



Antimicrobial Properties, Anti-Virulence Activities, and Physico-mechanical Characteristics of Orthodontic Adhesive Containing C-Phycocyanin: a Promising Application of Natural Products

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Abstract

Introduction: Antimicrobial orthodontic adhesives aim to reduce enamel demineralization, white spot lesions, and incipient tooth decay around bonded orthodontic brackets, but they should not imperil its mechanical properties.

Aim: To evaluate the antimicrobial and physico-mechanical properties of acrylic containing different concentrations of C-phycoyanin on *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*.

Materials and methods: The mechanical properties of acrylic resins were measured by flexural strength test after preparation of acrylic resin samples with concentrations of 1%, 2%, 5%, 7.5%, and 10% of C-phycoyanin. Then we evaluated the antimicrobial effects of acrylic resin containing the maximum concentration of C-phycoyanin with clinically acceptable flexural strength and the changes in expression of virulence factors.

Results: The highest and lowest means of flexural strength were obtained in acrylic resins containing 0% and 10% concentrations of C-phycoyanin at 50.2±4.5 and 30.1±3.3 MPa, respectively. Adding 1%, 2%, and 5% of C-phycoyanin showed no significant decrease in flexural strength ($p>0.05$). The maximum mean diameter of the growth inhibition zone was observed around discs containing 5% of C-phycoyanin. Until day 30 of the study, no microbial biofilms were formed on any acrylic disc. Only microbial biofilms of *C. albicans* were able to form on discs containing 5% of C-phycoyanin at 90 days. 5% C-phycoyanin could significantly decrease the expression levels of *gtfB*, *hsp16*, and *ALS9* 6.1-, 7.3-, and 3.9-fold, respectively.

Conclusions: It can be concluded that the most acceptable concentration of C-phycoyanin in acrylic resin is 5% based on the results of flexural strength tests and antimicrobial activities of acrylic resin containing various concentrations of C-phycoyanin.

Keywords

acrylic resins, antimicrobial activity, C-phycoyanin, flexural strength, gene expression

INTRODUCTION

Cold-cure acrylic resin is very important in dentistry as it is used in orthodontic devices such as functional devices, retainers, and temporary prostheses. These resins are often composed of methacrylates, especially methyl methacrylate and polyethyl methacrylate, and copolymers.^{1,2} Polymethyl methacrylate (PMMA) has good properties including ease of use and low cost. However, the use of this substance in dentistry is not without its drawbacks. One of the major drawbacks of acrylic resins in removable orthodontic plates is the potential of this material to accumulate plaque due to the absorption of nutrients through the surface pores of this material. Plaque accumulation results in decalcification of the tooth structure and eventually decay along with gingivitis.³⁻⁵ Thus, among the important side effects of such functional devices and retainers in orthodontics, the increased risk of biofilm accumulation of carcinogenic bacteria (*Streptococcus mutans* and *Lactobacillus acidophilus*) and the formation and spread of caries⁶⁻⁹, as well as fungal diseases such as denture stomatitis following the formation of fungal biofilm (*Candida albicans*)¹⁰ is expected.

The most common problem related to biofilm-forming microorganisms is their high resistance to antimicrobial compounds and even immune system factors. In addition, the bacteria present in the biofilm phase express different genes than the planktonic bacteria, which results in better adaptation of bacteria to the environmental conditions.¹¹

Therefore, according to the above mentioned, it is necessary to have acrylic resins with antimicrobial and anti-biofilm activities. Various methods have been proposed to solve the problem of microbial accumulation on acrylics of devices, one of which is the addition of antimicrobial agents to acrylics. Titanium dioxide (TiO₂), silicon dioxide (SiO₂), silver (Ag), zinc (Zn) and platinum (Pt) have been successfully added to acrylic resins and their antimicrobial properties have been observed.¹²⁻¹⁴ Since the above materials are of mineral and/or synthetic origin, biological concerns have been expressed about them.

Recently, marine algae have been widely regarded as a natural source of bio-products containing various biological properties. Seaweed *Spirulina*, which has a water-soluble blue pigment called C-phycoerythrin, has a variety of biological properties including antibacterial, antifungal, anti-virus, anti-tumor and anti-inflammatory properties.¹⁵⁻¹⁷

AIM

The aim of this study was to evaluate the antimicrobial, anti-biofilm and physico-mechanical properties of acrylic resins containing 1%, 2%, 5%, 7.5%, and 10% concentrations of C-phycoerythrin on *S. mutans*, *L. acidophilus*, and *C. albicans* microorganisms to obtain the most appropriate concentration used in orthodontics.

