloride			
	$\overline{x}$	Min	Max	σ	$\overline{x}$	Min	Max	σ
0’	0.2	0.18	0.21	0.01	0.4	0.4	0.42	0.01
15’	0.43	0.3	0.65	0.15	4.89	1.78	15.67	6.06
30’	0.71	0.48	1.16	0.26	14.63	2.89	45.31	17.5
45’	1.22	0.71	2.89	0.93	66.93	4.69	297.71	129.09
1h	1.25	0.82	2.38	0.64	70.55	6.26	312.43	135.32
2h	1.56	0.95	3.19	0.92	82.27	7.97	361.12	155.99
3h	1.9	1.27	3.86	1.1	91.36	9.21	397.72	171.41
4h	2.42	1.78	4.69	1.27	101.53	10.65	438.04	188.32
24h	3.33	2.06	7.24	2.21	165.27	16.44	709.83	304.79

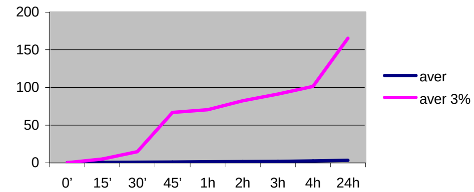

Diagram 6 – Average value of Cl^- in ChemFlex without and with the addition of 3% Benzalkonium chloride – concentration/ppm
Графикон 6 – Средна вредност на Cl^- во ChemFlex без и со додаток на 3% Benzalkonium chloride – концентрација/ppm

Table 9 – Табела 9

Determination of F^- in ChemFlex without and with the addition of 1% Benzalkonium chloride – concentration/ppm
Одредување на F^- во ChemFlex без и со додаток на 1% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				1% benzalkonium chloride			
	$\overline{x}$	Min	Max	σ	$\overline{x}$	Min	Max	σ
0'	0.24	0.17	0.28	0.04	0.14	0.12	0.16	0.02
15'	0.33	0.28	0.39	0.04	0.29	0.26	0.32	0.03
30'	0.41	0.36	0.5	0.05	0.38	0.33	0.46	0.05
45'	0.49	0.41	0.59	0.07	0.48	0.39	0.67	0.11
1h	0.75	0.64	0.93	0.12	0.79	0.62	1.06	0.18
2h	1.01	0.86	1.24	0.15	1.03	0.86	1.35	0.2
3h	1.22	1.01	1.47	0.18	1.15	0.93	1.41	0.18
4h	1.35	1.15	1.59	0.17	1.31	1.06	1.66	0.23
24h	3.04	2.62	3.21	0.26	2.87	2.62	2.96	0.15

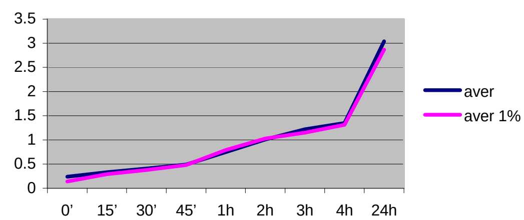

Diagram 7 – Average value of F^- in ChemFlex without and with an addition of 1% Benzalkonium chloride – concentration/ppm
Графикон 7 – Средна вредност на F^- во ChemFlex без и со додаток на 1% Benzalkonium chloride – концентрација/ppm

Table 10 – Табела 10

Determination of F^- in ChemFlex without and with the addition of 2% Benzalconium chloride – concentration/ppm
Одредување на F^- во ChemFlex без и со додаток на 2% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				2% benzalkonium chloride			
	$\overline{x}$	Min	Max	σ	$\overline{x}$	Min	Max	σ
0’	0.15	0.13	0.19	0.02	0.14	0.12	0.16	0.02
15’	0.41	0.31	0.52	0.11	0.43	0.41	0.44	0.02
30’	0.51	0.38	0.67	0.15	0.5	0.44	0.57	0.05
45’	0.6	0.48	0.73	0.12	0.59	0.55	0.64	0.04
1h	0.66	0.55	0.82	0.15	0.66	0.59	0.76	0.07
2h	1.02	0.82	1.3	0.23	0.99	0.89	1.15	0.11
3h	1.27	1.01	1.59	0.3	1.22	1.1	1.41	0.13
4h	1.48	1.24	1.8	0.3	1.44	1.3	1.66	0.17
24h	3.42	2.96	4.12	0.57	3.15	2.84	3.49	0.33

