



Comparative evaluation of antibacterial properties of eighth generation bonding agent modified with 0.12% Chitosan, 0.2% Chitosan and 7% Arginine against *Lactobacillus Fermentum*: An In-vitro study

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Abstract

Aim: this study investigated antibacterial properties of eighth generation bonding agent modified with 0.12% chitosan, 0.2% chitosan and 7% arginine against *lactobacillus fermentum*.

Materials and Methods: 0.12% and 2% (w/w) of chitosan was added to adhesive. 7%(w/w) arginine was added to adhesive. Experimental adhesive was prepared in a dark room under controlled temperature and humidity. A sterile 10 wells were coated with 15 μ l of 8th generation bonding agent, its modifications with 0.12% chitosan, 0.2% chitosan & 7% arginine respectively for each group. Then 10 μ l bacterial suspension of *lactobacilli* was placed on bonding agent for 1hr at 37°C. Afterwards BHI broth (235 μ l) was added to each well and gently mixed for 2 min. Group 1 – control group Group 2 - 8 th generation bonding agent modified with 0.12% chitosan. Group 3 – 8 th generation bonding agent modified with 0.2% chitosan. Group 4 – 8 th generation bonding agent modified with 7% of arginine. After 48 hours bacterial growth was examined by turbidimetric analysis using spectrophotometer.

Statistical analysis: One way Analysis of Variance (ANOVA) test was performed for intergroup comparison, followed by post hoc test.

Results: Amongst all the experimental groups, highest antibacterial activity (lowest turbidity) was shown by group 3- eighth generation bonding agent modified with 0.2% chitosan (Mean=463.5 \pm 37.04). There was no statistically significant difference between antibacterial properties of group 2 -modification with 0.12% chitosan and group 4- modification with 7% arginine. Of all the experimental groups lowest antibacterial properties was shown by group 1 -control group- eighth generation bonding agent (Mean=722.1 \pm 42.68)

Conclusion: Of all the experimental groups, Group 3 (8th generation bonding agent modified with 0.2% chitosan) showed highest antibacterial activity, i.e lowest turbidity. And Group 1-control group (eight generation bonding agent) showed lowest antibacterial activity, i.e highest turbidity.

Key words: Antibacterial properties, Arginine, chitosan, 8th generation bonding agent, Lactobacillus Fermentum.

Introduction

The oral microbial flora is rich and enormously varied. An imbalance in the microbial flora can lead to the production of acidic compounds which leads to caries that causes the destruction of the teeth.¹ Like streptococcus mutans, Lactobacilli have been accompanying dental caries for over a century. One of the dominant species include Lactobacillus fermentum.²

Secondary caries is the most common cause of restoration failure, seemingly linked with residual bacteria and microleakage.³ Thus the ability of the restorative material to eliminate these bacteria would be advantageous to prevent microleakage and secondary caries. Hence, one of the bioactive functions proposed for these materials is their anti-bacterial activity.⁴

Introduced in 2010, Eighth-generation adhesives combine etching, priming, and bonding in one bottle when used in the self-etch mode, but can also be used with a total-etch or selective-etch technique.⁵

The characteristic biodegradation of the interface between the tooth and the adhesive layer produces crevices that are readily colonized by caries pathogens. Those crevices can be the consequences of polymerization shrinkage and improper resin based composite layering.⁶ Although it is improbable that biofilms can be eliminated from the crevices, the manufacturing of novel dentin adhesives that can shift the microbial ecology from a disease to a health state is greatly desirable.⁷

In recent years, the use of natural nontoxic antimicrobials for the control and prevention of dental caries has occurred. Chitosan produced from chitin, a multipurpose hydrophilic polysaccharide, has a broad antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible.⁸ Also, arginine, a prebiotic amino acid, has been shown to affect the oral biofilms ecology.⁹

Experimental:

Preparation of adhesive:

1. Chitosan modified adhesive: 0.12% and 2% (w/w) of chitosan solution was added to adhesive. Chitosan solution was prepared by dissolving chitosan powder in 1%(v/v) acetic acid as per formula 1%=1gm/10ml.
2. Arginine modified adhesive: 7%(w/w) arginine was added to adhesive. Experimental adhesive was prepared in a dark room under controlled temperature and humidity.

Sample preparation:

A sterile 10 microtiter wells were coated with 15µl of 8th generation bonding agent, its modifications with 0.12% chitosan, 0.2% chitosan & 7% arginine respectively for each group. Then 10µl bacterial suspension of lactobacilli was placed on bonding agent for 1hr at 37°C. Afterwards BHI broth (235µl) was added to each well and gently mixed for 2 min.

Experimental groups:

Group 1 – control group (n=10)

Group 2 - 8th generation bonding agent modified with 0.12% chitosan. (n=10)

Group 3 – 8th generation bonding agent modified with 0.2% chitosan. (n=10)

Group 4 – 8th generation bonding agent modified with 7% of arginine. (n=10)

After 48 hours bacterial growth was examined by turbidimetric analysis using spectrophotometer.