MATERIALS AND METHODS

Preparation of acrylic samples for flexural strength measurement

Acrylic resin samples were manufactured in accordance with ISO:20795-1 (2008).¹⁸ To prepare acrylic samples with different concentrations of C-phycoerythrin, C-phycoerythrin (Sigma, Germany) was mixed in acrylic monomer (SR Triplex cold, Ivoclar Vivadent, Liechtenstein, Germany) at 5 concentrations 1%, 2%, 5%, 7.5%, and 10%. To prepare the 1% concentration, 0.03 g of C-phycoerythrin was added to 1 mL of monomer. To prepare the 2% concentration, 0.06 g of C-phycoerythrin was added to 1 mL of monomer. To prepare 5% concentration, 0.15 g of C-phycoerythrin was mixed with 1 mL of monomer. To prepare the 7.5% concentration, 0.25 g of C-phycoerythrin was added to 1 mL of monomer, and to prepare the 10% concentration, 0.3 g of C-phycoerythrin was mixed with 1 mL of monomer. Sonication with the power of 100 W and frequency of 30 kHz for 3 minutes was used to mix C-phycoerythrin in the monomer. According to ISO:20795-1 (2008)¹⁸, the metal model was first prepared in a larger dimension of 54×14×7.3 mm for the manufacture of acrylic resin samples containing C-phycoerythrin in dimensions of 5×10 (±0.2)×3.3 (±0.2) mm with smooth surface without porosity and precise angles and with acrylic shrinkage when setting. After the acrylic resins were set, the samples were removed from the mold and cut to the desired dimensions (Fig. 1).

For flexural strength testing based on the ISO:20795-1 (2008)¹⁸, prepared specimens were immersed in water at 37°C for 50±2 hours before testing to provide similar conditions to the oral environment. Each specimen was immediately placed in a ZWICK Z250 (Fig. 2). Then, the flexural strength of each specimen was calculated in megapascals (MPa).

Preparation of acrylic samples for measuring the antimicrobial activity

Following the method described above, acrylic samples containing selected C-phycoerythrin (acrylic resin containing the highest percentage of clinically acceptable flexural strength) were made for antimicrobial tests. The mixture of each concentration was poured into a pre-made mold with 5 mm in diameter. After polymerization, the samples were incubated at room temperature for 24 hours to fully set. The samples were finally extracted and polished using Exsys disc and Superfingrit disc (3 ME SPE, USA). After polishing, the specimens were 10 mm in diameter and 4 mm in thickness (Fig. 3). All samples were sterilized by gamma rays at a dose of at least 25 kGy at the Iranian Atomic Center.