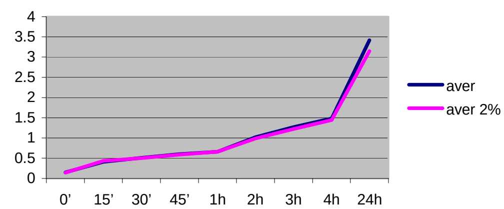

Diagram 8 – Average value of F^- in ChemFlex without and with an addition of 2% Benzalkonium chloride – concentration/ppm
Графикон 8 – Средна вредност на F^- во ChemFlex без и со додаток на 2% Benzalkonium chloride – концентрација/ppm

Table 11 – Табела 11

Determination of F^- in ChemFlex without and with the addition of 3% Benzalkonium chloride – concentration/ppm
Одредување на F^- во ChemFlex без и со додаток на 3% Benzalkonium chloride – концентрација/ppm

	Without benzalkonium chloride				3% benzalkonium chloride			
	$\overline{x}$	Min	Max	σ	$\overline{x}$	Min	Max	σ
0’	0.16	0.15	0.17	0.01	0.13	0.12	0.15	0.02
15’	0.37	0.32	0.39	0.03	0.31	0.29	0.36	0.03
30’	0.54	0.46	0.59	0.07	0.41	0.38	0.44	0.03
45’	0.57	0.52	0.62	0.05	0.5	0.44	0.57	0.05
1h	0.72	0.64	0.82	0.07	0.62	0.55	0.7	0.06
2h	1.02	0.93	1.15	0.1	0.89	0.79	0.93	0.06
3h	1.31	1.24	1.41	0.07	1.11	0.97	1.15	0.08
4h	1.53	1.47	1.66	0.09	1.37	1.19	1.41	0.1
24h	3.41	3.21	3.64	0.16	3.01	2.73	3.21	0.18

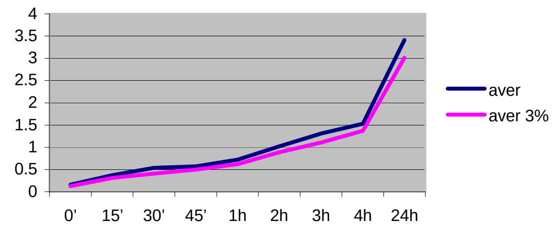

Diagram 9 – Average value of F^- in ChemFlex without and with an addition of 3% Benzalkonium chloride – concentration/ppm
Графикон 9 – Средна вредност на F^- во ChemFlex без и со додаток на 3% Benzalkonium chloride – концентрација/ppm

Table 12 – Табела 12

Student t-test for Cl^-
Студентов t-тест за Cl^-

1% benzalkonium chloride				2% benzalkonium chloride				3% benzalkonium chloride			
mV		ppm		mV		ppm		mV		ppm	
|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*
7.05	< 0.001	6.01	< 0.001	4.54	< 0.01	2.66	p < 0.03	20.93	< 0.001	27.58	< 0.001
1.28	> 0.2	1.02	> 0.2	5.52	< 0.001	1.76	p < 0.2	4.59	< 0.01	1.64	< 0.2
1.48	< 0.2	1.07	> 0.2	5.35	< 0.001	1.60	p < 0.2	5.23	< 0.001	1.77	< 0.2
1.54	< 0.2	1.21	> 0.2	4.94	< 0.01	1.47	p < 0.2	3.49	< 0.01	1.13	> 0.2
1.51	< 0.2	1.16	> 0.2	5.04	< 0.01	1.49	p < 0.2	3.62	< 0.01	1.14	> 0.2
1.59	< 0.2	1.18	> 0.2	4.94	< 0.01	1.48	p < 0.2	3.74	< 0.01	1.15	> 0.2
1.72	< 0.2	1.19	> 0.2	5.01	< 0.01	1.48	p < 0.2	3.72	< 0.01	1.16	> 0.2
1.77	< 0.2	1.14	> 0.2	4.89	< 0.01	1.50	p < 0.2	3.64	< 0.01	1.17	> 0.2
1.90	< 0.1	1.21	> 0.2	5.05	< 0.001	1.43	p < 0.2	3.89	< 0.01	1.18	> 0.2

