ORIGINAL PAPER

NEW FINDINGS ABOUT RELEASING OF CHLORIDE IONS AND QUATERNARY AMMONIUM COMPOUNDS FROM CONVENTIONAL AND EXPERIMENTAL GLASS IONOMERS

Aleksandar Dimkov[1], **Elizabeta Gjorgievska**[1], **Jasna Simonoska**[2]

[1]Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, Saints Cyril and Methodius University of Skopje, North Macedonia
[2]Department of Pediatric and Preventive Dentistry, Dental Clinic Center St. Panteleimon, Skopje, North Macedonia

ABSTRACT

Introduction: Glass ionomer cements (GICs), the most frequently used restorative materials in pediatric dentistry, may be used as medium for slow release of other active anti-microbial components apart from fluoride.

Objectives: The objectives of this study were to determine the level of released chloride ions and anti-microbial compounds by incorporating 2% anti-microbial agents into conventional and experimental GICs.

Material and methods: The study was carried out using a conventional glass ionomer cements ChemFlex and Fuji IX as well as experimental glass ionomer cement MP4 and anti-microbial compounds, such as cetylpyridinium chloride and benzalkonium chloride. A total of 36 specimens (4 mm × 6 mm) were prepared, including 6 specimens of each GIC integrated with 2% of anti-microbial agents, and other six samples of the same cement without any anti-microbial agents used as a control group. Amounts of released Cl– ions were determined with the use of an ion-selective electrode, and amounts of anti-microbial agents were determined by UV/VIS spectrophotometer. Measurements were performed at 9 successive time intervals starting from fifteenth minute and finishing after seven days.

Results: The results obtained show a continual release of both chloride ions and anti-microbial compounds from analyzed GICs that increased over time.

Conclusions: In comparison with conventional GICs, MP4 GIC produces a significantly higher release both of chloride ions and anti-microbial compounds.

Key words: benzalkonium chloride, cetylpyridinium chloride, anti-microbials, conventional GICs, MP4.

J Stoma 2024; 77, 2: 77-86
DOI: https://doi.org/10.5114/jos.2024.139877

INTRODUCTION

Glass-ionomer cements (GICs) are essential materials in clinical practice, especially in pediatric dentistry, due to their versatility, self-adhesion to enamel and dentine, and good bio-compatibility. They are very useful in full restorations application, particularly in children, liners and bases, fissure sealants and also, to a lesser extent, adhesives for orthodontic brackets and as cementitious sealers in endodontics. For more than 60 years, GICs have been used in restorative dentistry, especially in pedodontics. By the late 1960s, they began to develop as a result of early research conducted by Alan Wilson and Brian Kent in the Government Chemist's Laboratory in London [1, 2].

GICs have a list of advantages, including a similar thermal expansion coefficient of dental structures, adhering strongly to dental hard tissues, releasing fluoride for a considerable amount of time (at least five years), and being bio-compatible. When applied as bases in

JOURNAL OF STOMATOLOGY
CZASOPISMO STOMATOLOGICZNE

Address for correspondence: Prof. Aleksandar Dimkov, Pediatric and Preventive Dentistry, "Saints Cyril and Methodius" University of Skopje, Mother Theresa 17, 1000, Skopje, North Macedonia, e-mail: dimkovaleksandar@gmail.com

Received: 15.06.2023 • Accepted: 30.09.2023 • Published: 29.05.2024

deep cavities, their ability to release fluoride makes them excellent materials to substitute dentine [3-9]. Despite these benefits, traditional GICs still have several drawbacks, such as brittleness, low fracture resistance, and low resistance to abrasion and wear. Additionally, they have a limited working time and a very long setting time as well as even worse aesthetic properties than composite materials [10-14]. However, GICs are one of the most analyzed materials in dentistry. The PubMed database, the most relevant search engine in the field of medical sciences, lists about 8,000 papers for the query term "Glass Ionomer Cements" [15]. The release and uptake process is one of the most important properties of GICs [16-19]. In that direction, the release of fluoride ions and the property of GICs to act as a kind of reservoir for those ions, emphasize them as the main restorative materials, which participate in the process of remineralization of de-mineralized tooth surfaces [20-23]. However, another crucial characteristic of GICs is that the released fluoride ions act on local bacteria by decreasing cariogenic micro-organisms [24-30].

GICs may serve as models for the release of other active anti-microbial substances in addition to fluoride ions. Different anti-microbial chemicals have been added to conventional and resin-modified GICs to enhance their anti-bacterial properties. In a number of studies, the most frequently analyzed anti-microbial agent is chlorhexidine (CHX), described as a golden standard for anti-bacterial application. The addition of CHX in different combinations and different concentrations in conventional GICs increases anti-bacterial activity against cariogenic bacteria [31-36]. Unfortunately, there is not much published data on the use of other additional anti-microbial components in GICs.

The group of quaternary ammonium compounds consists of anti-microbial agents with proven anti-microbial activity. Only several papers described the incorporation of quaternary ammonium anti-microbial compounds, such as benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) [37-43]. As an active ingredient in oral antiseptics, CPC has a broad anti-bacterial spectrum, a potent bactericidal impact on Gram-positive bacteria and fungicidal effect on fungi [37-40]. BKC acts against bacteria, some viruses, fungi, and protozoa. Bacterial spores are considered to be resistant. Solutions are bacteriostatic and bactericidal activity, depending on their concentration. Gram-positive bacteria are more sensitive than Gram-negative ones [38, 39]. Both BKC and CPC contain chloride ions in their structure. Chloride exhibits anti-microbial properties through its inorganic and organic compounds, by rupturing chemical bonds in pathogens' molecules, such as bacteria and viruses, and destroying them. It has been demonstrated that chlorine-based inorganic compounds, such as sodium chloride, potassium chloride, chlorine dioxide, calcium hypochlorite, sodium hypochlorite, and lithium hypochlorite, have anti-bacterial activity [44-46]. Inorganic and organic chlorine compounds are also widely used in dentistry. The elimination of biofilm by chlorine compounds is an essential step for successful therapy of implant-related infections [47]. Several clinical studies have confirmed the efficacy of sodium hypochlorite and chlorine dioxide oral rinse in combating dental plaque and gingival inflammation [48]. Root canal irrigants using chlorine solutions play a significant role in eliminating micro-organisms, tissue dissolution, and removing debris and smear layer [49]. Treatment of candidiasis using chlorine dioxide, especially in geriatric patients, has also been proven [50].