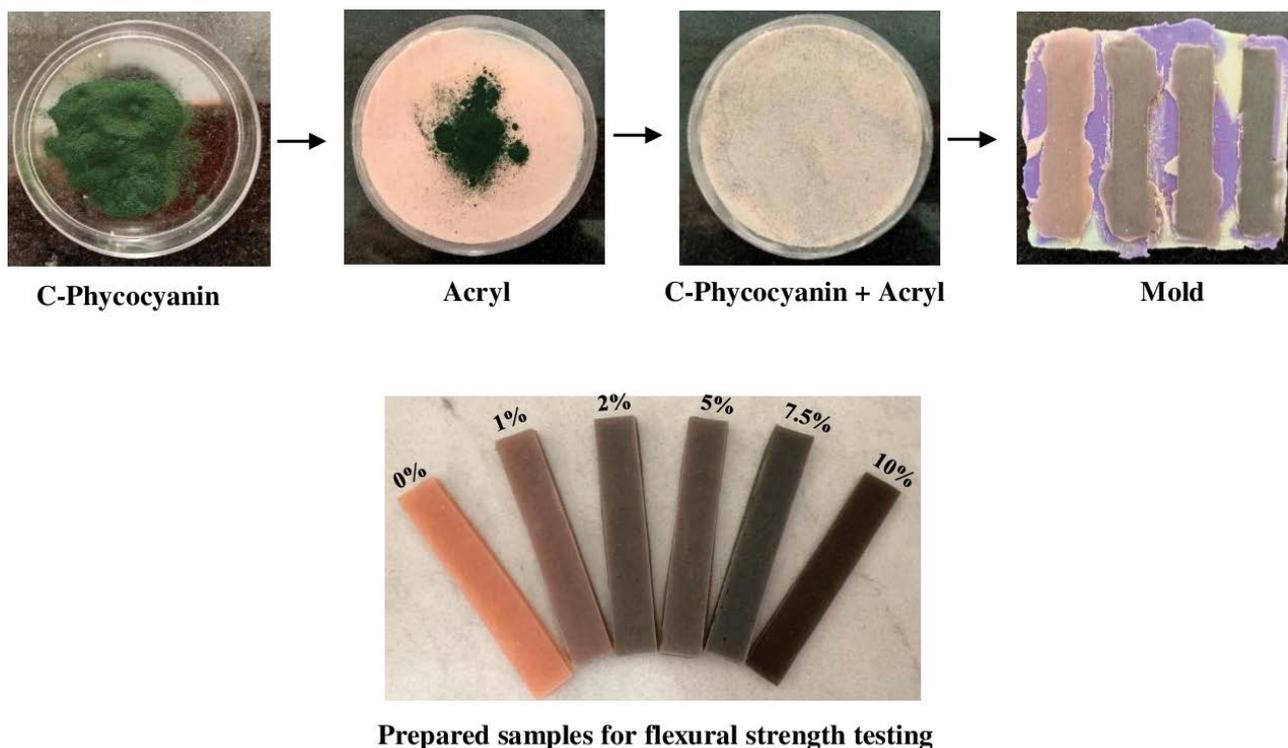


Figure 1. Preparation of acrylic samples with different concentrations of C-phycoyanin.



Figure 2. Zwick Roell for the flexural strength test.



Figure 3. Preparation of acrylic discs containing selected C-phycoyanin (containing the highest percentage of clinically acceptable flexural strength).

Microorganisms and growth conditions

Standard strains of *S. mutans* ATCC 35668, *L. acidophilus* ATCC 314 and *C. albicans* ATCC 14053 were prepared from Microbial Institute of Pasteur, Iran. *S. mutans* and *L. acidophilus* were incubated in Tryptic Soy Broth (TSB; Merck, Germany) in the presence of 5% CO₂ that was provided by gas-pack and *C. albicans* in aerobic conditions for 48 hours at 37°C.

Artificial aging on acrylic discs containing selected C-phycoyanin

One hundred fifty acrylic discs containing selective C-phycoyanin sterile in 24-well microplate wells containing 2 ml of sterile artificial saliva (0.2 g NaCl, 0.2 g KCl, 0.453 g CaCl₂ • 2H₂O, 0.345 g NaH₂PO₄ • 2H₂O, 0.0025 g Na₂S • g of urea in 1000 mL of DI water; pH 7) were stored at 35°C (Fig. 4). At 0, 15, 30, 60 and 90 days, the discs were removed from each well and used for antimicrobial and anti-biofilm experiments.

Evaluation of antimicrobial effects of acrylic discs containing selected C-phycoyanin

Antimicrobial effects of selected C-phycoyanin (acrylic resin containing maximum C-phycoyanin content with clinically acceptable flexural strength) were evaluated by two different tests:



Figure 4. Homemade aging progressor device.

1. Determine the antimicrobial effects of acrylic discs containing selected C-phycoyanin by Disc Agar Diffusion test (DAD):

The antimicrobial activity of acrylic discs containing C-phycoyanin was evaluated by diffusion of C-phycoyanin particles from acrylic discs through DAD test. In this test, suspension of half a McFarland from each of the microorganisms was prepared in Mulberry Hinton Broth (MHB; Merck, Germany) and spread on Mulberry Hinton Agar (MHA; Merck, Germany) using a sterilized swab. The acrylic discs were placed at 2 cm intervals and plates were incubated at 37°C for 24 hours under appropriate conditions based on the type of microorganism. After incubation, the diameter of the non-growing microorganisms was measured. In this test, acrylic discs without C-phycoyanin were considered as the control group.

2. Determination of the antimicrobial effects of acrylic discs containing selected C-phycoyanin on inhibition of microbial biofilm formation:

The discs were immersed in tubes containing microbial suspension at a concentration of 1.5×10^8 CFU/mL to form the biofilms of the studied microorganisms on acrylic discs containing selected C-phycoyanin and acrylic discs without C-phycoyanin as a control group. The samples were incubated for 48 hours at 37°C under the optimum conditions of each microorganism. After 48 hours, the acrylic discs were rinsed with sterile normal saline to remove the planktonic microorganisms from the discs. Afterward, the discs were sonicated in TSB for 5 minutes in ultrasonic W150 at 50 Hz (Bandelin, Germany) to isolate the microorganisms that formed the biofilm. Finally, the number of colonies of microorganisms was calculated based on the previous study.¹⁹

Assessment of the virulence-associated genes expression by quantitative real time PCR (qRT-PCR)

In the present study, the changes in gene expression were investigated following the isolation of microorganisms from acrylic discs containing 5% concentration of C-phycoyanin. Briefly, after isolation of each microorganism, the total RNA extraction was performed using the GeneAll Hybrid-R™ RNA purification kit (GeneAll Biotechnology Co. Ltd, Seoul, Korea) according to the manufacturer's instructions. The complementary DNA (cDNA) was synthesized through a Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, US) with random primers after the elimination of the residual genomic DNAs by RNase-free DNase I treatment (Thermo Scientific GmbH). The primers that were designed using the Primer3 software version 4.0 (<http://bioinfo.ut.ee/primer3/>) are presented in **Table 1**.