Table 13 – Табела 13

Student t-test for F^-
Студентов t-тест за F^-

1% benzalkonium chloride				2% benzalkonium chloride				3% benzalkonium chloride			
mV		ppm		mV		ppm		mV		ppm	
|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*	|*t*|	*p*
5.19	< 0.001	4.98	< 0.01	0.85	> 0.2	0.79	> 0.2	3.51	< 0.01	3.59	< 0.01
1.81	< 0.2	1.78	< 0.2	0.69	> 0.2	0.47	> 0.2	2.76	< 0.03	2.72	< 0.03
0.92	> 0.2	0.88	> 0.2	0.06	> 0.2	0.14	> 0.2	4.08	< 0.01	3.81	< 0.01
0.28	> 0.2	0.16	> 0.2	0.09	> 0.2	0.23	> 0.2	2.23	< 0.1	2.23	< 0.1
0.28	> 0.2	0.35	> 0.2	0.15	> 0.2	0.00	> 0.2	2.44	< 0.04	2.40	< 0.05
0.07	> 0.2	0.13	> 0.2	0.15	> 0.2	0.27	> 0.2	2.49	< 0.04	2.46	< 0.04
0.68	> 0.2	0.66	> 0.2	0.21	> 0.2	0.35	> 0.2	4.04	< 0.01	4.20	< 0.01
0.34	> 0.2	0.28	> 0.2	0.16	> 0.2	0.25	> 0.2	2.74	< 0.04	2.81	< 0.04
1.22	> 0.2	1.30	> 0.2	0.85	> 0.2	0.90	> 0.2	3.53	< 0.01	3.60	< 0.01

Discussion

The aim of this study was to perceive the possibility of incorporating an antimicrobial agent in a glass-ionomer cement. A very small number of studies dealing with this field could be found in the literature, so the results obtained here could be considered as pioneering attempts on the topic. There have been attempts to incorporate chlorhexidine in glass-ionomer cements with certain results, but the results referred mainly to the effect of the newly obtained cements on the bacterial oral cariogenic flora, and not to the precise analysis of the physical characteristics of the newly obtained cements and the concentration of ions released in the medium.

In order to determine the amount of ions (Cl^- and F^-) released in the medium (deionized water) containing the samples, measurements were carried out with chloride and fluoride specific ion-selective electrodes over various time intervals. The analysis of the release of chloride ions was conducted in order to see whether or not and in what concentration chloride ions are released from benzalkonium chloride, and thus to conclude on the possibility of the glass-ionomer cement having an antimicrobial effect. The common characteristic for all three percentages of benzalkonium chloride is that there is a difference in the concentration of chloride ions in the medium (deionized water) between the samples with and the samples without the addition of an antimicrobial agent.

In the samples with the addition of 1% of benzalkonium chloride the concentration of Cl^- ions rises drastically over the whole 24 hours long measurement interval; doubling its value in the first 15 minutes, and reaching a sevenfold value after 24 hours. In order to see whether the difference is statistically significant Student t-test was used. The results of the t-test for the values expressed in millivolts demonstrate that the difference between the average values of the samples without and with benzalkonium chloride are statistically significant for $p < 0.2$ at all intervals of measurement, with the exception of the measurements after 15 minutes where there is no significance, and for the values measured at time zero where the level of significance is for $p < 0.001$. Contrary to these results, the t-test applied on the concentrations expressed in parts per million shows significance only for the measurement at time zero ($p < 0.001$).

Values obtained with the statistical data processing in the case of 2% of benzalkonium chloride are interesting, because they exhibit statistical signifycance both for the measured values expressed in millivolts and for those calculated in parts per million, but with different levels of significance. The analysis of the results in mV exhibits a level of significance for values between $p < 0.01$ and $p < 0.001$ for all measurements (time intervals), in contrast to the results expressed in ppm, where there is a statistical significance, but for $p < 0.2$.

In the case of the statistical data processing for 3% incorporated benzalkonium chloride the results of the processing of 2% repeat, i.e. the level of significance for mV is for values between $p < 0.01$ and $p < 0.001$, whereas for the concentrations the difference is significant only for the first three measurements (0': $p < 0.01$, 15': $p < 0.2$ and 30': $p < 0.2$).

