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Antibacterial activity of selected glass ionomer cements

Aktywność przeciwbakteryjna wybranych cementów szkłojonomerowych

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

Introduction: The aim of the paper was to determine the antibacterial activity of four glass ionomer cements against bacteria of the genera *Streptococcus* and *Lactobacillus*.

Material and methods: Four capsulated glass ionomer cements were applied in the study: Fuji Triage (GC), Fuji IX (GC), Ketac Molar (3M Espe) and Ketac Silver (3M Espe). Four standard bacterial strains were used to assess the antibacterial activity of the studied cements: *Streptococcus mutans*, *S. sanguis*, *S. salivarius* and *Lactobacillus casei*. The antibacterial activity was determined by the agar diffusion method. The bacterial suspension was spread with a cotton swab on TSA plates. For each material six wells (7 mm diameter, 5 mm deep) were made with a cork borer. Each well was then filled with freshly prepared cements. The results were obtained by measuring the bacterial growth inhibition zone after 1, 2, 3 and 7 days.

Results: Fuji Triage cement inhibited the growth of all bacterial strains. Fuji IX cement demonstrated the most potent antibacterial activity against *S. sanguis.* Ketac Molar showed antibacterial activity against *S. sanguis* and *S. salivarius*, whereas Ketac Silver was efficient against *S. mutans* as well. Neither of the Ketac cements inhibited growth of the standard *L. casei* strain.

Discussion: Antibacterial activity of glass ionomer cements has attracted the interest of scientists in recent years. Most authors, including us, carried out experiments using the agar diffusion method and demonstrated antibacterial activity of glass ionomer cements. Different antibacterial activity of glass ionomer cements, observed in our study and studies of other authors, depended on the evaluated cement, bacterial strain and period of evaluation.

Key words: **glass ionomer • antibacterial activity • *Streptococcus mutans* • *S. salivarius* • *S. sanguis* • *Lactobacillus casei***

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Introduction

Procedures employed in the therapy of dental caries do not eliminate all the microorganisms from the cavity [3,8,21,22]. The bacteria left in the dentine and possible loss of marginal seal may lead to secondary caries, and consequently to diseases of the pulp [4,29,34]. During the preparation of the cavity it often turns out that total removal of decalcified dentine may cause pulp exposure. Many studies have proven that although demineralized dentine contains microorganisms, it can be temporarily left intact in order to prevent the pulp from being exposed [6,22]. Such treatment is acceptable, provided that only such materials and medicaments are chosen which possess antibacterial activity against cariogenic bacteria [23,31]. It has also been established that advantageous properties of glass ionomer cements, such as adhesion to dental tissues, biocompatibility and high fluoride release rate allow for the use of such materials in many clinical situations [2,14,15,36,39].

There are many glass ionomer cements available on the dental market. They may consist of two separately packaged ingredients, namely a powder and a fluid, which are mixed in the desired proportion by a physician before application. Some cements are delivered in capsules, which provides constant proportion of the components and may be of great significance when comparing properties of different preparations. According to some authors, the difference in powder/fluid ratio may influence the physical properties of the filling [28].

By modifying glass ionomer cements, the manufacturers strive not only to improve their mechanical and aesthetic properties, but according to a new approach to the treatment of carious disease, to increase their antibacterial activity as well. Therefore, it seems necessary to compare materials from this group with products that have been available on the dental market for many years.