Table 1. Mean flexural strength of acrylic resin components containing different concentrations of C-phycoyanin studied; 0.0%: No C-phycoyanin

Groups	MPa (mean ± SD)	P value
0%	50.2±4.5	-
1%	47.4±2.5	0.128
2%	43.5±2.3	0.091
5%	40.3±2.9	0.078
7.5%	34.7±3.0	0.042
10%	30.1±3.3	0.012

The qRT-PCR analysis was performed on a Line-GenEK Real-Time PCR system. The expression levels of luxI gene were calculated relative to the calibration sample (an endogenous control *16S rRNA*) to normalize the sample input. Eventually, the relative expression ratio was analyzed using the method proposed by Livak and Schmittgen.²⁰

Statistical analysis

SPSS v. 23 was used for data analysis. Descriptive statistics were used to describe the data and one way ANOVA, repeated measures analysis of variance, and post hoc Bonferroni test were used to analyze the data. The level of significance was set at $p < 0.05$.

RESULTS

Flexural strength measurement

The flexural strength of the study groups is shown in **Table 2**. As can be seen, the highest and the lowest mean

Table 2. The diameter of growth zone with acrylic discs containing different concentrations of C-phycoerythrin studied using DAD test

Strains	C-phycoerythrin concentrations (%)														
	1					2					5				
	Days														
	1	15	30	60	90	1	15	30	60	90	1	15	30	60	90
Growth inhibition zone (mm)					Growth inhibition zone (mm)					Growth inhibition zone (mm)					
<i>S. mutans</i>	7	7	6	6	5	10	10	10	9	6	12	12	11	10	6
<i>L. acidophilus</i>	7	7	7	6	6	10	10	10	9	7	12	12	11	10	7
<i>C. albicans</i>	5	5	5	5	4	9	9	8	6	5	11	11	11	11	5

flexural strengths belong to the control group at 50.2±4.5 MPa and 10% concentration of C-phycoerythrin at 30.1±3.3 MPa, respectively. The results also showed that the flexural strength of acrylic resin did not decrease significantly ($p<0.05$) by adding 1%, 2%, and 5% of C-phycoerythrin, while adding 7.5% and 10% of C-phycoerythrin significantly decreased flexural strength ($p>0.05$). Therefore, according to the results, 1%, 2%, and 5% concentrations of C-phycoerythrin were used for microbial tests with acceptable flexural strength.

Antimicrobial effect of C-phycoerythrin by DAD test

The antimicrobial effects created by the release of selected C-phycoerythrin particles from acrylic discs on MHA were measured by DAD test at 1, 15, 30, 60, and 90 days. According to **Table 3**, the maximum mean diameter of growth inhibition zone against all 3 microorganisms was observed around discs containing 5% of C-phycoerythrin. As can be seen in **Table 3**, the changes in the diameter of the growth zone are time-dependent. As the incubation time of the discs containing C-phycoerythrin increased, the diameter of the growth zone decreased, so that the growth rate of all microorganisms reduced at 90 days. According to the results, acrylic resins containing 5% of C-phycoerythrin were able to inhibit the growth of *S. mutans*, *L. acidophilus* and *C. albicans* more than other groups up to 30 days of study on MHA.

Anti-biofilm effects of acrylic discs containing selected C-phycoerythrin on inhibition of microbial biofilm formation

The anti-biofilm effects created by C-phycoerythrin in acrylic discs were measured at 1, 15, 30, 60 and 90 days. According to the results (**Table 4**), no microbial biofilm was formed on any acrylic disc at concentrations of 1%, 2%, and 5% of C-phycoerythrin by the 30th day of the study. Microbial biofilm from *S. mutans* and *L. acidophilus* strains were formed on the acrylic discs at 1% concentration on days 60

Table 4. The primer sequences in this study

Genes		5' to 3'
ALS9	F	CCATATTCAGAAACAAAGGGTTC
	R	AACTGAAACTGCTGGATTG
28S rRNA	F	TCGACGAGTCGAGTTGTTT
	R	AGCCCTTCCCTTTCAACAAT
hsp16	F	CGTGGCCGGTACTAGAAAAG
	R	TGCTTTGGTAGGGTGATGGT
ldhD	F	GTCGGTGTGTGGTACTGG
	R	TTAGCTGGAACGCTCTGGTAC
gtfB	F	TGTTGTTACTGCTAATGAAGAA
	R	GCTACTGATTGTCGTTACTG
16S rRNA	F	GCAGAAGGGGAGAGTGGAAAT
	R	GGCCTAACACCTAGCACTCA

Table 3. Microbial biofilm formed by the studied microorganisms on acrylic discs containing different concentrations of C-phycoerythrin