The question that should now be answered is why the statistical data we obtained demonstrate a huge difference in the release of chloride ions from samples without and with the addition of antimicrobial agent. One of the possible causes is most probably due to the instance of calibration of the chloride specific electrode that results with a mathematical equation for the conversion of milivolts to a concentration which is quite approximate. This can also be seen from the large standard deviations. Because the calibration curve is obtained by connecting the points of *de facto* measured milivolts (on the y-axis) and the points obtained from the previous dissolving (titration) of the solution for calibration (NaCl) (on the x-axis), which is nevertheless done manually, possibilities of the occurrence of errors are probable. That is why the statistical data processing conducting for the values in mV, which are in essence the data generated by the apparatus performing the measurements, are perhaps more valid than those obtained with the analysis of the results of the concentrations, i.e. the ppm-data which are obtained through an approximate mathematical equation derived from a perhaps not quite proper calibration curve.

As regards the statistical processing of the data regarding the release of fluoride ions, the results regarding the significance are almost identical for both the millivolts and for the concentration. Namely, the electrode that was used for the measurements of the F^- ions had been previously factory calibrated and was therefore far more accurate. That is why there are no differences (or they are minimal) regarding the levels of significance for the millivolts and for the ppms, which allows us in the discussion of the results to use the statistical data obtainned from the results expressed in concentration of ions, i.e. in ppms. In the determination of F^- for 1% benzalkonium chloride, except at the time instants 0' and 15', where there is a statistical significance (for $p < 0.01$, i.e. $p < 0.2$), at all other time instants there is no such significance. The differences in the levels of fluoride ions without and with 2% benzalkonium chloride exhibit no statistical significance, while the levels of significance in the case of 3% benzalkonium chloride are in the range from $p < 0.1$ to $p < 0.01$. From the obtained results and from their statistical processing we can conclude that concentrations of 1% and 2% of benzalkonium chloride have no impact on the release of fluoride ions in the medium (deionized water), whereas, on the other hand, the differences in the measurements for 3% of benzalkonium chloride are, although small, yet statistically significant.

The manner of incorporating the crystals of benzalkonium chloride, which was done manually by mixing the antimicrobial component with polyacrylic acid, and then also with the powdery part of the GIC, could also be the cause of errors in the values obtained. During this procedure a nonuniform distribution of the antimicrobial agent within the GIC probably occurs. In the samples with a higher concentration of the antimicrobial agent on the surface of the sample, the concentration of released chloride ions would be higher and vice versa. However, the fact that there is a release of the antimicrobial component, along with the increase of the released component in the medium over time, is a sufficient reason to conclude that the incorporation of benzalkonium chloride is possible.

Therefore, the null hypothesis that no differences appear between the samples with or without addition of the antimicrobial component, has to be partially rejected. – differences appear, but, obtaining a glass-ionomer cement with incorporated antimicrobial agents which have effects on the bacterial oral flora, and which at the same time does not lose the characteristic properties of conventional cements, will be a subject of our further comprehensive analyses, both chemical and microbiological.

REFERENCES

1. Adams D., Addy M. (1994): Mouthrinses. *Adv Dent Res;* 8(2): 291–301.

2. Anti-microbial agents and treatments with special reference to dental caries. International Health Care Foundation. *General Reviews*. Department of Cariology, Faculty of Odontology, Malmö University, Sweden, 1999.

3. Attar N., Onen A. (2002): Fluoride release and uptake characteristics of aestetic restorative materials *J Oral Rehab;* 29: 791–798.

4. Berg J.H. (1998): The continuum of restorative materials in pediatric dentistry – a review for the clinician *Pediatric Dentistry;* 20(2): 93–100.

5. Billington R.W., Williams J.A., Dorban A., Pearson G.J. (2004): Glass ionomer cement: evidence pointing to fluorine release in the form of monofluorophosphate in addition to fluoride ion. *Biomaterials;* 25: 3399–3402.

6. Block, SS. (1991): Disinfection, Sterilization and Preservation. Fourth Edition. Philadelphia – London: Lea & Febiger.