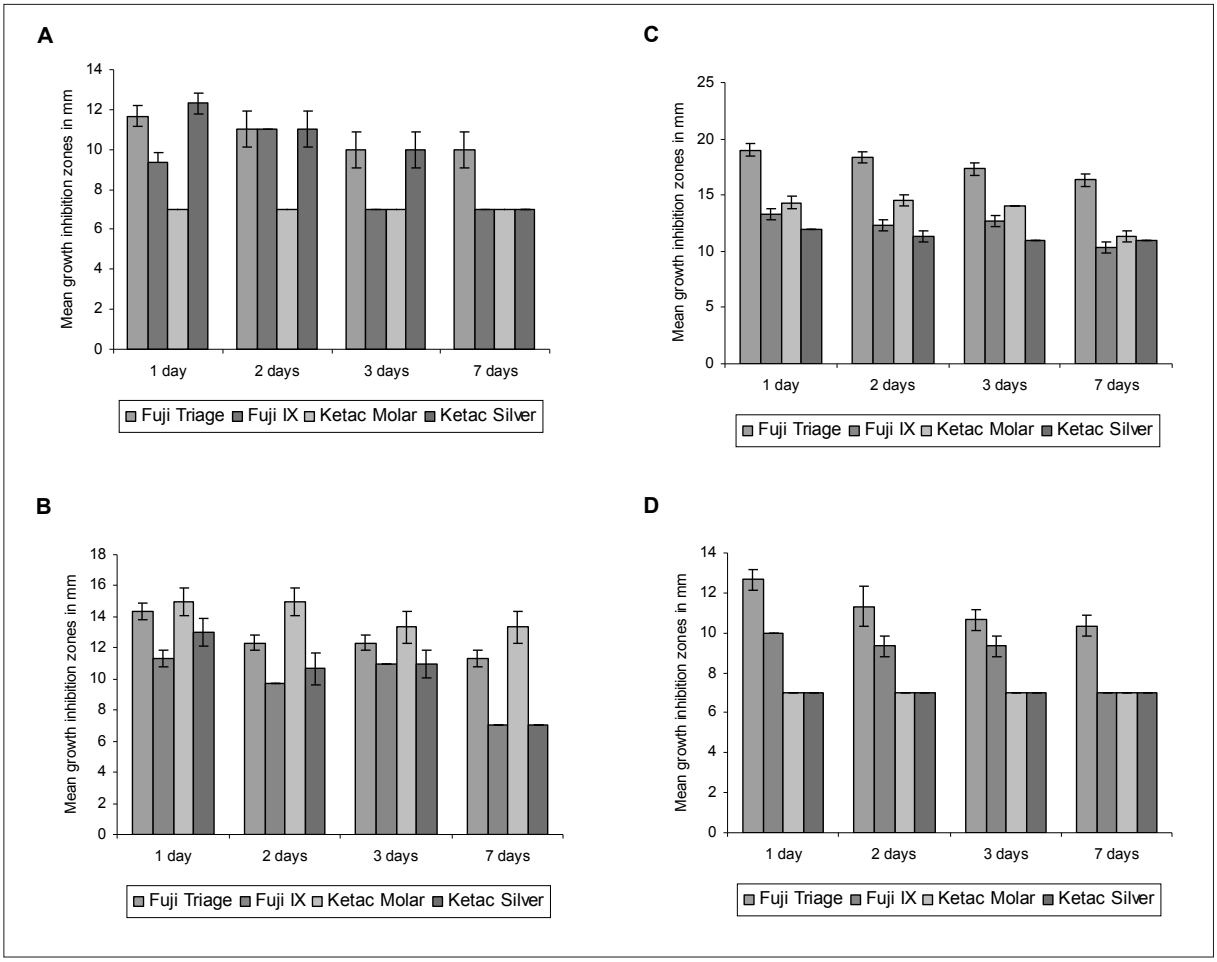

Fig. 1. Mean growth inhibition zones (in mm) of *S. mutans* (A), *S. salivarius* (B), *S. sanguis* (C) and *L. casei* (D) by four tested glass ionomer cements

Thus, the aim of this study was to determine antibacterial activity of four capsulated glass ionomer cements used to restore caries cavities.

Materials and methods

Four capsulated glass ionomer cements – Fuji Triage (GC), Fuji IX (GC), Ketac Molar (3M Espe), and Ketac Silver (3M Espe) – were included in the study (Table 1). Each material was prepared immediately before application according to the manufacturers' instructions.

The experiment was carried out using four different reference bacterial strains: *Streptococcus mutans ATCC 35668*, *Streptococcus sanguis ATCC 10556*, *Streptococcus salivarius ATCC 13419*, and *Lactobacillus casei ATCC 393.*

Bacteria were cultured on Columbia agar plates supplemented with 5% sheep blood (Emapol). The *Streptococci* were incubated for 18 hours at 37°C in an aerobic atmosphere, and *L. casei* in an atmosphere with 5% carbon dioxide.

Antibacterial activity of the studied restorative cements against reference strains was determined by an agar diffusion method using solid tryptic-soy agar medium (TSA, Tryptic Soy Agar, Oxoid) [11]. After an 18-hour incubation period, suspensions of McFarland 0.5 were prepared in 0.85% NaCl [30].