OBJECTIVES

The idea for this study developed from the knowledge that glass ionomer cements are frequently used in pediatric dentistry, and may be employed as a medium for delayed and slow release of additional active anti-bacterial components apart from fluoride.

The objectives of the study were to determine the level of released chloride ions from conventional GICs in a medium consisting of deionized water over different time periods. Also, to determine the level of released chloride ions from the experimental glass-ionomer cement with 2% anti-microbial agents in a medium consisting of deionized water over different time periods, and to measure the quantity of released anti-microbial components from the conventional and experimental GICs, in which 2% concentrations of anti-microbial agents were previously incorporated.

The study's null hypothesis was that neither conventional nor experimental GICs release chloride ions or anti-microbial agents.

MATERIAL AND METHODS

The study was performed using conventional glass ionomer cements, i.e., ChemFlex (ex. Dentsply, Germany) and Fuji IX (ex. GC, Japan), and experimental glass ionomer cement MP4 (Pilkington's Ltd., UK).

Anti-microbial compounds used were cetylpyridinium chloride (CPC; Sigma-Aldrich, Dorset, UK) and benzalkonium chloride (BKC; ex Fluka, Germany). The composition of each cement is presented in Table 1.

PREPARATION OF SAMPLES AND INCORPORATION OF ANTI-MICROBIAL COMPONENTS

Specimens were prepared in compliance with the 1989 British Standards Institution Dental Glass Ionomer Cements Specifications. Anti-microbial compounds, BKC and CPC, were first incorporated into a glass ionomer cement's polyacrylic acid by mixing, and then the powder was added gradually to previously prepared acid and

TABLE 1. Glass ionomer cements used in the study – classification and composition

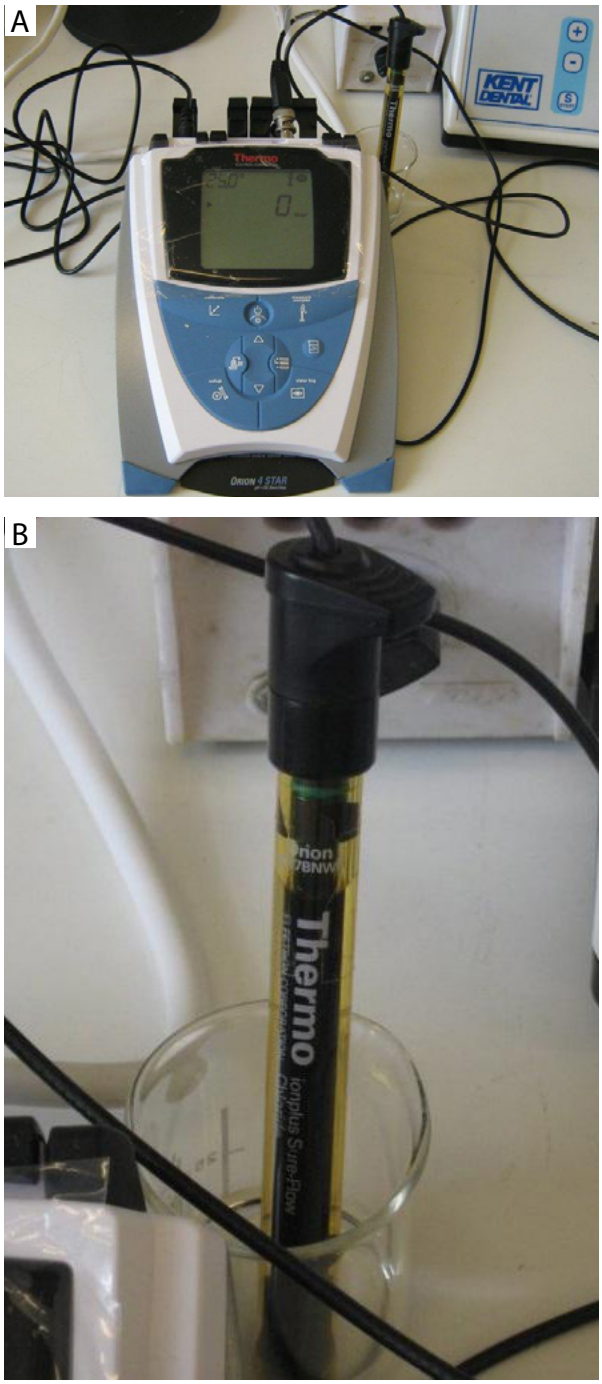
Material	Classification	Composition	Manufacturer
Fuji IX GP	Conventional glass ionomer cement	Silicon, aluminum, sodium, fluorine, phosphorus, strontium	GC Int., Tokyo, Japan
ChemFlex	Conventional glass ionomer cement	Strontium, aluminum, fluoride, silicate, tartaric acid, pigments, polyacrylic acid	Dentsply De Trey, Konstanz, Germany
MP4	Experimental glass ionomer cement	Silicon dioxide (SiO_2) 28% Aluminum (Al_2O_3) 35% Calcium oxide (CaO_2) 26% Sodium oxide (NaO) 11%	Pilkington's Ltd., UK

anti-microbial compound mixture, while care was taken to mix them together until complete saturation. Then, this freshly prepared paste was poured into metal molds (4 mm in diameter and 6 mm in height). The molds were put in specialized G-clamps after being sealed on both sides by metal plates. Anti-microbial agents were used in precise amounts of 2% of the cements' weight. By measuring specified proportion of the anti-microbial agent with an analytical scale (Mettler AE 200), the concentration (weight) of BKC and CPC was calculated. Previous investigations have established that the amount of anti-microbial agents at 2% was equal to 0.0044 grams of GIC ChemFlex, 0.0064 grams of GIC Fuji IX, and 0.0054 grams of experimental GIC MP4. After that, the samples were kept for an hour in an incubator at 37°C (maturation time). The specimens were removed from the clamps and molds after being removed from the incubator, and were kept individually in distinctly labeled plastic tubes with 5 ml of deionized water at a temperature of 22-24°C and air humidity of 40-50%.