Statistical analysis:

Statistical analysis was performed using Statistical Package for Social science (SPSS) version 21 for Windows (SPSSInc Chicago, IL). Descriptive quantitative data was expressed in mean and standard deviation respectively. Data normality was checked by using Shapiro – Wilk test. Confidence interval was set at 95% and probability of alpha error (level of significance) set at 5%. Power of the study was set at 80%. Overall intergroup comparison among four groups in relation to antibacterial activity was done using One-way Anova ‘F’ test followed by Tukey’s post hoc test for pairwise intergroup comparison between each group.

Results:

Table 1: Turbidimetric analysis of eighth generation bonding agent modified with 0.12% chitosan, 0.2% chitosan and 7% arginine against lactobacillus fermentum of each sample by turbidimetric analysis in the form of light absorbance unit

Sr no.	Group 1 (Control)	Group 2 (0.12% Chitosan)	Group 3 (0.2% Chitosan)	Group 4 (7% Arginine)
1	730	503	417	503
2	731	521	409	518
3	812	472	426	479
4	696	439	435	441
5	687	503	419	457
6	647	448	406	442
7	743	419	423	424
8	722	446	406	438
9	735	472	416	462
10	718	412	396	418
AVERAGE	722.1	463.5	415.3	458.2

Table 2: Descriptive statistics of turbidimetric analysis of eighth generation bonding agent modified with 0.12% chitosan, 0.2% chitosan and 7% arginine against lactobacillus fermentum

	Mean	SD	SE	Minimum	Maximum
Group 1 (Control)	722.1	42.68	13.49	647.0	812.0
Group 2 (0.12% Chitosan)	463.5	37.04	11.71	412.0	512.0
Group 3 (0.2% Chitosan)	415.3	11.37	3.59	396.0	435.0
Group 4 (7% Arginine)	466.2	23.85	3.17	424.0	518.0

Table 3: Comparative statistics of a turbidimetric analysis of eighth generation bonding agent modified with 0.12% chitosan, 0.2% chitosan and 7% arginine against lactobacillus fermentum

	Mean	SD	One way Anova F test	P value, Significance
Group 1 (Control)	722.1	42.68	F = 177.47	p < 0.001**
Group 2 (0.12% Chitosan)	463.5	37.04		
Group 3 (0.2% Chitosan)	415.3	11.37		
Group 4 (7% Arginine)	466.2	23.85		
Tukey's post hoc test for pairwise comparison				
Group	Comparison Group	Mean Difference	P value, Significance	
Group 1 (Control) vs	Group 2 (0.12% Chitosan)	258.6	p<0.001**	
	Group 3 (0.2% Chitosan)	306.8	p<0.001**	
	Group 4 (7% Arginine)	263.9	p<0.001**	
Group 2 (0.12% Chitosan) vs	Group 3 (0.2% Chitosan)	48.2	p=0.013*	
	Group 4 (7% Arginine)	5.3	p=0.984	
Group 3 (0.2% Chitosan) vs	Group 4 (7% Arginine)	14.38	p =0.032*	

*p<0.05 – significant difference **p<0.001 – highly significant difference

Amongst all the experimental groups, highest antibacterial activity (lowest turbidity) was shown by group 3- eighth generation bonding agent modified with 0.2% chitosan (Mean=463.5±37.04).

There was no statistically significant difference (p>0.05) between antibacterial properties of group 2 -modification with 0.12% chitosan and group 4- modification with 7% arginine.

Of all the experimental groups lowest antibacterial properties was shown by group 1 -control group- eighth generation bonding agent (Mean=722.1±42.68).

Discussion:

There are chances of restoration failure after placement due to the causes like residual bacteria and microleakage. The crevices in previous restorations together with frequent acidification enhances the colonization of aciduric microflora including *Lactobacillus fermentum*. So the antibacterial activity of bonding system is an important factor.¹⁰

8th generation bonding agent uses Nano-sized fillers which increases the penetration of resin monomers and hybrid layer thickness improving the mechanical properties of adhesion.¹¹ In this study, 8th generation bonding agent was modified with 0.12% chitosan, 0.2% chitosan and 7% arginine.

Amongst the all group 1 (control group) showed the least antibacterial activity, i.e highest turbidity. Adhesive system contains HEMA which partly aided antibacterial properties reducing the pH. By some previous studies it is not acidic enough to reach the lethal pH level for certain bacteria.^{12,13}

Among all the tested groups highest antimicrobial activity was shown by group 3 (8th generation bonding agent modified with 0.2% chitosan), followed by group 4 (modification with 7% arginine).

chitosan's antibacterial characteristics is may be due to the collaboration between positively charged chitosan and negatively charged bacterial cell which could change the bacterial cell permeability leading to escape of intercellular substances and cell death. So as the concentration of chitosan in adhesive increases, its antibacterial activity increases.¹⁴

Arginine entering in mouth can be metabolized by certain bacteria via arginine deiminase pathway (ADI pathway) to yield ammonia which neutralizes glycolytic acids and maintains relatively neutral environmental pH which can be less favourable to oral microflora.¹⁵

There was no statistically significant difference between antimicrobial activity of group 2 (0.12% chitosan) and group 4 (7% arginine).

This result suggests that both 0.12% chitosan and 7% arginine has almost similar antibacterial activity on lactobacillus.

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