Wells of 7 mm in diameter and 5 mm deep were cut in the agar with a cork borer, six for each material. The bottom of each well was sealed with 10 µl of liquid TSA. Bacterial suspension was spread on the medium surface using cotton swabs and then wells were filled with *ex tempore* prepared cements. The plates were left at room temperature for 30 minutes and then incubated at 37°C for 7 days. In order to control the growth of standard

Table 1. Materials tested in the study

Materials	Batch number	Manufacturer
Fuji Triage Capsule	0806051	GC Corporation, Tokio, Japan
Fuji IX GP Capsule	0904201	GC Corporation, Tokio, Japan
Ketac™ Molar Aplicap™	366335	3M ESPE AG, Seefeld, Germany
Ketac™ Silver Aplicap™	328538	3M ESPE AG, Seefeld, Germany

Table 2. Mean growth inhibition zones in mm of tested bacteria (SD)

Days	Material	*S. mutans*	*S. salivarius*	*S. sanguis*	*L. casei*
		mean (SD)	mean (SD)	mean (SD)	mean (SD)
1	Fuji Triage	11.67 (0.52)	14.33[a] (0.52)	19.00 (0.52)	12.67 (0.52)
	Fuji IX	9.33[a] (0.52)	11.33 (0.52)	13.33 (0.52)	10.00 (0.00)
	Ketac Molar	7.00 (0.00)	15.00[a] (0.89)	14.33 (0.52)	7.00[a] (0.00)
	Ketac Silver	12.33[a] (0.52)	13.00 (0.89)	12.00 (0.00)	7.00[a] (0.00)
2	Fuji Triage	11.00[b,c] (0.89)	12.33 (0.52)	18.33 (0.52)	11.33 (1.03)
	Fuji IX	11.00[b,d] (0.00)	9.67[b] (0.00)	12.33 (0.52)	9.33 (0.52)
	Ketac Molar	7.00 (0.00)	15.00 (0.89)	14.50 (0.55)	7.00[b] (0.00)
	Ketac Silver	11.00[c,d] (0.89)	10.67[b] (1.03)	11.33 (0.52)	7.00[b] (0.00)
3	Fuji Triage	10.00[e] (0.89)	12.33[c] (0.52)	17.33 (0.52)	10.67 (0.52)
	Fuji IX	7.00[f] (0.00)	11.00[d] (0.00)	12.67 (0.52)	9.33 (0.52)
	Ketac Molar	7.00[f] (0.00)	13.33[c] (1.03)	14.00 (0.00)	7.00[c] (0.00)
	Ketac Silver	10.00[e] (0.89)	11.00[d] (0.89)	11.00 (0.00)	7.00[c] (0.00)
7	Fuji Triage	10.00 (0.89)	11.33 (0.50)	16.33 (0.52)	10.33 (0.52)
	Fuji IX	7.00[g,h] (0.00)	7.00[e] (0.00)	10.33[a] (0.52)	7.00[d,e] (0.00)
	Ketac Molar	7.00[g,i] (0.00)	13.33 (1.03)	11.33[b] (0.52)	7.00[d,f] (0.00)
	Ketac Silver	7.00[h,i] (0.00)	7.00[e] (0.00)	11.00[a,b] (0.00)	7.00[e,f] (0.00)

Mean values in the columns indicate no significant differences ($p>0.05$) in post hoc Tukey test

strains, the microorganisms were additionally cultured on plain TSA medium.

The results were obtained by measuring the diameter of microbial inhibition zones (in millimeters, including the diameter of the well) after the first, second, third and seventh day of incubation. No growth inhibition was defined as the diameter of the well, which is 7 mm in our study.

All results were subjected to statistical analysis (post hoc Tukey test). Statistical significance was set at $p < 0.05$.

Results

Figure 1A demonstrates the activity of the studied cements against the *S. mutans* strain. Fuji Triage cement showed the most stable antibacterial activity, persisting for 7 days, while Ketac Silver exhibited a shorter duration of antibacterial activity (up to 3 days). The antibacterial activity of Fuji IX lasted for only 2 days, whereas Ketac Molar exhibited no growth inhibition during the entire study period.

The growth of *S. salivarius* (fig. 1B) was inhibited by each of the studied cements on the first day of culture. The antibacterial effect of each cement remained at a high level until day three. Ketac Molar and Fuji Triage were the only cements that inhibited the growth of *S. salivarius* on day 7.

S. sanguis turned out to be the most susceptible strain to the studied glass ionomer cements (fig. 1C). Four cements retained antibacterial activity against this strain until day 7. Fuji Triage cement demonstrated the highest antibacterial activity during the entire study period when compared with other cements.

Fuji Triage turned out to be the most effective. It inhibited growth of *Lactobacillus* during the entire study period. Fuji IX exhibited poorer, lasting for only 3 days, activity against this strain. Both Ketac cements did not inhibit the growth of *L. casei* (fig. 1D).

The mean bacterial growth inhibition zones induced by studied cements, standard deviations (SD) and lack of statistical significance are presented in Table 2.

Discussion

The primary goal of each treatment is to preserve or restore the function of the damaged organ by arresting the disease process itself or preventing its recurrence. The same applies to caries.

