Photodiagnosis and Photodynamic Therapy 27 (2019) 210–215

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

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

Maryam Pourhajibagher[a], Roghayeh Ghorbanzadeh[b], Abbas Bahador[c,*]

[a] Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Orthodontics, Shahed University, Tehran, Iran
[c] Oral Microbiology Laboratory, Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Cariogenic microorganism
Orthodontic adhesive
Acrylic resin
Undaria pinnatifida
Photodynamic therapy
Antimicrobial Photodynamic Therapy

ABSTRACT

Background: This study sought to evaluates the efficiency of anti-microbial activity of acrylic resins doped with different concentrations of *Undaria pinnatifida* after activation with light-emitting diode (LED) at producing photodynamic damage to multispecies biofilm-producing microbiome.

Material and methods: In this study, bioinformatics tools and computer simulation molecular modeling were used to evaluate the capacity of ferredoxin (FDX), an electron acceptor in metabolic pathways of *U. pinnatifida,* which can discharge electrons produced from photo-excited chlorophyll-a (Chl-a) by LED irradiation. Acrylic resin discs containing different concentration of *U. pinnatifida* (0, 0.5, 1, and 2%) were fabricated and were subjected to LED irradiation immediately before each experiment. After continuously rinsed (up to 30 days), the antimicrobial activity of acrylic resins doped with *U. pinnatifida* following photo-activation was determined by disc agar diffusion, biofilm formation inhibition, and eluted component assays versus bacterial species linked to caries that constitute a mixed biofilm including *Streptococcus mutans*, *S. sanguinis*, and *Lactobacillus acidophilus*, as well as *Candida albicans* as main etiology of candidal stomatitis.

Results: Modeling and a virtual screening analysis of FDX indicated that it is a stable protein with an iron-sulfur center that can discharge electrons produced from photo-excited Chl-a and transfers them to FDX–$NADP^+$ reductase for $NADP^+$ reduction in photosystem I, which is essential in the Calvin cycle for carbon assimilation. FDX acts as an electron transfer agent in the redox reactions. The results showed that growth inhibition zones were not seen around acrylic resin discs in any group. In biofilm test, the colony counts of all test microorganisms significantly decreased (36%–87%) by an increase in the percentage of *U. pinnatifida* in acrylic resins after photo-activation ($P < 0.05$). Acrylic resins doped with 2% wt. *U. pinnatifida* following photo-activation using LED was inhibited biofilm formation by the test microorganisms, up to 30 days of rinsing.

Conclusion: Based on the results presented here, an acrylic resin containing *U. pinnatifida*, even at the lowest concentration, following photo-activation using LED have antimicrobial properties against planktonic and biofilm forms of the cariogenic microorganisms as well as *C. albicans*.

1. Introduction

Orthodontic treatments have become very popular due to improving oral function or health, appearance, and social acceptance [1]. Polymethyl-methacrylate (PMMA) resins are the most popular material used in orthodontic treatments for construction of removable appliances and retainers [2]. Although PMMA resins have the desired properties for use in orthodontics, the porous surface and irregularities of removable appliances and retainers made of PMMA resins are the facilitator factors in the accumulation of microorganisms that can cause the broad majority of oral problems including acrylic resin contact stomatitis, gingival inflammation, and dental caries [3,4].

It has been revealed that *Streptococcus* spp. has been involved in early dental enamel demineralization [5] and *Lactobacilli* spp. are associated with dental plaque progression and cavitation [6]. Increased levels of *S. mutans*, *S. sanguinis,* and *Lactobacilli* spp., has been reported

* Corresponding author at: Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Keshavarz Blvd, 100 Poursina Ave., Tehran, 14167-53955 Iran.
E-mail address: abahador@sina.tums.ac.ir (A. Bahador).

https://doi.org/10.1016/j.pdpdt.2019.05.039
Received 24 January 2019; Received in revised form 29 May 2019; Accepted 31 May 2019
Available online 08 June 2019
1572-1000/