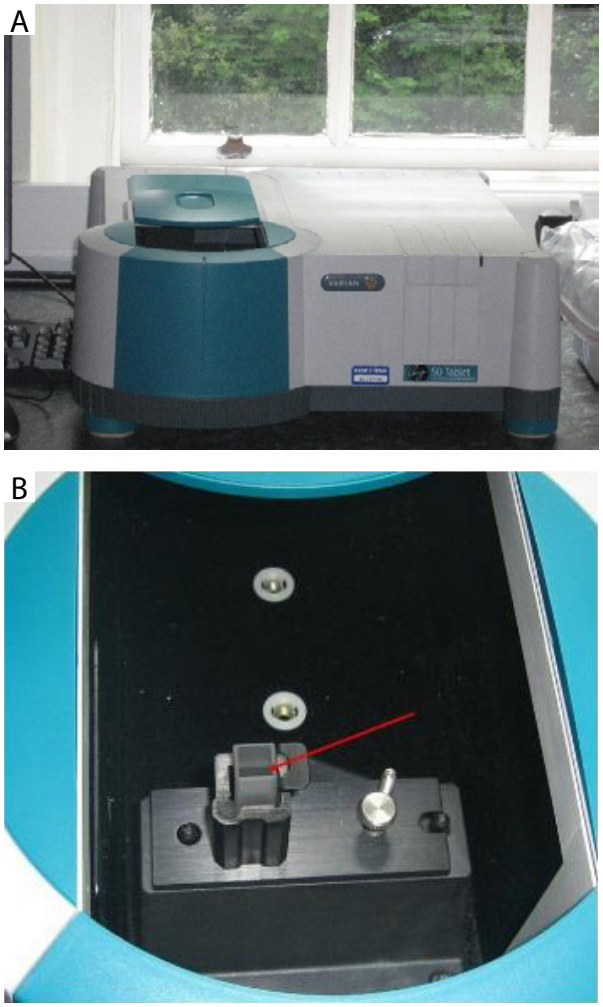
The amounts of released Cl^- ions were determined using ORION 4-star pH meter and an ion-selective electrode (ISE, Benchtop Thermo Electron Corporation, USA) optimized for chloride ion detection (Figure 1). Prior to use, an electrode was calibrated using standards with molarities that were within the range of ions' concentrations to be measured (0.1, 1.0, 10.0, 100.0, and 1,000.0 ppm). The release of chloride ions was done in a water medium of deionized water. Then, the ion-selective electrode was subsequently sunk in marked plastic laboratory tubes for chloride ions determination. Prior to each subsequent measurement, the plastic tubes were shaken for a few seconds to obtain a uniform distribution of released ions. Levels of released chloride ions were determined at different time intervals, as follows: immediately, after 15 min, 30 min, 45 min, 1 hour, 2 hours, 3 hours, 4 hours, 24 hours, and after 7 days (Figures 1 A, B).

DETERMINATION OF AMOUNTS OF ANTI-MICROBIAL AGENTS USING SPECTROPHOTOMETER

Concentrations of the anti-microbial agents (quaternary compounds) were determined using Varian Cary 50 tablet UV/VIS spectrophotometer (Agilent Technol-



FIGURES 1. ORION 4-star pH. **A**) ISE unit and **B**) ion-selective electrode



FIGURES 2. A) UV/VIS spectrophotometer and **B**) quartz glass cuvette.

ogies Inc., USA). CPC and BKC solutions with pre-set concentrations were used to calibrate the spectrophotometer. The UV spectrophotometer was calibrated to detect BKC at a maximum absorption wavelength of 214 nm, and CPC at a maximum absorption wavelength of 259 nm. Before each sample was measured, the plastic tube was shaken for 10 sec in order to disperse the settled anti-microbial organic compound uniformly throughout the medium (settling is caused by the high molecular weight of quaternary ammonium compounds). A portion of deionized water was poured from the plastic tube into a cuvette, and the cuvette was placed into the spectrophotometer. The instrument was connected to a computer that used specialized software, and displayed the results of measurements in absorbance units (AU). Once the measurement of each sample was done, the water was poured back into the tube. Re-zeroing was performed when changing the anti-microbial agent. Measurements were carried out over a period of 9 sequential time intervals, including 15 min, 30 min, 45 min, 1 h, 2 hrs., 3 hrs., 4 hrs., 24 hrs., and 7 days. The deionized water, in which the samples were kept was unchanged during whole time. Six samples of each GIC and both anti-microbial compounds were utilized to create a total of 36 specimens. Additionally, six samples of identical cements without any anti-microbial agents were created to serve as a control group (Figure 2).

One-way ANOVA test was applied for statistical analysis, and Mann-Whitney *U* test and post-hoc Tukey's test were performed for honest significant difference (HSD).

RESULTS

CHEMICAL ANALYSES WITH CONVENTIONAL GLASS IONOMER CEMENTS

The average values of released chloride ions from all the groups showed a gradual increase during the investigated periods, and this was especially emphasized in the groups of GICs with anti-microbial agents, particularly in the combination of anti-microbial agents with GIC ChemFlex. The variance analysis (ANOVA) revealed that all three groups showed statistically significant differences between average values across the study period.

According to Tukey's HSD test, GIC ChemFlex without anti-microbial agents demonstrated statistical insignificance only in one-time interval. The average value differences between all-time intervals in the group of ChemFlex with 2% BKC were statistically significant, except between the noted time intervals. The group of ChemFlex and 2% CPC demonstrated statistically significant differences in the average values between almost all-time intervals. In contrast to GIC ChemFlex, Fuji IX cement with no additional anti-microbial component showed a statistical significance in the majority of time periods. The combination of Fuji IX and 2% BKC was statistically insignificant in more time intervals than the combination of the same cement incorporated with 2% CPC, which had a high degree of statistical significance (Table 2).