Strains	C-phycoerythrin concentrations (%)															Control
	1					2					5					
	Days															
	1	15	30	60	90	1	15	30	60	90	1	15	30	60	90	
CFU/mL±SD×10 ²					CFU/mL±SD×10 ²					CFU/mL±SD×10 ²					CFU/mL±SD×10 ²	
<i>S. mutans</i>	NG	NG	NG	1.18±1.4	2.35±1.4	NG	NG	NG	NG	1.89±1.1	NG	NG	NG	NG	NG	6.88±3.5
<i>L. acidophilus</i>	NG	NG	NG	1.04±1.3	2.08±0.8	NG	NG	NG	NG	1.45±1.4	NG	NG	NG	NG	NG	6.54±4.2
<i>C. albicans</i>	NG	NG	NG	2.41±0.6	3.12±1.2	NG	NG	NG	1.16±1.6	2.13±1.8	NG	NG	NG	NG	1.33±1.14	6.23±4.1

and 90 and on acrylic discs at 2% concentration of C-phycoyanin on day 60. However, *C. albicans* was able to form microbial biofilm on day 60 of study on 1, 2 and 5% acrylic discs and on day 90 of study on acrylic discs containing 5% concentration of C-phycoyanin.

Anti-biofilm effects of acrylic discs containing selected C-phycoyanin on the biofilm-associated gene expression levels

5% concentration of C-phycoyanin reduced significantly the expression of *gtfB*, *hsp16*, and *ALS9* genes when compared to the control group. As mentioned before in the results of this study, Fig. 5 shows that the expressions of *gtfB* in *S. mutans*, *hsp16* in *L. acidophilus*, and *ALS9* in *C. albicans* were decreased to approximately 6.1-, 7.3-, and 3.9-fold, respectively. As the results reveal, the gene expression profiling was reduced in *L. acidophilus* cells with the greatest reduction, which was approximately 1.2- and 3.4-fold higher than *gtfB* and *ALS9* in *S. mutans* and *C. albicans*, respectively. According to the results, the level of *hsp16* gene expression compared with *ALS9* was significantly different ($p < 0.05$), while there was no remarkable difference in gene expression of *hsp16* compared with *gtfB* ($p > 0.05$).

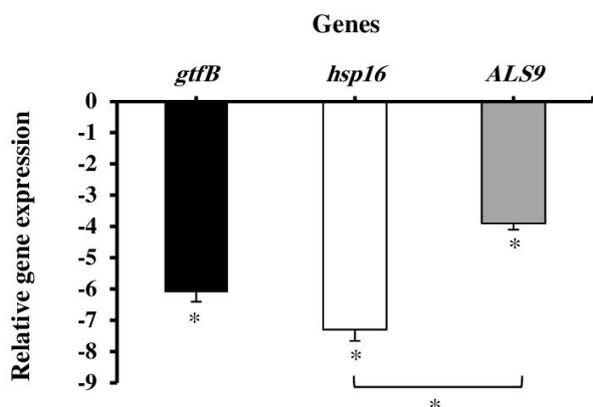


Figure 5. The relative fold change in mRNA expression following isolation of microorganisms from acrylic discs containing 5% concentration of C-phycoyanin. *Significantly different from the control group, $p < 0.05$.

DISCUSSION

Acrylic resins are widely used in orthodontic plates. However, the incidence of stress fractures, which are usually the middle part of the orthodontic plaque, mainly due to the low mechanical strength of acrylic resins, has long been a clinical problem.¹ In recent years, much attention has been paid to the use of antimicrobial compounds in acrylic.^{21,22} In addition to studying the antimicrobial properties, it is important to know the effects of these antimicrobial compounds on the physical properties of acrylic. Various stu-

dies have investigated the effects of adding nanoparticles as antimicrobial compounds to acrylic.²¹⁻²⁵ Results of studies on adding nanoparticles such as Ag to acrylic suggested that these nanoparticles increase the flexural strength of Selecta Plus acrylic, but excessive addition decreases the flexural strength.²³ In contrast, by adding Ag nanoparticles to Rapid acrylic, the flexural strength initially decreases but again increases to the initial flexural strength.²³ Therefore, the effect of adding Ag nanoparticles on the flexural strength of acrylic depends on the type of acrylic and the percentage of the addition of nanoparticles. Another study has shown that adding TiO₂ and/or SiO₂ nanoparticles to the acrylic resins reduce the flexural strength and is directly related to the dose of the nanoparticles.²⁴ The difference in flexural strength between acrylic containing TiO₂ and SiO₂ nanoparticles was not statistically significant. However, acrylic resin with TiO₂ exhibits less strength than SiO₂.²⁴

To the best of our knowledge, the effects of adding natural products as antimicrobial compounds to acrylic resins have not been studied so far. Therefore, in this study, antimicrobial, anti-biofilm, and physico-mechanical characteristics of orthodontic adhesive containing C-phycoyanin were investigated.