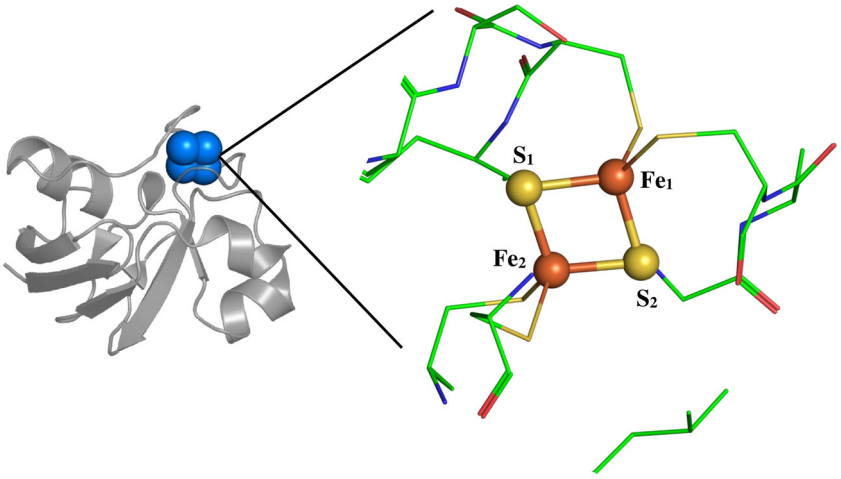

Fig. 1. Modeled spatial configuration of the FDX protein in *U. pinnatifida.*

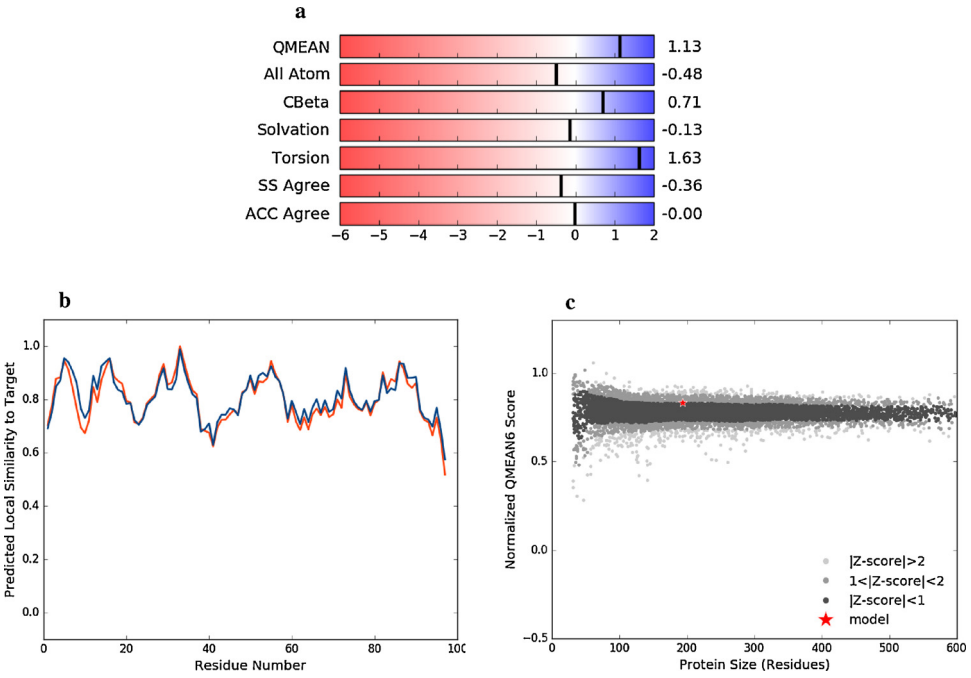

Fig. 2. Assessment of the quality of FDX. a. Global quality of the entire model by QMEAN score; b. Local quality estimate; c. Local per-residue analysis of different regions within a model.

after application of removable orthodontic appliances and retainers [7]. In addition, it has been shown that adhesion and colonization of acrylic resins in removable appliances and retainers by *Candida albicans* may cause inflammation in the mucosa, which is associated with an increased probability of candidal stomatitis. [8]. Recently, several kinds of research have accomplished to reduce and/ or eliminate the microbial contamination from removable orthodontic appliances base acrylic resin without affecting the physico-mechanical properties of removable orthodontic appliances and retainers [2–4,9]. Accordingly, incorporation of antimicrobial agents in orthodontic acrylic resin to plaque biofilm reduction is highly effective [10]. Despite the potencies of these procedures in control of microbial biofilms, stringent compliance of the patient's oral hygiene is essential. One of the main aims of the current orthodontic treatment is to improve therapeutic performance by using new materials or surface coatings, in order to reduce or inhibit the biofilm formation of cariogenic microorganisms as well as *C. albicans* on around removable orthodontic appliances and retainers [11].