UV SPECTROPHOTOMETRIC ANALYSES WITH CONVENTIONAL GLASS IONOMER CEMENTS

According to the variance analysis, all the groups showed statistically significant differences in average values over analyzed time ($p < 0.05$). Except for the combination of ChemFlex with an additional 2% BKC, there was substantial significant reliance according to Tukey's HSD test (Table 3).

CHEMICAL ANALYSES WITH EXPERIMENTAL GLASS IONOMER CEMENT MP4

The statistical analyses carried out with ANOVA test showed that there was a statistically highly significant

TABLE 2. Release of Cl^- ions from conventional GICs without and with 2% incorporated anti-microbial agents (data obtained in ppm)

Time	ChemFlex, average ± (SD)	ChemFlex + 2% BKC, average ± (SD)	ChemFlex + 2% CPC, average ± (SD)	Fuji IX, average ± (SD)	Fuji IX + 2% BKC, average ± (SD)	Fuji IX + 2% CPC, average ± (SD)
0′	0.07 (0.04)	0.40 (0.19)	0.06 (0.02)	0.17 (0.02)	14.13 (2.14)	0.13 (0.02)
15′	0.14 (0.19)	2.21 (0.28)	2.35 (4.15)	0.53 (0.10)	30.28 (4.13)	1.03 (0.72)
30′	0.22 (0.21)	3.86 (0.98)	9.39 (4.05)	0.69 (0.14)	33.75 (2.44)	1.84 (2.03)
45′	0.31 (0.27)	5.55 (1.45)	16.02 (4.42)	0.44 (0.13)	34.62 (2.15)	4.83 (3.39)
1 h	0.75 (0.68)	7.06 (1.77)	20.02 (3.84)	1.77 (0.99)	46.73 (6.56)	3.63 (3.25)
2 h	3.72 (4.91)	10.92 (2.09)	34.47 (4.88)	13.23 (1.60)	82.10 (9.91)	23.83 (3.02)
3 h	15.18 (7.37)	18.31 (4.48)	53.07 (7.65)	12.39 (3.23)	70.33 (12.55)	26.23 (3.44)
4 h	20.40 (8.23)	23.69 (2.88)	42.78 (6.20)	11.66 (4.97)	115.82 (22.62)	43.05 (5.90)
24 h	8.43 (7.07)	16.51 (2.02)	25.48 (3.71)	3.11 (2.30)	42.42 (8.12)	22.83 (3.56)
7 d	28.47 (1.66)	122.00 (2.26)	130.17 (23.08)	63.43 (10.27)	90.62 (30.40)	43.15 (14.59)
p-value	0.00	0.00	0.00	0.00	0.00	0.00
Tukey HSD test	Insignificant ($p > 0.05$) 4 h: 3 h, 7 d	Insignificant ($p > 0.05$) 0′: 15′, 30′ 15′: 45′ 30′: 1 h 1 h: 2 h 3 h: 24 h	Significant ($p < 0.05$) 0′, 15′: 2 h, 3 h, 4 h, 24 h 30′, 45′: 4 h, 24 h 1 h: 24 h 24 h: all time intervals	Significant ($p < 0.05$) 0′, 15′, 30′, 45′ 1 h, 24 h: 2 h, 3 h, 4 h, 7 d 7 d: all time intervals	Insignificant ($p > 0.05$) 0′: 15′, 30′, 45′, 1 h 45′: 1 h 2 h: 3 h, 24 h 4 h: 7 d 24 h: 1 h	Significant ($p < 0.05$) 0′: 1 h, 2 h, 3 h, 4 h, 24 h, 7 d 15′, 30′, 45′, 24 h: 2 h, 3 h, 4 h, 7 d 1 h: 2 h, 4 h, 7 d 2 h: 4 h, 24 h 3 h: 4 h

TABLE 3. Release of 2% anti-microbial agents incorporated into conventional GICs (data obtained in absorbance units)

Time	ChemFlex + BKC, average ± (SD)	ChemFlex + CPC, average ± (SD)	Fuji IX + BKC, average ± (SD)	Fuji IX + CPC, average ± (SD)
15′	0.01 (0.00)	0.03 (0.01)	0.15 (0.05)	0.09 (0.05)
30′	0.01 (0.00)	0.03 (0.00)	0.15 (0.04)	0.09 (0.04)
45′	0.02 (0.00)	0.03 (0.00)	0.14 (0.04)	0.12 (0.04)
1 h	0.02 (0.01)	0.03 (0.00)	0.18 (0.05)	0.12 (0.03)
2 h	0.04 (0.00)	0.04 (0.01)	0.20 (0.04)	0.14 (0.04)
3 h	0.05 (0.00)	0.04 (0.01)	0.22 (0.04)	0.16 (0.04)
4 h	0.08 (0.00)	0.05 (0.02)	0.21 (0.04)	0.17 (0.04)
24 h	0.07 (0.02)	0.06 (0.03)	0.22 (0.04)	0.18 (0.04)
7 d	0.16 (0.02)	0.11 (0.05)	0.28 (0.04)	0.23 (0.05)
p-value	0.000000	0.000047	0.000012	0.000009
Tukey's HSD test	Insignificant ($p > 0.05$) 15′: 30′ 15′: 45′ 30′: 45′	Significant ($p < 0.05$) 7 d: 15′, 30′, 45′, 1 h, 2 h, 3 h, 4 h, 24 h	Significant ($p < 0.05$) 7 d: 15′, 30′, 45′,1 h 4 h: 24 h	Significant ($p < 0.05$) 7 d: 15′, 30′, 45′, 1 h, 2 h 15′: 24 h 30′: 24 h

difference in the average values for $p = 0.00000$. To provide a clearer picture of the findings of statistical analysis using Tukey's HSD test, statistically insignificant differences were only reported for both combinations and all time intervals. According to Mann-Whitney *U* test, the difference in the average values between MP4 + CPC 2% and MP4 + BKC 2% was statistically significant ($p < 0.05$) between the average values for all time intervals, except at zero time and at fourth hour (Table 4).

