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Fluoride release and antibacterial activity of selected dental materials

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

The aim of the study was to assess the fluoride ion release and antibacterial activities of the glassionomer cement Fuji IX and the compomer (composite modified polyacid) Dyract AP.

Fluoride ion release was measured using direct potentiometry with an Orion fluoride ion selective electrode. The measurement was carried out after 1, 4, 7, 14, 30, and 60 days of storage in phosphate buffer at pH 6.8. The antibacterial activity of the materials was evaluated against the bacteria *Streptococcus mutans ATCC 35668*, *Streptococcus salivarius ATCC 13419*, *Streptococcus sanguis ATCC 10556*, and *Lactobacillus casei subsp. casei ATCC 393*. The agar diffusion test was applied. The material specimens were assessed twice: after setting and seven days later. Zones of bacterial growth inhibition were measured in millimeters after 24 hours.

The results of the study showed that both materials released ion fluoride, with a higher emission of Fuji IX than Dyract AP. The highest level of emission was observed on the seventh day of the study in both materials. After 24 hours of bonding there was inhibition of bacterial growth by Fuji IX, whereas Dyract AP did not show similar activity. On the eighth day after polymerization, Dyract AP was significantly more active towards *Streptococcus sanguis* and *salivarius*.

Key words: **fluoride release • antibacterial activity • dental materials**

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Introduction

Acidogenic bacteria play the main role in the development of dental caries [7,46]. Treatment procedures using various methods of processing the defects do not always eliminate all the microorganisms from the carious focus [4,27,32,45]. The presence of bacteria in the dental tissue left after treatment or their invasion through a microcrack that can sometimes occur between the tooth and its filling can result in secondary caries [34]. Therefore, antibacterial action is a desired feature of materials used for dental fillings. Many new filling materials characterized by fluoride ion release were developed towards the end of the 20th century [11,15,16,38,42].

Aim

The aim of the study was to assess the fluoride ion release and antibacterial activity of two groups of dental material used as permanent fillings in carious defects.

Material and Methods

The glassionomer cement Fuji IX (GC Japan) and the compomer (composite modified polyacid) Dyract AP underwent examination. The studies were carried out in two stages. In the first part, fluoride ion release from the selected materials was determined to establish peak fluoride ion release. The material was hardened in forms prepared according to the manufacturers' recommendations. The samples were disc-shaped, 13.5 mm in diameter and 1 mm thick. After polishing, the filling samples were air-conditioned for 24 hours at 37°C. Then they were placed in 10 ml of a phosphate buffer solution of pH 6.8 in polyethylene containers and the temperature was maintained at 37°C. Fluoride ion release was measured using direct potentiometry with an Orion fluoride ion selective electrode. The measurement was carried out after 1, 4, 7, 14, 30, and 60 days of storage in the contact solution. Each time, three solutions were taken for examination from consecutive material containers. Ten ml of TISAB buffer was added to the analyzed solutions before the examination in order to stabilize the pH and eliminate the influence of foreign ions during the examination.

In the second part of the study, the antibacterial activity of the above materials was assessed. The samples were prepared under aseptic conditions. Semicircular forms 9 mm in diameter for fixing the application resin (Kerr) were used. Fuji IX cement was prepared manually according to the manufacturer's recommendations. Two-layered polymerization with a Heliolux lamp II (Vivadent) was applied in the case of the compomer Dyract AP. The consecutive samples were then weighed. Table 1 shows the mean masses of the material samples. Statistically non-significant differences in sample mass were due to the fact that the matrixes used were not professionally calibrated. Each time, 12 samples of both materials were prepared and half of them were placed separately in screw-capped containers with 5 ml of phosphate buffer of pH 6.8 and stored for seven days in a thermostatic chamber at 37°C. The samples were put on microbiological media immediately after their hardening and after seven days at the time of the highest level of fluoride ion emission.