Chlorophyll-a (Chl-a) is a photoactive natural pigment present in *Undaria pinnatifida* [12], a brown seaweed microalga, which has a significant attraction as a natural source of bioactive molecules with a wide range of pharmaceutical activities including inhibition of microbial growth [12,13]. The photobiological properties of Chl-a, have been intensively investigated during the last few years [13]. It has been shown that fucoxanthin-chlorophyll a/c-protein complexes (FCPc) in the thylakoid membranes from several brown algae including *U. pinnatifida* work as harvesting light and transferring pigment [14,15]. As a promising photosensitizer, Chl-a in *U. pinnatifida* can use in the context of PDT due to its excellent photosensitizing properties. Chl-a has a high extinction coefficient at 660 nm and good singlet oxygen production. Several studies reported that, with appropriate light illumination, it can generate singlet oxygen as a toxic ROS molecule leading to antimicrobial activity [16–19]. Ferredoxins (FDXs), iron–sulfur proteins, are the last electron acceptor that acts as an electron transfer agent from sunlight-excited chlorophyll to reducing the enzyme NADP + reductase. The recent study revealed that in FDXs-limiting conditions including light stress, the ROS level will be increased. [20–22].

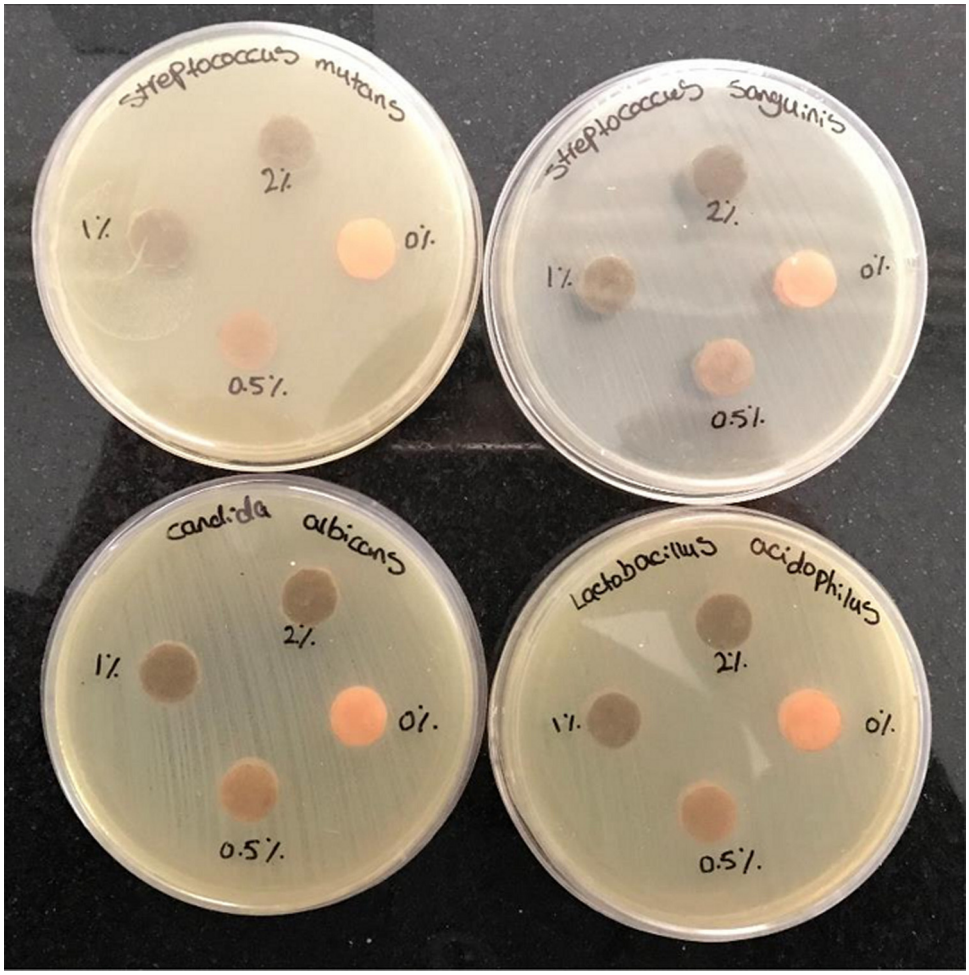

Fig. 3. Disc agar diffusion test of acrylic resin doped with different concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation against multispecies cariogenic microorganisms.

