Antimicrobial action of various polyacrylic acids on streptococcus mutans and actinomyces viscosus

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Summary

The aim of this study was to determine the antimicrobial effects of various polyacrylic acids (PAA, E9, Copolymer) on *Streptococcus mutans* Ingbritt NCTC 10449 and *Actinomyces viscosus* NCTC 9935. Petri dishes were filled with sterile agar and 5 mm in diameter wells were produced into the agar plates. The acid solutions were placed into each well in the agar plates. Plates were incubated in a high CO_2 atmosphere at 37°C for 48 hours. After the incubation period, the plates were observed for zones of bacterial inhibition around each well. The sizes of the zones were measured in millimeters with a dial calliper. For statistical analysis of the findings, Mann-Whitney U Test was used.
The results showed that copolymer had got the most inhibitory effect against *Streptococcus mutans* in both dialyzed and non-dialyzed form when it was compared with other polyacrylic acids used in the study. The differences between the inhibitory effects of polyacrylic acids against Streptococcus mutans and *Actinomyces viscosus* were found statistically significant ($p < 0,05$). In conclusion, the acid solutions evaluated in this study showed different inhibitory effects depending on structural properties. Non-dialyzed forms of the acids were found more effective on both microorganisms than dialyzed form. *Actinomyces viscosus* was more sensitive against acid solutions than *Streptococcus mutans.*
Keywords: polyacrylic acids, antimicrobial effect, glass-ionomer cements, *Streptococcus mutans, Actinomyces viscosus.*

Introduction

Microorganisms, which can enter a tooth restoration interface through micro leakage, have been implicated by numerous investigators as causes of postoperative sensitivity, recurrent caries and pulpal inflammation [1,2,3]. Various dental materials are being developed in attempt to solve some of these problems. The antimicrobial properties of one such material, glass-ionomer cement, have been confirmed [4,5]. This antimicrobial activity appears to be caused by fluoride release [4,5,6] or low pH levels on setting [7,8,9] because of the structure of the polyacrylic acid in the cement.

The acidity of glass-ionomer cements is caused by polyalkenoic acids and other acidic components. Polyalkenoic acids appear to be a key factor in antibiosis [7,8] and are present in all formulations of these cements, in either or both the liquid and the powder components [10]. Maltz and Emilson [11] confirmed that low pH enhanced the bactericidal effect of the fluoride. Therefore the purpose of this study was to evaluate the antimicrobial activity of various dialyzed and non-dialyzed polyacrylic acids on *Streptococcus mutans* and *Actinomyces viscosus.*

Material and Methods

Three different experimental polyacrylic acid solutions at 10% concentrations in both dialyzed and nondialyzed forms were tested to determine their ability to inhibit the growth of *Streptococcus mutans* and *Actinomyces viscosus (Table 1).* Plates were used for inhibition of bacterial growth by acid solutions.

The antibacterial activities of the materials (n = 6) were evaluated using the standard agar diffusion methodology. The antibacterial activi-

ties of the above materials were investigated against *Streptococcus mutans* Ingbritt NCTC 10449 and *Actinomyces viscosus* NCTC 9935.

A total of 24 agar plates were evenly divided into two bacteria groups: *Streptococcus mutans* Ingbritt NCTC 10449 and *Actinomyces viscosus* NCTC 9935 and inoculated with each species. Cultures were transferred into a glass tube containing approximately 10 ml of sterile water using a sterile inoculating loop. A cotton wool swab was dipped into the tube and, spreading the bacteria over the agar medium and 0.1 ml quantities were streaked across the surface of the plate in two directions and then spread. Wells of 5 mm in diameter were made in the agar using a sterile cork borer. Powders of the acids were then dissolved in distilled water to obtain 10% concentration solutions and were placed into each well in the agar plates using with a sterile syringe. All procedures were performed using aseptic techniques.

Immediately after placement of the materials into the wells, the plates were incubated in a high CO_2 atmosphere at 37°C for 48 h. The inhibition zone diameters surrounding the material, a measure of the susceptibility of the microorganisms and antibacterial power of the materials, were measured with a dial calliper.