Flexural strength test was performed to investigate the effects of adding the C-phycoyanin at different concentrations of 1%, 2%, 5%, 7.5%, and 10% to the orthodontic adhesive on the mechanical properties of acrylic resins used in orthodontics. The results of our study showed that adding 1%, 2%, and 5% of C-phycoyanin showed no significant decrease ($p < 0.05$) in flexural strength compared to C-phycoyanin-free acrylic, while adding 7.5% and 10% of C-phycoyanin significantly decreased flexural strength ($p > 0.05$).

In addition to investigating the mechanical properties, the antimicrobial activities of acrylic resin in combination with C-phycoyanin against *S. mutans*, *L. acidophilus*, and *C. albicans* were also evaluated. The reason for the selection of these microorganisms is due to their primary role in biofilm formation, caries, sometimes gingivitis, and prosthetic stomatitis in patients using acrylic devices.^{26,27} These microorganisms are abundant on the teeth and mucous surfaces of the oral cavity. Many strains are highly acidogenic and some are acid resistant. This group begins to grow and implant immediately after the first teeth appear in the children's oral cavity and is found in abundance in dental plaque injuries and their plaque content increases with dietary sucrose intake. It has been found to play an important role in the development of dental and oral diseases.²⁸ In the present study, the results of the DAD test showed the development of a growth inhibition zone, which means that C-phycoyanin was able to be transported directly to the adjacent acrylic regions by diffusion into the agar medium. Therefore, the direct release of C-phycoyanin can lead to antimicrobial activity from areas where there is direct contact between the acrylic plaque and the tooth. In this study, the maximum mean diameter of growth inhibition zone against all three microorganisms (*S. mutans*, *L. acidophilus*,

and *C. albicans*) was observed around discs containing 5% concentration of C-phycoyanin. The results of the DAD test in this study are in contrast to the results reported in a study by Sodager et al.²⁵ which investigated the antimicrobial properties of curcumin nanoparticles in orthodontic composite - the researchers observed no growth inhibition zone in the DAD test. In another study²⁹ the growth inhibition zone was not displayed by adding 5% chitosan/zinc oxide nanoparticles, and growth inhibition zone was observed only by adding 10% concentration of these nanoparticles. The results of the DAD test in this study are in line with the results reported in a study by Gligorijević et al.³⁰ which evaluated the antimicrobial activity of Ag nanoparticles on triplex acrylic self-cure. The researchers observed a growth inhibition zone in DAD test against *Staphylococcus aureus* at all concentrations of 2%, 5%, 10%, and 100%.³⁰

In the current study, the inhibitory effect of microbial biofilm formation was also investigated. Biofilms have been shown to play a major role in causing dental caries and periodontal diseases.²⁸ Biofilms are defined as the concentrated accumulation of interconnected or surface-bound microorganisms encapsulated in self-produced extracellular polymeric material (EPS).²⁵ The importance of biofilm inhibition testing is that it demonstrated that bacteria in the form of biofilms are four times more resistant to antimicrobial compounds than planktonic form at a similar density.³¹ Therefore, the bacteria in the form of biofilm on the tooth create a much more resistant microbial accumulation that can stimulate the onset of decay and accelerate its development. In the present study, inhibition testing of microbial biofilm formation on discs containing different concentrations of C-phycoyanin over 90 days of the study showed that the microbial biofilm formation ability of each microorganism was significantly increased by the addition of C-phycoyanin to acrylic resins, so that no biofilms were observed at 30 days on any acrylic disc containing 1%, 2%, and 5% concentrations of C-phycoyanin. But over time, microbial biofilm from *S. mutans* and *L. acidophilus* strains were prepared on acrylic discs at 1% concentration at 60 and 90 days and on acrylic discs at 2% concentration of C-phycoyanin at 60 days. However, *C. albicans* was able to form microbial biofilms on 60 days of study on acrylic discs containing 1%, 2%, and 5% concentration and at 90 days of study on acrylic discs containing 5% concentration of C-phycoyanin.

The regular process of microbial biofilm reduction with increasing concentration of antimicrobial agents does not apply to the results of Sodagar et al.²⁵ There was no significant inhibition of biofilm formation in their study by adding nano-curcumin particles to the composite with increasing concentrations from 1% to 2% and 10%.²⁵ In a study by Marra et al.³², the microbial biofilms of *S. mutans* and *S. aureus* were systematically attenuated with increasing concentration of poly (2-tert-butylaminoethyl) methacrylate. In this study, the formed microbial biofilms by *S. mutans* and *L. acidophilus* were inhibited for up to 90 days and *C. albicans* for up to 60 days with increasing concentration of

C-phycoyanin. Additionally, as revealed by the results of this study, the expression of *gtfB*, *hsp16*, and *ALS9* genes was downregulated (6.1-, 7.3-, and 3.9-fold, respectively) when exposed to 5% C-phycoyanin. Collectively, this observation clearly reveals that 5% concentration of C-phycoyanin is able to reduce the biofilm formation ability of *S. mutans*, *L. acidophilus*, and *C. albicans* strains.