Table 1. Mean mass of examined samples assessed microbiologically

	Kind of material	
	Dyract AP	Fuji IX
	Mean mass of samples in g	
1st examination	0.40	0.41
2nd examination	0.39	0.37
3rd examination	0.39	0.36
4th examination	0.38	0.38

An adapted agar diffusion test, used for the assessment of antibiotic activity, was applied in the microbiological studies [53]. The following standard strains/lines were used for the evaluation: *Streptococcus mutans ATCC 35668*, *Streptococcus salivarius ATCC 13419*, *Streptococcus sanguis ATCC 10556*, and *Lactobacillus casei subsp. casei ATCC393*. The strains were grown on BHI medium (Oxoid) for 18 hours at 37°C and afterwards suspensions with densities of 0.5 on the McFarland scale were prepared. The suspensions were diluted 100-fold in 0.9% NaCl. Agar ISO-SENSITEST (Oxoid) with 5% ram blood was inoculated with 0.1 ml of the suspension using a cotton swab and then the prepared dental material was put on the plates. The plates with streptococci were incubated in air with additional CO_2 and those with *Lactobacillus* in an anaerobic atmosphere for 18 hours at 37°C. After 24 hours, the zones of bacterial growth inhibition were measured in mm. Lack of growth inhibition was determined in the way described by Vermeersch et al. [47], i.e. by the size of the sample diameter, which was 9 mm in our study and not 0 as in other authors' reports. The statistical analysis was carried out with the t-student test for pairs, with the level of significance set at $p<0.05$.

Results

The results of fluoride release are presented in Figure 1 as fluoride released from 1 mm^2 of dental material as a function of time, and they confirmed the release of fluoride ions by the commercial materials used as permanent dental fillings. Both the glassionomer cement Fuji IX and the compomer Dyract AP showed accumulative increases in the amount of fluoride in the solutions. The studies revealed that the highest level of fluoride emission to the solution in both materials was observed on the seventh day of the examinations, with simultaneous stabilization of its level in the solution. The solution with the sample of Fuji IX filling had a higher level of fluoride ions and approximately 0.7 µg of them were released from 1 mm^2 of sample surface. A slightly smaller amount of F^- (0.5 $\mu g/mm^2$) was released from the compomer. Decreases in the amounts of fluoride in the solutions were observed after a month of sample storage.

Antimicrobial activity is presented in Table 2. The glassionomer cement Fuji IX showed antibacterial activity towards all the species of microorganisms 24 hours after material binding. The highest zone of increased inhibition was observed in Streptococcus mutans (15.83) and the lowest

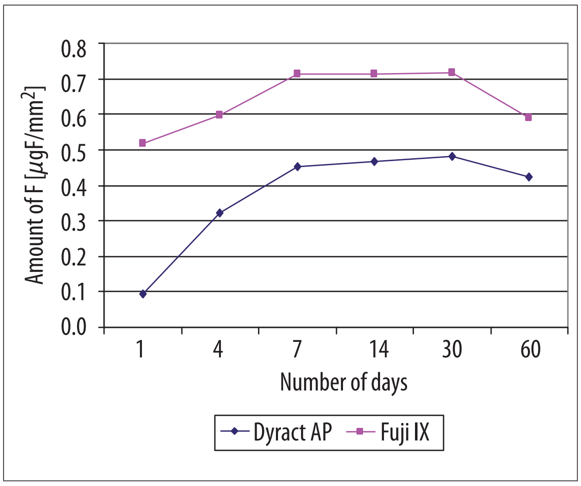

Figure 1. Amount of fluoride released in relation to time of storage in contact solution

in Streptococcus sanguis (11.83). Growth inhibition zones measured on the seventh day after material binding showed another relationship. Fuji IX inhibited the growth of *Streptococcus sanguis* and *Streptococcus salivarius*, although the differences were not statistically significant compared with the first 24 hours. There was no growth inhibition observed in *Streptococcus mutans* and *Lactobacillus casei*, which means that the material's biological activity statistically significantly decreased ($p<0.00035$, $p<0.0018$, respectively). Dyract AP (compomer) did not inhibit test bacterial growth in the first 24 hours after polymerization. A significant increase in activity towards *Streptococcus sanguis* and *Streptococcus salivarius* ($p<0.04$, $p<0.004$, respectively) was observed after eight days.