Well diameters and inhibition zone diameters were measured for each plate at the same point and subtracted one from the other. To compensate for any irregularity in the shape of the zone, the diameters were measured twice, the second measurement at right angle to the first. The inhibition zone diameter was the difference between the average of these diameters and well diameter.

The diffusibility on the agar gel of each polyacrylic acid was determined by color change, the acid turning the initially violet agar gel to yellow. After comparison of inhibition zones and diffusion zones of each acid, it was shown that they were similar. Differences in inhibition zone diameters were analyzed using a Mann-Whitney U test and ANOVA-Kruskal Wallis Test.

Results

In the *S. mutans* group, in both dialyzed and non-dialyzed form, the inhibition zone diameters of Copolymer were bigger than E9 and PAA. In the *A. viscosus* group, the diameters of inhibition zones of dialyzed E9 and dialyzed Copolymer were bigger than dialyzed PAA's and those of non-dialyzed E9 and non-dialyzed PAA were

Table 1. Materials Used (10% acid solutions)

Materials	Type	Property
PAA	Polyacrylic acid	Nondialyzed
PAA	Polyacrylic acid	Dialyzed
E9	Polyacrylic acid	Nondialyzed
E9	Polyacrylic acid	Dialyzed
Copolymer	Polyacrylic acid, maleic acid	Nondialyzed
Copolymer	Polyacrylic acid, maleic acid	Dialyzed

Table 2. Inhibition zone diameters, mean and standard deviation (cm) of various polyacrylic acids

		S. mutans (n = 6) X ± SD	A. viscosus (n = 6) X ± SD
Dialyzed	E9	1.11 ± 0.006	1.61 ± 0.008
	PAA	1.10 ± 0.006	1.51 ± 0.007
	Copolymer	1.22 ± 0.008	1.60 ± 0.006
Nondialyzed	E9	1.14 ± 0.008	1.73 ± 0.008
	PAA	1.15 ± 0.008	1.74 ± 0.01
	Copolymer	1.44 ± 0.006	1.68 ± 0.009

X: Mean; SD: Standard Deviation

Table 3. The comparison of the materials used in the study

	Dialyzed		Nondialyzed	
	X^2	p	X^2	p
E9 $_{(S-A)}$	12.98	0.002	12.10	0.002
PAA $_{(S-A)}$	12.99	0.002	12.56	0.002

Table 4. Statistical comparison of the two materials

		Dialyzed p	Nondialyzed p
	E9-PAA	p = 0.171*	p = 0.604*
S.mutans	E9-Copolymer	p = 0.003	p = 0.003
	PAA-Copolymer	p = 0.003	p = 0.003
	E9-PAA	p = 0.003	p = 0.155*
A.viscosus	E9-Copolymer	p = 0.150*	p = 0.003
	PAA-Copolymer	p = 0.003	p = 0.004

* not significant

Table 5. Significance of differences in the inhibition zone diameters for dialyzed and nondialyzed polyacrylic acid: Streptococcus mutans vs Actinomyces viscosus

	Dialyzed	Nondialyzed
	p	p
E9 $_{(S-A)}$	0.003	0.003
PAA $_{(S-A)}$	0.003	0.004
Copolimer $_{(S-A)}$	0.003	0.003

p < 0,05: Statistical significant difference; (S-A): *S. mutans* vs. *A. viscosus* for same polyacrylic acid.

Table 6. Significance of differences in the inhibition zone diameters for Streptococcus mutans and Actinomyces viscosus: Dialyzed vs nondialyzed polyacrylic acids

	S. mutans	A. viscosus
	p	p
E9 $_{(D-ND)}$	0.003	0.003
PAA $_{(D-ND)}$	0.003	0.004
Copolimer $_{(D-ND)}$	0.003	0.003

p < 0,05: Statistical significant differencem; (D-ND): Dialyzed vs. nondialyzed form of same polyacrylic acid

higher than those of non-dialyzed Copolymer *(Table 2).*

Table 2, 3 and *4* show the inhibition zone diameters and significance of differences for each acid and bacteria group. They show that Copolymer had a significantly higher inhibitory effect against *S. mutans* in both dialyzed and non-dialyzed form than any of the other polyacrylic acids (p = 0.003).