UV SPECTROPHOTOMETRIC ANALYSES WITH EXPERIMENTAL GLASS IONOMER CEMENT MP4

The ANOVA test showed that the differences in average values were highly statistically significant ($p = 0.00000$). Individual comparisons of the average value differences (Tukey's HSD test) in combination of MP4 + CPC indicated statistical significance for $p < 0.05$ only in the time intervals mentioned, but the combination of MP4 + BKC showed a statistically negligible difference across all time intervals. The difference in the average values between MP4 and both anti-microbials was statistically significant over all time periods for $p < 0.05$, according to Mann-Whitney *U* test (Table 5).

TABLE 4. Release of Cl^- ions from experimental GIC MP4 integrated with 2% anti-microbial agents (data obtained in ppm)

Time	MP4 + CPC 2%, average ± (SD)	MP4 + BKC 2%, average ± (SD)	MP4 + CPC 2% MP4 + BKC 2%, Mann-Whitney *U* test (*p*-level)
0′	0.42 (0.20)	0.35 (0.02)	0.748774
15′	20.46 (3.76)	43.00 (8.89)	0.003948
30′	32.58 (4.58)	80.25 (17.48)	0.003948
45′	40.13 (7.18)	93.41 (17.71)	0.003948
1 h	43.50 (7.26)	108.18 (17.41)	0.003948
2 h	80.51 (15.55)	139.00 (17.73)	0.003948
3 h	89.35 (17.53)	137.33 (12.11)	0.003948
4 h	118.83 (22.05)	123.50 (10.56)	0.630954
24 h	161.66 (29.22)	54.76 (3.52)	0.003948
7 d	75.86 (6.83)	58.55 (3.36)	0.003948
p-value	0.000000	0.000000	
Tukey's HSD test	Insignificant ($p > 0.05$) 0′: 1 h 7 d: 2 h 7 d: 3 h	Insignificant ($p > 0.05$) 15′: 24 h 15′: 7 d 45′: 1 h	

COMPARATIVE ANALYSES BETWEEN ANALYZED GLASS IONOMER CEMENTS

The average values of released chloride ions and anti-microbial agents in samples prepared from all glass ionomer cements separately integrated with 2% anti-bacterial agents, are shown in Tables 6 and 7. The applied statistical analysis of variance revealed the existence of statistically significant differences between the GICs in all time periods ($p < 0.05$). Tukey's HSD test showed that the combinations of the three GICs with both BKC and CPC resulted in a statistically significant difference in the average values ($p < 0.05$).

DISCUSSION

The determination of chloride ions was carried out with an ion-selective electrode in a medium of deionized water, in which the GICs samples, without and with the addition of 2% concentration of anti-microbial compounds, were stored during ten time periods encompassing a week.

The purpose of this investigation was to determine if the GICs have an anti-bacterial impact because chlorine, the primary component of anti-microbial compounds

TABLE 5. Release of 2% anti-microbial agents incorporated into experimental GIC MP4 (data obtained in absorbance units)

Time	MP4 + CPC, average ± (SD)	MP4 + BKC, average ± (SD)	MP4 + CPC MP4 + BKC, Mann-Whitney *U* test (p-level)
15′	0.16 (0.03)	1.61 (1.22)	0.003948
30′	0.17 (0.03)	1.69 (1.15)	0.003948
45′	0.18 (0.03)	1.75 (1.10)	0.003948
1 h	0.20 (0.04)	1.80 (1.06)	0.003948
2 h	0.22 (0.05)	1.87 (1.01)	0.003948
3 h	0.26 (0.05)	1.94 (0.94)	0.003948
4 h	0.28 (0.06)	2.01 (0.90)	0.003948
24 h	0.33 (0.08)	2.47 (0.88)	0.003948
7 d	0.71 (0.16)	2.75 (0.71)	0.003948
p-value	0.000000	0.563817	
Tukey's HSD test	Significant ($p < 0.05$) 7 d: all others 24 h: 15′, 30′, 45′	Insignificant ($p > 0.05$)	

TABLE 6. Release of Cl^- ions from all GICs integrated with 2% anti-microbial agents (data obtained in ppm)

Time	ChemFlex + BKC	Fuji IX + BKC	MP4 + BKC	ANOVA ($p < 0.05$)	ChemFlex + CPC	Fuji IX + CPC	MP4 + CPC	ANOVA ($p < 0.05$)
0′	0.40	14.13	0.35	0.000000	0.06	0.13	0.42	0.000214
15′	2.21	30.28	43.00	0.000000	2.35	1.03	20.46	0.000000
30′	3.86	33.75	80.25	0.000000	9.39	1.84	32.58	0.000000
45′	5.55	34.62	93.41	0.000000	16.02	4.83	40.13	0.000000
1 h	7.06	46.73	108.18	0.000000	20.02	3.63	43.50	0.000000
2 h	10.92	82.10	139.00	0.000000	34.47	23.83	80.51	0.000000
3 h	18.31	70.33	137.30	0.000000	53.07	26.23	89.35	0.000000
4 h	23.69	115.82	123.50	0.000000	42.78	43.05	118.83	0.000000
24 h	16.51	42.42	54.76	0.000000	25.48	22.83	161.66	0.000000
7 d	122.00	90.62	58.55	0.000072	130.17	43.15	75.86	0.000001
Tukey's HSD test	Significant ($p < 0.000$)				Significant ($p < 0.00$)			

TABLE 7. Release of 2% anti-microbial agents from all GICs (data obtained in absorbance units)