Discussion

The issues examined in this study are current and interesting to many authors. Studies on fluoride release from materials for permanent fillings of carious defects have been presented both in Polish [1,2,10,14,23–25,28,30,39,49–51] and foreign literature [3,12,13,17,18,22,40,41,43,48,52,54]. Despite the relatively abundant literature concerning the issue, comparison of the results is not easy. Fluoride release, irrespective of the kind of examined material, depends on many factors; sample size, the amount, composition, temperature, and pH of the contact solution, as well as sample load are significant. The authors of published reports used various sample sizes (from 1.7 mm to 20 mm in diameter), various kinds and amounts of contact solutions, and many measurement methods [1,3,10,12,14,17,18,22,25,28,30,39–41,43,48,50–52,54]. The comparison of study results is also difficult due to the fact that there are many fillings releasing fluoride and the choice of material depends on the investigator and his experience.

Our experiment was to evaluate the conventional glassionomer cement Fuji IX (GC Japan) and the compomer Dyract AP (Dentsply). The materials have undergone clinical assessment [31]. The results confirm fluoride release by both materials, with higher emission by Fuji IX than by Dyract AP. A similar relationship was obtained by Xu et al. [54], who examined fluoride release and compression endurance of 15 commercial filling materials. However, we could not find other studies that considered the materials chosen for our examination. On the other hand, we should stress that all authors who are involved with the issue confirm that conventional glassionomer cements release more fluoride than compomers [3,40,43,48–50,54]. This is probably connected with their chemical compositions and thus the mechanism of fluoride release. Fuji IX is a conventional glassionomer cement with a large powder/fluid ratio. It contains calcium, aluminum, fluoride, and silicon glass [54]. Dyract AP is a compomer which contains strontium, aluminum, fluoride, and silicon glass as well as strontium fluoride embedded in UDMA resin [41,54]. The mechanism of fluoride release from both glassionomer cement and compomer has not yet been explained. According to most authors, conventional glassionomer cements release fluoride from glass particles [9,35,36,40,48,54]. This happens through diffusion and immediately reaches a maximum in the first 24 hours. Then the emission decreases gradually and persists for a long period of time [3,13,14,48]. In the case of compomers, light polymerization causes resin binding and, despite the progressive acid-base reaction inside the material (similarly to that in glassionomer cements), fluoride release is slower [8,12,40,41,48].

Table 2. Mean growth-inhibition of test bacteria in mm

Kind of bacteria	No. of samples	Growth inhibition zones in mm				Statistical analysis
		Fuji IX		Dyract AP		
		I day	VIII day	I day	VIII day	
Streptococcus mutans	6	15.83*	9.00*	9.00	9.00	p<0.00035
Streptococcus sanguis	6	11.83	10.66	9.00*	10.50*	p<0.044
Streptococcus salivarius	6	12.16	10.83	9.00*	10.60*	p<0.0041
Lactobacillus casei subsp. casei	6	12.33*	9.00*	9.00	9.00	p<0.0018

According to some authors [12,14,40,41,48], the level of fluoride emission does not depend on ion concentration in the material, but on its external diffusion. This is related to material hydrophilia, as the ions are released from the side of the matrix to which water diffuses. The water content in eight different filling materials was measured by Attin et al. [cited as 40]. They showed that the water content in composite and compomer was small (0.3 and 1.6%, respectively) after 28 days. On the other hand, the water content in conventional glassionomer cement and resin-modified glassionomer was 4.8–10.3%. Many authors explain the differences in fluoride release among the examined materials by the mechanism of water sorption [3,12,17,37,40,41,48,54].