CONCLUSIONS

Based on the results of flexural strength and antimicrobial and anti-biofilm activities of acrylic resin containing various concentrations of C-phycoyanin against *S. mutans*, *L. acidophilus*, and *C. albicans*, it can be concluded that the most acceptable concentration of C-phycoyanin is 5% in acrylic resins used in orthodontics.

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Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Rice CA. A history of dental polymers. Aust Prosthodont J 1994; 8:47-54.
2. Sodagar A, Bahador A, Khalil S, et al. The effect of TiO₂ and SiO₂ nanoparticles on flexural strength of poly (methyl methacrylate) acrylic resins. J Prosthodont Res 2013; 57(1):15-9.
3. Lessa FC, Enoki C, Ito IY, et al. In-vivo evaluation of the bacterial contamination and disinfection of acrylic baseplates of removable orthodontic appliances. Am J Orthod Dentofacial Orthop 2007; 131(6):705.e11-7.
4. Morgan TD, Wilson M. The effects of surface roughness and type of denture acrylic on biofilm formation by Streptococcus oralis in a constant depth film fermentor. J Appl Microbiol 2001; 91(1):47-53.
5. Duymus ZY, Yanikoglu ND. The investigation of solubility values, water sorption and bond strength of auto-polymerising and heat-polymerising acrylic resin materials. Eur J Prosthodont Restor Dent 2006; 14(3):116-20.
6. Kohler B, Bjarnason S. Mutans streptococci, lactobacilli and caries prevalence in 11- and 12-year-old Icelandic children. Community Dent Oral Epidemiol 1987; 15(6):332-5.
7. Caufield PW, Dasanayake AP, Li Y, et al. Natural history of Streptococcus sanguinis in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun 2000; 68(7):4018-23.
8. Badet C, Thebaud NB. Ecology of lactobacilli in the oral cavity: a review of literature. Open Microbiol J 2008; 2:38-48.

9. Moulana Z, Ghasem Pour M, Asghar Pour F, et al. The frequency of streptococcus mutans and lactobacillus spp.in 3-5-year-old children with and without dental caries. *Medical Laboratory Journal* 2013; 7(1):29–34.
10. Salerno C, Pascale M, Contaldo M, et al. Candida-associated denture stomatitis. *Med Oral Patol Oral Cir Bucal* 2011; 16(2):139–43.
11. Bahador A, Pourakbari B, Ghorbanzadeh R, et al. Anti-bacterial effects of polymethylmethacrylate with in situ generated silver nanoparticles on primary colonizers of human dental plaque and cariogenic bacteria. *Annual Research & Review in Biology* 2014; 4(10):1587–601.
12. Sodagar A, Khalil S, Kassae MZ, et al. Antimicrobial properties of poly (methyl methacrylate) acrylic resins incorporated with silicon dioxide and titanium dioxide nanoparticles on cariogenic bacteria. *J Orthod Sci* 2016; 5(1):7–13.
13. Cierech M, Wojnarowicz J, Szmigiel D, et al. Preparation and characterization of ZnO-PMMA resin nanocomposites for denture bases. *Acta Bioeng Biomech* 2016; 18(2):31–41.
14. Nam KY. Characterization and bacterial anti-adherent effect on modified PMMA denture acrylic resin containing platinum nanoparticles. *J Adv Prosthodont* 2014; 6(3):207–14.
15. Pattarayan D, Rajarajan D, Ayyanar S, et al. C-phycoyanin suppresses transforming growth factor- β 1-induced epithelial mesenchymal transition in human epithelial cells. *Pharmacol Rep* 2017; 69(3):426–31.
16. Pourhajibagher M, Chiniforush N, Bahador A. Antimicrobial action of photoactivated C-phycoyanin against *Enterococcus faecalis* biofilms: Attenuation of quorum-sensing system. *Photodiagnosis Photodyn Ther* 2019; 28:286–291.
17. Shanmugam A, Sigamani S, Venkatachalam H, et al. Antibacterial activity of extracted phycocyanin from *Oscillatoria* sp. *J App Pharm Sci* 2017; 7(03):062–067.
18. Nguyen LG, Kopperud HM, Øilo M. Water sorption and solubility of polyamide denture base materials. *Acta Biomater Odontol Scand* 2017; 3(1):47–52.
19. Pourhajibagher M, Salehi Vaziri A, Takzaree N, et al. Physico-mechanical and antimicrobial properties of an orthodontic adhesive containing cationic curcumin doped zinc oxide nanoparticles subjected to photodynamic therapy. *Photodiagnosis Photodyn Ther* 2019; 25:239–46.
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ Method. *Methods* 2001; 25(4):402–8.
21. Pourhajibagher M, Ghorbanzadeh R, Bahador A. Antimicrobial properties of acrylic resins doped with *Undaria pinnatifida* exposed to light-emitting diode: In silico and in vitro assessments on multispecies biofilm-producing microbiota. *Photodiagnosis Photodyn Ther* 2019; 27:210–5.
22. Gong SQ, Epasinghe DJ, Zhou B, et al. Effect of water-aging on the antimicrobial activities of an ORMOSIL-containing orthodontic acrylic resin. *Acta Biomater* 2013; 9(6):6964–73.
23. Sodagar A, Kassae MZ, Akhavan A, et al. Effect of silver nanoparticles on flexural strength of acrylic resins. *J Prosthodont Res* 2012; 56(2):120–4.
24. Bruckner DA, Colonna P. Nomenclature for aerobic and facultative bacteria. *Clin Infect Dis* 1997; 25:1–10.
25. Sodagar A, Bahador A, Pourhajibagher M, et al. Effect of addition of curcumin nanoparticles on antimicrobial property and shear bond strength of orthodontic composite to bovine enamel. *Journal of dentistry (Tehran, Iran)* 2016; 13:373–80.
26. Ravikumar S, Anburajan L, Meena B. Antibacterial activity of *Ulva reticulata* from southwest coast of Kanyakumari, India. *J Coast Life Med* 2016; 4(3):246–7.
27. Pérez M, Falqué E, Domínguez H. Antimicrobial action of compounds from marine seaweed. *Marine drugs* 2016; 14(3):52–61.
28. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. *J Dent Edu* 2001; 65:1028–37.
29. Mirhashemi A, Bahador A, Kassae M, et al. Antimicrobial effect of nano-zinc oxide and nano-chitosan particles in dental composite used in orthodontics. *J Med Bacteriol* 2016; (3-4):1–10.
30. Gligorijević N, Kostić M, Tačić A, et al. Antimicrobial properties of acrylic resins for dentures impregnated with silver nanoparticles. *Acta Stomatologica Naissi* 2017; 33(75):1696–702.
31. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. *Virulence* 2011; 2(5):435–44.
32. Marra J, Paleari AG, Rodriguez LS, et al. Compagnoni MA. Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. *J Appl Oral Sci* 2012; 20(6):643–8.