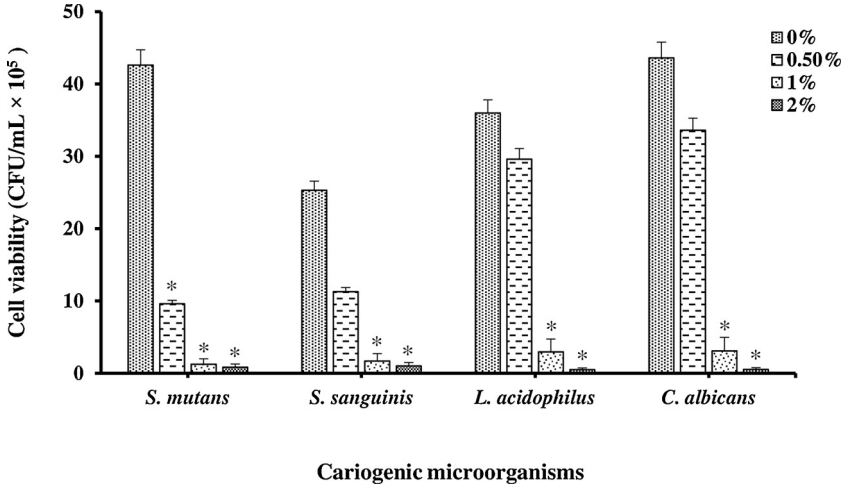

Fig. 4. Biofilm inhibition assay of acrylic resin doped with different concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation against multispecies cariogenic microorganisms.

According as mentioned in unique features of *U. pinnatifida*, the aim of the present study was therefore to evaluate potency of acrylic resins containing *U. pinnatifida* following photo-activation against multi-species cariogenic biofilm-producing bacteria (including *S. mutans*, *S. sanguinis*, and L. *acidophilus*) and *C. albicans*. In this study, our hypothesis is that there is a statistically significant increase in antimicrobial activity of the acrylic resins doped with *U. pinnatifida* submitted to light activation against multispecies cariogenic biofilm-producing bacteria. Under the null hypothesis, this increase is insignificant.

Table 1
Eluted component assay of acrylic resins doped with *U. pinnatifida* following photo-activation against multispecies cariogenic microorganisms.