In our study we used an accumulative method of fluoride release evaluation and the measurement of emission was started after 24 hours. The highest level of fluoride ion in the solution occurred on the seventh day and was 0.7 $\mu g/mm^2$ for Fuji IX and 0.5 $\mu g/mm^2$ for Dyract AP. This level was maintained up to the 30^{th} day and then decreased in both materials. However, not all authors assess the issue unanimously. Sales et al. [41] and Vermeersch et al. [48] obtained results similar to ours in both glassionomer cements and compomers. However, a different relationship was observed by Show [43], who monitored fluoride release for six months. He found a slow increase in fluoride emission over time. Hattab's [18] and Mount's [35] results concerning glassionomer cements were similar to ours, while Forster's [14] and Wilson's [52] were different, as they suggested slow long-term increases in fluoride release from the materials. As far as compomers are concerned, the reports do not entirely confirm our results. Some investigators [12,40,54] found a stabilization of fluoride release slightly later, with emission subsequently decreasing.

Evaluation of the fluoride released by Fuji IX and Dyract AP was not the only aim of the examination. We also determined the antibacterial activities of both materials and the evaluation depended on the peak of their emission. Therefore the microbiological examination was conducted twice: immediately after material binding and seven days later.

Most reports are of short-term evaluations (24 or 48 hours after material binding) [5,11,15,16,20,21,26,29,33,42,47], while Perez et al. [38] and Botelho [6] conducted longer observations. In our study we used the agar diffusion method, similarly to most investigators [6,11,15,16,20,21,26,33,42, 47], although lately there have been reports suggesting the use of other techniques of assessment [5,29,38].

The results demonstrated that Fuji IX cement inhibited the growth of all the test bacteria for the 24 hours after fixing, while Dyract AP did not show such activity. Most reports, except for Botelho's studies [6], stress the antibacterial properties of glassionomer cements [5,11,20,21,42,47]. In the case of compomers, opinions differ. Some authors confirm our results [21,47], while others claim that compomers, like glassionomer cements, inhibit caries-producing bacterial growth [16,26,33,38].

The antibacterial activity of the dental materials used in the treatment of caries can have many causes. Chemical composition, low pH during binding, and the release of fluoride and other ions are of great importance [5,11,15, 20,21,26,31,36,42,47]. Our results suggest that the level of released fluoride can influence the ability of inhibiting caries-producing bacteria. Dyract AP, which did not show these abilities in the first 24 hours, inhibited the growth of *Streptococcus sanguis* and *Streptococcus salivarius* seven days after polymerization (i.e. after obtaining the maximum of fluoride ion release), like Fuji IX. Antibacterial effect against *Streptococcus mutans* and *Lactobacillus casei* was not present in either material. A lack of growth inhibition of some bacteria by fluoride-releasing materials was also observed by other authors [15,20,33,42]. This can occur due to bacterial species susceptibility to fluoride ion, as determined by some authors [19,44]. Yotis and Brennan observed significant differences in fluoride binding by oral bacteria. According to some authors this is the result of various ion levels and the susceptibility of sites that bind fluoride in bacteria cells. De Schepper [11] and Herrera [20] suggest that fluoride activity on bacterial cells depends not only on the amount of the ion, but also on the material's pH during binding. As shown in some studies, glassionomer cements are characterized by low pH during binding, which is maintained from several minutes to 24 hours [11,15,42,47]. The relationship between the antibacterial activity of fluoride ions and pH was also observed by Sikorska-Jaroszyńska [44], according to whom the MIC (minimum inhibition concentration) is 50 times lower in an acidic environment than in a neutral one. This relationship can explain the result concerning Fuji IX, which inhibited the growth of all the test bacteria species in the first 24 hours after binding, and after 8 days only that of *Streptococcus sanguis* and salivarius.

Our *in vitro* studies confirmed fluoride release and antibacterial activity of the selected materials against some bacterial species. However, we agree with other authors' opinions [5,20] that long-term clinical studies should be conducted in order to verify the results.

Conclusions

1. Both of the examined dental materials release fluoride ions which have an effect on their antibacterial activity.

2. Due to their properties, both Fuji IX and Dyract AP can be recommended as permanent fillings of carious defects, taking into consideration the prophylaxis of secondary caries.

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