The basic stage of its invasive treatment is the removal of carious tissues. Many authors report that the removal of carious dentine according to Black's criteria does not completely eliminate bacterial flora from the carious cavity [3,8,21,22]. Caries recurrence may also be caused by microleakage enabling the microorganisms to get into the gap between the filling and dental tissues [4,29,34]. The results of clinical and laboratory studies indicate that it is clinically insignificant if the number of bacteria left in the cavity after its preparation is lower than CFU/ml<10^2, [3,20,21,22] provided that the filling is properly sealed. The threat for dentine pulp complex is therefore avoided. Banerjee et al. concluded that it is advisable to use restorative materials that provide a long-term seal and antibacterial activity against cariogenic strains [3].

Antibacterial properties of lining and permanent restorative materials are described in the available literature. The sterility was achieved in only 61.4% of cavities filled with calcium hydroxide based preparations and 81.8% of cavities filled with zinc oxide-eugenol cements [23]. Due to their poor durability, high solubility and unsatisfactory marginal seal, such materials may only be used as the first lining layer [7,23]. Composite materials and amalgam offer many advantages and are recommended as a permanent filling for caries cavities. According to the results of some studies, the amalgam shows antibacterial potential, while composite resins do not exhibit such activity against cariogenic strains [5,7,18,32].

Antibacterial activity of glass-ionomer cements has attracted the interest of scientists in recent years [7,9,12,13,17,18,24,26,33,36,37,38]. The methodology of studies concerning the antimicrobial properties of dental materials presented in the literature varies greatly, which in turn hampers the comparison of study results. Most authors, including us, have carried out their experiments using the agar diffusion method, [9,12,13,17,18,24,26,35,37,38], although some suggested other techniques e.g. DCT (Direct Contact Test) [7,24,33]. The study period varied as well. Most of them were short-term studies (24 or 48 hours), and only a few were conducted over a longer period of time [9,24,26,33].

Our 7-day study revealed that Fuji Triage cement exhibited the best antibacterial activity. However, our results cannot be compared with other studies because the material has become available on the dental market only relatively recently.

Fuji IX cement demonstrated the best growth inhibition activity against *S. sanguis*. Growth inhibition of *L. casei* and *S. salivarius* was observed until day 3, whereas the growth of *S. mutans* was inhibited only until the second day of the experiment. Similarly high activity of this material against *S. sanguis* was observed by Marczuk-Kolada [26]. She reported no growth inhibition zone for *S. mutans* and *L. casei* after 8 days, which was confirmed in our study as well [26].

We observed that the antibacterial activity of Ketac Molar cement was limited to *S. salivarius* and *S. sanguis* only. Other authors reported that it inhibited the

growth of *S. mutans* as well [7]. They compared Ketac Molar cement with zinc oxide-eugenol cement and demonstrated that antibacterial activity of the former lasted for only two days, whereas the latter was effective during the entire seven-day study period [7]. Similar growth inhibition of *S. mutans* by studied glass ionomers was noted in these experiments as well (Ketac Molar and Photac Fil) [7].

The lack of growth inhibition of *L. casei* by Ketac Silver cement observed by other authors was confirmed in our study as well [17,25].

Many factors may influence the antibacterial activity of glass-ionomer cements, including chemical composition, release of fluoride and other ions, and low pH value during setting [7,12,17,18,26,35,38]. The literature concludes that each of the studied glass ionomers releases fluoride ions, yet the release rate varies [1,19]. Varying growth inhibition patterns may result from microbial species-specific sensitivity to fluoride ions [10,16,27]. Yotis and Brenan [40] observed significant differences in fluoride ion binding by bacteria colonizing the oral cavity. According to them, it is related to the number and affinity of ion binding sites in bacterial cells. De Schepper [12] and Herrera [17] suggested that the efficiency of fluoride ions depends not only on their amount, but also on the pH value of the material during setting. The results of the studies indicate that the fluoride activity against cariogenic bacteria increases in an acidic environment [27]. Glass ionomer cements have a low pH value during setting, lasting from several minutes to 24 hours [12,35,38].

According to the current state of knowledge, fluoride-releasing adhesives, including glass ionomer cements, should be considered as an important group of restorative materials [18,29]. Many authors emphasize the significance of their antibacterial activity, resulting largely from the release of fluoride ions [3,7]. Recent interest in this problem has resulted in reports assessing the properties of glass ionomers containing other antibacterial substances e.g. chlorhexidine [9,37]. Perhaps, what we are witnessing today is the emergence of a completely new direction in the development of restorative materials.

In conclusion, the four evaluated glass ionomer cements demonstrated antibacterial properties. Growth inhibition was different for each cement, bacterial strain and study period. From the standpoint of prophylaxis of secondary caries, glass ionomer cements can be recommended for temporary and permanent caries cavity filling.

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