Groups	Concentrations of *U. pinnatifida*	*Streptococcus mutans*			*Streptococcus sanguinis*			*Lactobacillus acidophilus*			*Candida albicans*		
		Mean of CFU/mL ± SD	% of reduction	P value	Mean of CFU/mL ± SD	% of reduction	P value	Mean of CFU/mL ± SD	% of reduction	P value	Mean of CFU/mL ± SD	% of reduction	P value
Day 3	0	$36.3 \times 10^5 \pm 4.9$	–	–	$41.0 \times 10^5 \pm 2.6$	–	–	$50.3 \times 10^5 \pm 2.5$	–	–	$73.6 \times 10^5 \pm 6.6$	–	–
	0.5	$27.6 \times 10^5 \pm 2.3$	25	0.17	$30.6 \times 10^5 \pm 4.7$	27	0.08	$32.3 \times 10^5 \pm 5.1$	36	0.01	$49.3 \times 10^5 \pm 5.1$	33	0.00
	1	$20.0 \times 10^5 \pm 3.0$	45	0.05	$22.3 \times 10^5 \pm 3.05$	47	0.00	$23.6 \times 10^5 \pm 3.05$	54	0.01	$30.6 \times 10^5 \pm 7.2$	59	0.03
	2	$11.0 \times 10^5 \pm 2.0$	70	0.01	$12.6 \times 10^5 \pm 2.5$	71	0.01	$18.3 \times 10^5 \pm 2.5$	64	0.00	$22.0 \times 10^5 \pm 2.0$	70	0.00
Day 7	0	$39.6 \times 10^5 \pm 3.2$	–	–	$45.3 \times 10^5 \pm 4.5$	–	–	$72.3 \times 10^5 \pm 3.7$	–	–	$80.0 \times 10^5 \pm 6.0$	–	–
	0.5	$23.3 \times 10^5 \pm 3.5$	42	0.02	$29.6 \times 10^5 \pm 2.0$	36	0.02	$30.0 \times 10^5 \pm 5.0$	59	0.00	$42.3 \times 10^5 \pm 8.3$	48	0.01
	1	$16.6 \times 10^5 \pm 2.08$	59	0.00	$17.6 \times 10^5 \pm 2.08$	63	0.01	$22.3 \times 10^5 \pm 2.5$	70	0.00	$25.3 \times 10^5 \pm 3.2$	69	0.00
	2	$10.3 \times 10^5 \pm 1.5$	75	0.00	$15.0 \times 10^5 \pm 2$	67	0.01	$16.3 \times 10^5 \pm 1.1$	78	0.00	$18.0 \times 10^5 \pm 3.6$	78	0.00
Day 15	0	$45.0 \times 10^5 \pm 3.6$	–	–	$50.3 \times 10^5 \pm 5.6$	–	–	$83.0 \times 10^5 \pm 7.0$	–	–	$91.3 \times 10^5 \pm 4.1$	–	–
	0.5	$19.0 \times 10^5 \pm 2.0$	58	0.00	$22.3 \times 10^5 \pm 2.08$	56	0.03	$26.6 \times 10^5 \pm 5.8$	68	0.01	$39.6 \times 10^5 \pm 8.5$	58	0.00
	1	$15.0 \times 10^5 \pm 2.0$	67	0.00	$14.6 \times 10^5 \pm 2.08$	72	0.01	$19.3 \times 10^5 \pm 0.5$	78	0.00	$22.3 \times 10^5 \pm 1.5$	76	0.00
	2	$6.6 \times 10^5 \pm 3.2$	87	0.00	$11.6 \times 10^5 \pm 2.08$	78	0.02	$13.3 \times 10^5 \pm 1.5$	85	0.00	$15.0 \times 10^5 \pm 4.5$	84	0.00
Day 30	0	$19.0 \times 10^6 \pm 3.0$	–	–	$27.6 \times 10^6 \pm 2.5$	–	–	$40.3 \times 10^6 \pm 4.9$	–	–	$55.6 \times 10^6 \pm 7.5$	–	–
	0.5	$13.0 \times 10^6 \pm 2.6$	32	0.05	$18.6 \times 10^6 \pm 1.5$	34	0.04	$25.0 \times 10^6 \pm 2.0$	38	0.01	$39.6 \times 10^6 \pm 6.5$	30	0.04
	1	$9.3 \times 10^6 \pm 1.5$	53	0.04	$11.0 \times 10^6 \pm 2.6$	60	0.00	$16.6 \times 10^6 \pm 3.05$	60	0.00	$27.0 \times 10^6 \pm 2.64$	51	0.03
	2	$17.6 \times 10^6 \pm 3.05$	63	0.03	$10.3 \times 10^6 \pm 1.5$	60	0.01	$14.0 \times 10^6 \pm 2.0$	65	0.02	$19.0 \times 10^6 \pm 1.0$	66	0.01

2. Materials and methods

2.1. Target site selection in U. Pinnatifida using in silico analysis

In this study, several bioinformatics tools and computer simulation molecular modeling were used to predict the molecular modeling, and structure validation of FDX in *U. pinnatifida* as one of the target sites. The National Center for Biological Information (NCBI; available at http://www.ncbi.nlm.nih.gov) was used to retrieve the amino acid sequences of FDX. Alignment with a Protein-Basic Local Alignment Search Tool (BLAST; https://www.ncbi.nlm.nih.gov/blast/) was conducted in Protein Data Bank (PDB) entries to find an appropriate template to FDX. The physicochemical properties of FDX were performed using UniProt (http://www.expasy.org/tools/) and Expasy ProtParam servers (http://www.expasy.org/cgi-bin/protpraram). The three-dimensional structure of the FDX was obtained from http://www.ncbi.nlm.nih.gov/Structure/VAST. In addition, the FDX was analyzed as the target based on the Global Model Quality Estimation (GMQE) and Qualitative Model Energy ANalysis (QMEAN) scores.