7. Botelho MG. (2003): Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in a glass ionomer cements. *Caries Research;* 37: 108–114.

8. Charles CH., Pan PC., Sturdivant L., Vincent JW. (2000): In vivo antimicrobial activity of an essential oil-containing mouthrinse on interproximal plaque bacteria. *J Clin Dent;* 11(4): 94–7.

9. Charles CH., Sharma NC., Galustians HJ., Qaqish J., McGuire JA., Vincent JW. (2001): Comparative efficacy of an antiseptic mouthrinse and an antiplaque/antigingivitis dentifrice. A six-month clinical trial. *J Am Dent Assoc;* 132(5): 670–5.

10. Ciancio S. (1994): Expanded and future uses of mouthrinses. *J Am Dent Assoc;* 125 Suppl 2: 29S–32S.

11. DePaola LG., Minah GE., Overholser CD., Meiller TF., Charles CH., Harper DS., McAlary M. (1996): Effect of an antiseptic mouthrinse on salivary microbiota. *Am J Dent;* 9(3): 93–5.

12. Dimkov A. Mikrobioloski aspekti na hemiski i mehanicki sredstva za oralna higiena kaj skolski deca Magisterski trud 2003.

13. Dionysopoulos P., Kotsanos N., Pataridou A. (2003): Fluoride release and uptake by four new fluoride releasing restorative materials *J Oral Rehab;* 30(9): 866–872.

14. Featherstone JDB. **(**2000): The science and practice of caries prevention. *J Am Dent Assoc*.

15. Fine DH., Furgang D., Lieb R., Korik I., Vincent JW., Barnett ML. (1996): Effects of sublethal exposure to an antiseptic mouthrinse on representative plaque bacteria. *J Clin Periodontol;* 23(5): 444–51.

16. Hengtrakool C., Pearson G.J., Wilson M. (2006): Interaction bettween GIC and S.sanguis biofilms: Antibacterial properties and changes of surface hardness *J Dentistry;* 34: 588–597.

17. Jedrychowski JR., Caputo AA., Kerper S. **(**1983): Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. *J Oral Rehabil;* 10(5): 373–81.

18. Kubert D., Rubin M., Barnett ML., Vincent JW. (1993): Antiseptic mouthrinse – induced microbial cell surface alterations. *Am J Dent;* 6(6): 277–9.

19. Leunga D., Spratt D.A., Pratten J., Gulabivala K., Mordan N.J., Young M.N. **(**2005): Chlorhexidine-releasing methacrylate dental composite materials. *Biomaterials;* 26: 7145–7153.

20. Marsh PD. (1993): Antimicrobial strategies in the prevention of dental caries. *Caries Res;* 27 Suppl 1: 72–6

21. McLean J.W., Nicholson J.W., Wilson A.D. (1994): Proposed nomenclature for glass-ionomer dental cements and related materials. *Quintessence Int;* 25(9): 587–589.

22. Netuschil L., Weiger R., Preisler R., Brecx M. (1995): Plaque bacteria counts and vitality during chlorhexidine, Meridol and Listerine mouthrinses. *Eur J Oral Sci;* 103(6): 355–61.

23. Nicholson J.W. (2000): Adhesive dental materials and their durability. *Int J Adhesion Adhesives;* 20: 11–16.

24. Nicholson J.W. (1998): Chemistry of glass-ionomer cements: a review. *Biomaterials;* 19: 485–494.

25. Palmer G., Jones FH., Billington RW., Pearson GJ. **(**2004): Chlorhexidine release from an experimental glass ionomer cement. *Biomaterials;* 25: 5423–5431.

26. Pitten FA., Kramer A. **(**2001): Efficacy of cetylpyridinium chloride used as oropharingeal antiseptic. *Arzneimittelforschung;* 51(7): 588–95.

27. Radford JR., Beighton D., Nugent Z., Jackson RJ. (1997): Effect of use of 0.05% cetylpyridinium chloride mouthwash on normal oral flora. *J Dent;* 25(1): 35–40.

28. Ribeiro J., Ericson D. **(**1991): In vitro antibacterial effect of chlorhexidine added to glass-ionomer cements. *Scand J Dent Res;* 99(6): 533–40.

29. Sanders B.J., Gregory R.L., Moore K., Avery D.R. (2002): Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine *J Oral Rehab;* 29: 553–558.