Time	ChemFlex + BKC	Fuji IX + BKC	MP4 + BKC	ANOVA ($p < 0.05$)	ChemFlex + CPC	Fuji IX + CPC	MP4 + CPC	ANOVA ($p < 0.05$)
15′	0.01	0.15	1.61	0.002140	0.03	0.09	0.16	0.000045
30′	0.01	0.15	1.69	0.000863	0.03	0.09	0.17	0.000001
45′	0.02	0.14	1.75	0.000382	0.03	0.12	0.18	0.000001
1 h	0.02	0.18	1.80	0.000237	0.03	0.12	0.20	0.000000
2 h	0.04	0.20	1.87	0.000101	0.04	0.14	0.22	0.000001
3 h	0.05	0.22	1.94	0.000034	0.04	0.16	0.26	0.000000
4 h	0.08	0.21	2.01	0.000015	0.05	0.17	0.28	0.000002
24 h	0.07	0.22	2.47	0.000001	0.06	0.18	0.33	0.000003
7 d	0.16	0.28	2.75	0.000000	0.11	0.23	0.71	0.000000
Tukey HSD test	Significant ($p < 0.00$)				Significant ($p < 0.000$)			

BKC and CPC, has been proven to have such an effect. The results on the release of chloride ions from conventional GICs containing 2% anti-microbial agents indicated a continuous release of these ions over the course of a week, which is likely caused by chlorides in the anti-microbial compounds. However, chloride ion release occurs from the GICs with no anti-microbial compounds incorporated. Additionally, a continuous release of chloride ions was noted during all investigated periods. There have been several research on the release of elements from conventional cements. However, not a single study on the release of chloride ions from GICs, either in the references or in the Medline database, has been found. Additionally, manufacturers do not mention chloride ions in compositions of the examined cements in their specifications. This raises a question of where the chloride ions in the measured medium came from. Even when the cumulative release impact is examined, the amount of released chloride ions keeps increasing, providing proof of their ongoing release. Given that the application of a particular ISE does not demonstrate the existence of chloride ions in a medium devoid of cement, it is obvious that these concentrations are not caused by the medium itself (deionized water). The potential of an external (aero) introduction or secondary contamination of the medium, is also ruled out for two reasons: the concentrations of chlorine in the lab's air were brought to zero, and the test tubes holding the samples and medium were shut after each measurement. The integration of ions from the air into a water medium in a very short amount of time would be doubtful, even if there was a precise calculation of the chlorine content in the air.

The cumulative effects of released anti-microbial compounds incorporated into conventional GICs were

also performed in a medium of deionized water. The cumulative impact of released anti-microbial compounds is crucial, especially in situations where oral hygiene is lacking and dental plaque is persistently abundant. On the other hand, the cumulative effect of the modified "anti-microbial cements" applied in the cavities, which release anti-microbial compounds toward the interior of the cavity, i.e., at the interfaces between the restorations and the dentine, is of no lesser importance. The improvement of anti-microbial properties of GICs, without a loss in their chemical and physical characteristics, is of permanent interest in dental medical science. The incorporation of compounds with far stronger anti-microbial characteristics is still questionable with respect to the effect on the basic characteristics of the used GIC. A number of studies described the possibility of incorporating chlorhexidine [31-36]. It was the first anti-microbial compound incorporated into GICs. Data from the literature on the possibility of incorporating other compounds, especially from the group of quaternary ammonium compounds, is insignificant. The incorporation and release of anti-bacterial substances cetylpyridinium chloride and benzalkonium chloride are discussed in this work. Due to their chemistry, i.e., large and heavy organic chains, their determination with ion-selective electrodes is impossible, and therefore the analysis was conducted with the use of ultraviolet-visible spectrophotometry. This procedure is appropriate for the detection of organic compounds contained in a medium, which were the target of the analysis.

There are few literature data describing the incorporation and release of other anti-microbial agents from other GICs prepared in a different way, in samples with different sizes, and using a different methodology and equipment [37-43]. Attempts by incorporating CPC in other restorative materials, such as resin matrix, have been made. Although the two restorative materials are incomparable, some similarities can be seen in the obtained results. The release of CPC was conducted in a water medium, the amount of the released CPC was determined with the use of a UV/VIS spectrophotometer, and the analyzed intervals were 1, 3, 5, and 7 days [42].

This study also included chemical analyses with the experimental glass ionomer cement MP4, developed by Pilkington's Ltd., UK. Being a cement with no fluoride, MP4 is mainly used in laboratories for experiments on the incorporation and release of fluoride ions, but it is also used as a medical cement [5]. The intention was to see the possibility of the release of chloride ions and anti-microbial compounds of BKC and CPC from the MP4 experimental GIC as well as to compare the values obtained with the conventional GICs.

The outcomes of the release of chloride ions demonstrated their continual release over the investigated periods, until the seventh day for the CPC compound, i.e., until the 24th hour for the BKC compound, when a drastic drop in the average values occurred. The results of the possibility for the release of anti-microbial compounds from MP4 into a water medium point to a constant slight increase in the average values of the released compounds. The differences in the average values at all time intervals were higher for the combination of MP4 + BKC, which was also statistically confirmed (Mann-Whitney test). For the combination of MP4 + CPC, the analysis of the variance revealed statistically significant variations between the average values in the studied period; however, this was not the case for the second tested compound. To achieve a comprehensive understanding of the experimental GIC MP4's properties regarding the release of chloride ions, i.e., anti-microbial chemicals, the findings were compared to the results for conventional cements. According to the findings on the release of chloride ions between the conventional cements and the experimental cement, the release of ions was significantly greater for the MP4 + BKC combination until the fourth hour, and for the MP4 + CPC combination until the 24th hour. All of the examined intervals' resultant average value differences were statistically very significant (ANOVA). Differences in the values obtained from the combination of the MP4 cement with the two studied compounds with regard to the conventional cements were statistically significant, although at different levels of significance, according to the results acquired using Tukey's test.

The comparative analysis of the data obtained on the release of anti-microbial compounds (UV-analyses) shows a drastically higher release of the BKC compound from the experimental cement at all time intervals with respect to the conventional cements, and a significantly higher release of the CPC compound. As no experiments with the incorporation of anti-microbial compounds in experimental GIC MP4 have been performed to date, and regarding the results obtained from our comparative analysis with conventional cements, numerous questions arise. Does the proportional composition of compounds constituting the cement, or the absence of fluoride ions in the composition, or perhaps the polyacrylic and tartaric acids, which are in this case mixed within the powder of the cement, contribute to the significant differences in conventional cements? Considering the fact that no analyses have been done so far of the release of chloride ions, i.e., of anti-microbial compounds, further research is needed in order to get answers to these issues.