Comparing between bacteria groups, the mean inhibition zone diameters of all the polyacrylic acids were significantly larger in the S. mutans than those in the A. viscosus group *(Table 5)* (p < 0.05). The inhibition zone diameters were significantly larger for the non-dialyzed than the dialyzed form of each polyacrylic acid in each bacterial group *(Table 6)* (p < 0.05).

Discussion

The antibacterial property of glass-ionomer cements play an important role in their successful use in restorative and endodontic dentistry. Luglie et al. [12] found that in the majority of

cases, prepared cavities, even if ready for restoration, are still septic. Other studies have reported the presence of bacteria in dentine tubules and cementum even after treatment [13,14,15]. To prevent the growth of these bacteria, endodontic filling materials should have an antibacterial effect; hence, this investigation of the antibacterial activities of the various polyacrylic acids found in glass-ionomer cements.

Smith and Ruse [16], investigating the initial acidity of glass-ionomer cements, reported the initial pH of these materials to be below 3. Woolfard [10] reported the surface pH of glass-ionomers to remain low for the first hour of setting. Mc Comb and Ericson found a simple correlation between surface pH of these materials and antibacterial activity [9]. De Schepper et al. [7,8], investigating the effectiveness of freshly mixed glass-ionomer cements and light-cured glass-ionomers against *Streptococcus mutans*, reported that antibacterial activity was dependent upon a lower pH.

Our findings are consistent with the findings of these studies. The polyacrylic acids were dialyzed in this study. The process of separation of the low molecular weight compounds of a structure from high molecular weight ones using a semi permeable membrane is described as dialization [17].

The findings showed that all of the polyacrylic acid solutions were also effective against both bacteria. The diameter of the inhibition zones produced by the solutions was greater for *Actinomyces viscosus* than for *Streptococcus mutans.* The results are not surprising, as previous studies have reported aciduric properties from Streptococcus mutans [18]. So it can be thought that Actinomyces viscosus is a more acid-sensitive microorganism than *Streptococcus mutans.* A more careful examination of the inhibition zone sizes produced by the polyacrylic acid solutions evaluated in this study reveals a greater sensitivity of Actinomyces viscosus to the factors responsible for the antibacterial activity of the solutions. The less aciduric nature of this microorganism compared to that of *Streptococcus mutans* [19,20] confirmed the greatest importance of acidity to the antibacterial properties of the dental materials.

Copolymer had the highest inhibitory effect against Streptococcus mutans in both dialyzed and non-dialyzed form, which we attribute to its acidic significantly different copolymeric structure.

Since *Actinomyces viscosus* was more sensitive to low pH, inhibition zones of polyacid solutions against *Actinomyces viscosus* were determined as being bigger than inhibition zones against Streptococcus mutans. The differences between inhibition of *Streptococcus mutans* and *Actinomyces viscosus* by dialyzed or non-dialyzed polyacids used in this study were found statistically significant. In addition to this, there were statistical significant differences between inhibition effects of non-dialyzed and dialyzed polyacids against *Streptococcus mutans* or Actinomyces viscosus. The results of the study showed that the differences between the diameters of inhibition zones produced by non-dialyzed and dialyzed polyacids against *Streptococcus mutans* were determined less than other microorganism's. So, it was decided that generally, acidic condition was more effective on inhibition of *Actinomyces viscosus* than inhibition of *Streptococcus mutans* and nondialised polyacids have got more inhibitory effect on both *Streptococcus mutans* and *Actinomyces viscosus* than dialyzed polyacids but *Actinomyces viscosus* was more sensitive to non-dialyzed polyacid solutions than *Streptococcus mutans.* According to these findings, it appears that the high molecular weight compounds have more antibacterial effects on *Streptococcus mutans* and *Actinomyces viscosus* than low molecular weight ones.

In our opinion, the findings of this study will be able to give manufacturers some ideas about antibacterial properties of produced materials, which have low or high molecular weights and different structural properties.

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