Антимикробные свойства, антивирулентная активность и физико-механические характеристики ортодонтического клея, содержащего С-фикоцианин: перспективное применение натуральных продуктов

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Резюме

Введение: Антимикробные ортодонтические адгезивы направлены на уменьшение деминерализации эмали, белых пятен и начального разрушения зубов вокруг несъёмных ортодонтических скоб, но они не должны ухудшать его механические свойства.

Цель: Оценить антимикробные и физико-механические свойства акрила, содержащего различные концентрации С-фикоцианина, в отношении *Streptococcus mutans*, *Lactobacillus acidophilus* и *Candida albicans*.

Материалы и методы: Механические свойства акриловых смол были измерены с помощью испытания на прочность на изгиб после приготовления образцов акриловой смолы с концентрациями 1%, 2%, 5%, 7.5% и 10% С-фикоцианина. Затем мы оценили антимикробный эффект акриловой смолы, содержащей максимальную концентрацию фикоцианина, с клинически приемлемой прочностью на изгиб и изменениями в экспрессии фактора вирулентности.

Результаты: Самые высокие и самые низкие значения прочности на изгиб были достигнуты с акриловыми смолами, содержащими концентрации С-фикоцианина 0% и 10% при 50.2 ± 4.5 и 30.1 ± 3.3 МПа, соответственно. Добавление 1%, 2% и 5% С-фикоцианина не показало значительного снижения прочности на изгиб ($p > 0.05$). Максимальный средний диаметр зоны задержки роста наблюдался вокруг дисков, содержащих 5% С-фикоцианина. К 30 дню исследования микробные биоплёнки не образовывались ни на одном акриловом диске. Только микробные биоплёнки из *C. albicans* образовывались на дисках, содержащих 5% С-фикоцианина, на 90-й день. 5% С-фикоцианин может значительно снизить уровни экспрессии *gtfB*, *hsp16* и *ALS9* в 6.1-, 7.3- и 3.9-раза соответственно.

Заключение: Можно сделать вывод, что наиболее приемлемая концентрация С-фикоцианина в акриловой смоле составляет 5% на основании результатов исследования прочности на изгиб и антимикробной активности акриловой смолы, содержащей различные концентрации С-фикоцианина.

Ключевые слова

акриловые смолы, антимикробная активность, С-фикоцианин, прочность на изгиб, экспрессия генов