CONCLUSIONS

Based on the study and obtained data, we may conclude that: conventional GICs without incorporated anti-microbial compounds release chloride ions at all time periods, and the concentration increases with time. Experimental GIC MP4 integrated with 2% anti-microbial agents releases a high level of chloride ions. Both conventional and experimental GICs containing 2% anti-

microbials release anti-microbial agents that are proportionate to a time period. Fuji IX GIC releases more chloride ions on average than ChemFlex. In comparison with conventional GICs, MP4 GIC produces a significantly higher release both of chloride ions and antimicrobial compounds.

DISCLOSURES

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

References

1. Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. J Funct Biomater 2016; 28: 16. DOI: 10.3390/jfb7030016.
2. Hill R. Glass ionomer polyalkenoate cements and related materials: past, present and future. Br Dent J 2022; 232: 653-657.
3. Berg JH, Croll TP. Glass ionomer restorative cement systems: an update. Pediatr Dent 2015; 37: 116-124.
4. Nicholson JW, Czarnecka B. The biocompatibility of resin-modified glass-ionomer cements for dentistry. Dent Mater 2008; 24: 1702-1708.
5. Fierascu RC. Incorporation of nanomaterials in glass ionomer cements-recent developments and future perspectives: a narrative review. Nanomaterials (Basel) 2022; 12: 3827. DOI: 10.3390/nano12213827.
6. Upadhya NP. Glass ionomer cement – the different generations. Trends in Biomaterials & Artficial Organs 2005; 18: 158-165.
7. Sainath Reddy TH, Venkatesh KV, Mani R. Comparative evaluation of three different glass ionomer cements. Indian J Dent Res 2021; 32: 485-488.
8. Kucukyilmaz E, Savas S, Kavrik F, Yasa B, Botsali MS. Fluoride release/recharging ability and bond strength of glass ionomer cements to sound and caries-affected dentin. Niger J Clin Pract 2017; 20: 226-234.
9. Rolim FG, de Araújo Lima AD, Lima Campos IC, de Sousa Ferreira R, da Cunha Oliveira-Júnior C, Gomes Prado VLG, et al. Fluoride release of fresh and aged glass ionomer cements after recharging with high-fluoride dentifrice. Int J Dent 2019; 2019: 9785364. DOI: 10.1155/2019/9785364.
10. de Lima Navarro MF, Pascotto RC, Borges AFS, Soares CJ, Prócida Raggio D, Rios D, et al. Consensus on glass-ionomer cement thresholds for restorative indications. J Dent 2021; 107: 103609. DOI: 10.1016/j.jdent.2021.103609.
11. Culbertson BM. Glass-ionomer dental restoratives. Prog Polym Sci 2001; 26: 577-604.
12. Nicholson JW. Maturation processes in glass-ionomer dental cements. Acta Biomater Odontol Scand 2018; 4: 63-71.
13. Wetzel R, Eckardt O, Biehl P, Brauer DS, Schacher FH. Effect of poly(acrylic acid) architecture on setting and mechanical properties of glass ionomer cements. Dent Mater 2020; 36: 377-386.
14. Algera TJ, Kleverlaan CJ, Prahl-Andersen B, Feilzer AJ. The influence of environmental conditions on the material properties of setting glass-ionomer cements. Dent Mater 2006; 22: 852-856.
15. https://pubmed.ncbi.nlm.nih.gov/?term=glass+ionomer+cements (Accessed: 10.05.2023).
16. Cildir SK, Sandalli N. Fluoride release/uptake of glass-ionomer cements and polyacid-modified composite resins. Dent Mater J 2005; 24: 92-97.
17. Shahid S, Billington RW, Pearson GJ. The role of glass composition in the behaviour of glass acetic acid and glass lactic acid cements. J Mater Sci Mater Med 2008; 19: 541-545.
18. Dziuk Y, Chhatwani S, Möhlhenrich SC, Tulka S, Naumova EA, Danesh G. Fluoride release from two types of fluoride-containing orthodontic adhesives: Conventional versus resin-modified glass ionomer cements – an in vitro study. PLoS One 2021; 16: e0247716. DOI: 10.1371/journal.pone.0247716.
19. Morales-Valenzuela AA, Scougall-Vilchis RJ, Lara-Carrillo E, Garcia-Contreras R, Salmeron-Valdes EN, Aguillón-Sol L. Comparison of fluoride release in conventional glass-ionomer cements with a new mechanical mixing cement. Oral Health Prev Dent 2020; 18: 319-323.
20. Brenes-Alvarado A, Cury JA. Fluoride release from glass ionomer cement and resin-modified glass ionomer cement materials under conditions mimicking the caries process. Oper Dent 2021; 46: 457-466.
21. Panpisut P, Monmaturapoj N, Srion A, Angkananuwat C, Krajangta N, Panthumvanit P. The effect of powder to liquid ratio on physical properties and fluoride release of glass ionomer cements containing pre-reacted spherical glass fillers. Dent Mater J 2020; 39: 563-570.
22. Williams JA, Briggs E, Billington RW, Pearson GJ. The effects of adding fluoride compounds to a fluoride-free glass ionomer cement on subsequent fluoride and sodium release. Biomaterials 2003; 24: 1301-1308.
23. Hafshejani TM, Zamanian A, Venugopal JR, Rezvani Z, Sefat F, Saeb MR, et al. Antibacterial glass-ionomer cement restorative materials: a critical review on the current status of extended release formulations. J Control Release 2017; 262: 317-328.
24. Hengtrakool C, Pearson GJ, Wilson M. Interaction between GIC and S. sanguis biofilms: antibacterial properties and changes of surface hardness. J Dent 2006; 34: 588-597.
25. Roshan NM, Shigli AL, Deshpande SD. Microbiological evaluation of salivary Streptococcus mutans from children of age 5-7 years, pre- and post-atraumatic restorative treatment. Contemp Clin Dent 2010; 1: 94-97.
26. Vermeersch G, Leloup G, Delmée M, Vreven J. Antibacterial activity of glass-ionomer cements, compomers and resin composites: relationship between acidity and material setting phase. J Oral Rehabil 2005; 32: 368-374.
27. Luczaj-Cepowicz E, Marczuk-Kolada G, Zalewska A, Pawińska M, Leszczyńska K. Antibacterial activity of selected glass ionomer cements. Postepy Hig Med Dosw (Online) 2014; 68: 23-28.
28. Makarla S, Venugopal R, Bavle RM, Selvan AK, Muniswamappa SS, Dinesh R. Determining the best anti-microbial properties of dental cements used for pulp capping procedures using deep dentinal carious material. J Oral Maxillofac Pathol 2023; 27: 239. DOI: 10.4103/jomfp.jomfp_109_21.
29. Sagmak S, Bahsi E, Ozcan N, Satici O. Comparative evaluation of antimicrobial efficacy and fluoride release of seven different glass-ionomer-based restorative materials. Oral Health Prev Dent 2020; 18: 521-528.
30. Nakajo K, Imazato S, Takahashi Y, Kiba W, Ebisu S, Takahashi N. Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci. Dent Mater 2009; 25: 703-708.
31. Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM. Chlorhexidine-releasing methacrylate dental composite materials. Biomaterials 2005; 26: 7145-7153.
32. da Mota Martins V, Paranhos LR, de Oliveira MN, Maia LC, Machado AC, Santos-Filho PCF. Does the addition of chlorhexidine to glass ionomer cements influence its antimicrobial effect and survival rate? A systematic review. Eur Arch Paediatr Dent 2022; 23: 365-379.
33. Yadiki JV, Jampanapalli SR, Konda S, Inguva HC, Chimata VK. Comparative evaluation of the antimicrobial properties of glass ionomer cements with and without chlorhexidine gluconate. Int J Clin Pediatr Dent 2016; 9: 99-103.
34. Mobarak EH, Shabayek MM, El-Deeb HA, Mulder J, Hassan FM, Van der Sanden WJM, Frencken JE. Survival of occlusal ART restorations using high-viscosity glass-ionomer with and without