30. Shiu-yin Cho., Ansgar C.C. (1999): A review of glass-ionomer restorations in the primary dentition. *J Can Dent Assoc;* 65: 491–5.

31. Takahashi Y., Imazato S., Kaneshiro AV., Ebisu S., Frencken JE., Tay FR. (2006): Antibacterial effects and physical properties of glass-ionomer cements containning chlorhexidine for the ART approach. *Dent Mater;* 22(7): 647–52.

32. Vermeersch G., Leloup G., Delmee M., Vreven J. (2005): Antibacterial activity of glass-ionomer cements, compomers and resin composites: relationship between acidity and material setting phase. *J Oral Rehab;* 32: 368–374.

33. Williams J.A., Billington R.W., Pearson G.J. (1999): Comparison of ion release from glass ionomer cement as a function of the method of incorporation of added ions. *Biomaterials;* 20: 589–594.

34. Williams J.A., Briggs E., Billington R.W., Pearson G.J. (2003): The effects of adding fluoride compounds to a fluoride-free glass-ionomer cement on a subsequent fluoride and sodium release. *Biomaterials;* 24: 1301–1308.

35. Yoshihara A., Sakuma S., Kobayashi S., Miyazaki H. (2001): Antimicrobial effect of fluoride mouthrinse on mutans streptococci and lactobacilli in saliva. *Pediatr Dent;* 23(2): 113–7.

36. Young A. D. (2006): Treating Caries Chemically: Fact and Fiction ADA Continuing Education Recognition Program.

Резиме

МОЖНОСТИ ЗА ИНКОРПОРИРАЊЕ АНТИМИКРОБНИ КОМПОНЕНТИ ВО ГЛАС-ЈОНОМЕР ЦЕМЕНТИ

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Покрај ослободувањето на флуоридните јони, ГЈЦ-и потенцијално можат да бидат искористени како матрици за ослободување на други активни антимикробни состојки. Целта на оваа студија беше да ја согледаме можноста од инкорпорација на антимикробно соединение во глас-јономер цемент, како и влијанието од неговото вградување врз физичките карактеристики на самиот цемент. За реализација на поставената цел го користевме комерцијално достапниот глас-јономер цемент ChemFlex, материјал кој наоѓа широка примена во реставративната стоматологија. Беа изготвени по 5 примероци од комерцијалниот глас-јономер цемент ChemFlex без додавање на антимикробно соединение – контролна група; по пет примерока од истиот овој цемент, но со додавање на различни концентрации на – Benzalkonium Chloride – 1%, 2% и 3% – вкупно 15 примероци. Беа мерени времето на работа, разликата во тежината на примероците пред и по завршувањето на анализите и концентрацијата на испуштени јони – флуориди и хлор во одредени временски интервали. Резултатите од испитувањата на времето на работа на овој цемент без вградена антимикробна компонента е исто со она дадено од производителот. Вградениот 1% бензалкониум хлорид не влијае врз времето на работа, односно времето на работа е идентично со комерцијалниот ГЈЦ. Минималните отстапувања кои се јавуваат кај 2%, односно 3% се во границите на нормалата и не се сметаат за значајна разлика. Мерењата на тежината на примероците со и без инкорпориран бензалкониум хлорид пред и по 24 часа покажуваат дека антимикробната компонента не влијае врз процесот на примање на течност. За да ја одредиме количината на ослободените јони (Cl^- и F^-) во медиумот (дејонизирана вода) во кои беа поставени примероците, извршивме мерења со јон-селективни електроди специфични за хлор и флуор во тек на различни временски интервали. Анализата на ослободувањето на хлор ја спроведовме со цел да се види дали и во која концентрација доаѓа до испуштање на хлорот како дел од бензалкониум хлоридот, а аналогно на тоа и на можноста од антимикробно дејство на самиот глас-јономер цемент. Она што е заедничко за секоја од трите процентуелни застапености на бензалкониум хлоридот е дека постои разлика во концентрацијата на хлоридните јони од примероците со и без додаток на антимикробното средство.

Клучни зборови: глас-јономер цементи, benzalkonium chloride, јон-селективни електроди, флуор, хлор, антимикробни соединенија.

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