chlorhexidine: a 2-year split-mouth quadruple-blind randomized controlled clinical trial. J Adv Res 2019; 17: 117-123.
35. Mittal S, Soni H, Sharma DK, Mittal K, Pathania V, Sharma S. Comparative evaluation of the antibacterial and physical properties of conventional glass ionomer cement containing chlorhexidine and antibiotics. J Int Soc Prev Community Dent 2015; 5: 268-275.
36. Palmer G, Jones FH, Billington RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomaterials 2004; 25: 5423-5431.
37. Hamid N, Telgi RL, Tirth A, Tandon V, Chandra S, Chaturvedi RK. Titanium dioxide nanoparticles and cetylpyridinium chloride enriched glass-ionomer restorative cement: a comparative study assessing compressive strength and antibacterial activity. J Clin Pediatr Dent 2019; 43: 42-45.
38. Dimkov A, Gjorgievska E, Nicholson JW, Kaftandzieva A. Antibacterial effects of conventional glass ionomer cement. Bratisl Lek Listy 2016; 117: 31-35.
39. Dimkov A, Nicholson WJ, Gjorgievska E, Booth S. Compressive strength and setting time determination of glass-ionomer cements incorporated with cetylpyridinium chloride and benzalkonium chloride. Prilozi 2012; 33: 243-263.
40. Botelho MG. Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in a glass ionomer cement. Caries Res 2003; 37: 108-114.
41. Tüzüner T, Ulusu T. Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement. J Appl Oral Sci 2012; 20: 45-49.
42. Namba N, Yoshida Y, Nagaoka N, Takashima S, Matsuura-Yoshimoto K, Maeda H, et al. Antibacterial effect of bactericide immobilized in resin matrix. Dent Mater 2009; 25: 424-430.
43. Kurt A, Tüzüner T, Baygın Ö. Antibacterial characteristics of glass ionomer cements containing antibacterial agents: an in vitro study. Eur Arch Paediatr Dent 2021; 22: 49-56.
44. Bautista-Gallego J, Arroyo-López FN, Durán-Quintana MC, Garrido-Fernandez A. Individual effects of sodium, potassium, calcium, and magnesium chloride salts on Lactobacillus pentosus and Saccharomyces cerevisiae growth. J Food Prot 2008; 71: 1412-1421.
45. Lee SS, Suprono MS, Stephens J, Withers SA, Oyoyo U, Li Y. A randomized, blinded, clinical investigation of breath odor reduction efficacy of a stabilized chlorine-dioxide containing flavored mouthwash. Am J Dent 2021; 34: 195-200.
46. Robert O. Young chlorine dioxide (ClO2) as a non-toxic antimicrobial agent for virus, bacteria and yast (Candida albicans). Int J Vaccines Vaccin 2016; 2: 00052. DOI: 10.15406/ijvv.2016.02.00052.
47. Venkei A, Eördegh G, Turzó K, Urbán E, Ungvári K. A simplified in vitro model for investigation of the antimicrobial efficacy of various antiseptic agents to prevent peri-implantitis. Acta Microbiol Immunol Hung 2020; 67: 127-132.
48. Kerémi B, Márta K, Farkas K, Czumbel LM, Tóth B, Szakács Z, et al. Effects of chlorine dioxide on oral hygiene – a systematic review and meta-analysis. Curr Pharm Des 2020; 26: 3015-3025.
49. Ragnarsson KT, Rechenberg DK, Attin T, Zehnder M. Available chlorine consumption from NaOCl solutions passively placed in instrumented human root canals. Int Endod J 2015; 48: 435-440.
50. Mohammad AR, Giannini PJ, Preshaw PM, Alliger H. Clinical and microbiological efficacy of chlorine dioxide in the management of chronic atrophic candidiasis: an open study. Int Dent J 2004; 54: 154-158.