2.2. Specimen fabrication

Acrylic resin discs (8 mm in diameter and 2 mm thick) with 0.5%, 1%, and 2% concentrations of *U. pinnatifida* (Sigma-Aldrich Co., Ltd., Dorset, UK) were prepared. Initially, a metal mold was used to make disc-shaped acrylic resin patterns. To achieve 0.5% concentration, 0.02 g of *U. pinnatifida* powder (Sigma-Aldrich Co., Ltd., Dorset, United Kingdom) mixed into each milliliter of monomer acrylic liquid (Selecta Plus, Dentsply Company, UK). The components were homogenized in an ultrasonic set (Bandelin SONOPULS ultrasonic homogenizer, Germany) for 5 min by the power of 100 W and a frequency of 30 kHz. Then 2 g PMMA (Selecta Plus, Dentsply Company, UK) was added to the mixture. Eventually, the mixture was packed into metal molds and allowed to set thoroughly. To obtain 1% and 2% concentrations, 0.04 and 0.08 g of *U. pinnatifida* powder were used, respectively. The acrylic resin without *U. pinnatifida* was used as a control (0%). Afterward, the discs were de-molded, polished, and sterilized by 25 kGy irradiation according to ISO 11135:1994 for medical devices [23] prior to the tests.

2.3. Light source

The equipment used in this study was a blue light (435 ± 20 nm; DY400-4, Denjoy Dental Co., Ltd., Shenzhen, China) with an output power of 1000–1400 mW/cm^2. The specimens have received a dose of 300–420 J/cm^2 during irradiation. The probe of blue light was held 1 mm above the top surfaces of the specimens. The diameter of the irradiated area (acrylic resin discs) was as the same as the diameter of the blue light probe (*i.e.* 8 mm).

2.4. Test microorganisms and growth conditions

Standard strains of *S. mutans* ATCC 35668, *S. sanguinis* ATCC 10556, L. *acidophilus* ATCC 314, and *C. albicans* ATCC 14053 (obtained from Iranian Biological Resource Center, Tehran, Iran) were cultured in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany). The strains were incubated in an anaerobic atmosphere at 37 °C for 48 h except for *C. albicans* which was incubated in an aerobic condition. To examine the antimicrobial efficacy of acrylic resin doped with *U. pinnatifida* following photo-activation, the test microbial suspension of approximately 1.5×10^8 colony forming units (CFU)/mL was prepared using both spectrophotometry (optical density [OD] 600 nm: 0.08–0.13) and colony counting.

2.5. Antimicrobial testing

2.5.1. Disc agar diffusion test

Based on the Clinical Laboratory Standards Institute (CLSI) guideline [24], disc agar diffusion method was performed by spreading the microbial inoculums of approximately 1–2 × 10^8 CFU/mL to the surface of BHI agar (Merck, Darmstadt, Germany) plates by a sterile swab. Acrylic resin discs containing a different concentration of *U. pinnatifida* following photo-activation were then placed on the inoculated BHI agar surface with 2 cm distance from each other. After incubation of plates in growth conditions depending on the type of microorganism for 24 h at 37 °C, the diameter of growth inhibition zones was measured.

2.5.2. Biofilm inhibition test

For the biofilm inhibition tests, acrylic resin discs containing a different concentration of *U. pinnatifida* following photo-activation were placed in the sterile tubes and microbial suspension with a concentration of 1.5 × 10^8 CFU/mL was added to each tube. The tubes were then incubated in growth conditions associated with each microorganism that mentioned above at 37 °C for 48 h in order for the biofilm to form. After that, discs were rinsed in phosphate-buffered saline (PBS; pH 7.4) for 1 min to eliminate planktonic microbial cells. To isolate biofilm-producing microorganisms, acrylic resin discs were exposed to sonication and vortexed. The obtained microbial suspension was serially diluted and spread-cultured in BHI agar. The microbial colonies were then counted using the previous study [25].

2.5.3. Eluted component test

Acrylic resin discs containing a different concentration of *U. pinnatifida* following photo-activation were placed in tubes containing 1 mL of normal saline. After 3, 7, 15 and 30 days, 100 µL of normal saline was transferred to the tube containing 100 µL of the microbial suspension with a final concentration of 1.5 × 10^5 CFU/mL. The tubes were placed in a shaking incubator with 120 rpm for 24 h at 37 °C. The obtained suspension was serially diluted and spread-cultured in BHI agar. The microbial colony counts were determined as mentioned in the previous section.

2.6. Statistical analysis

Data were entered in statistical package for social sciences (SPSS) software version 25 and analyzed using Two-way analysis of variance (ANOVA) and Bonferroni Post Hoc tests at a considered statistically significant of $P < 0.05$.

3. Results

3.1. Sequence retrieval, hierarchical structure and physicochemical indices of FDX

NCBI GenBank database showed that FDX (YP_009182605) has 97 amino acids that its estimated structure weight was 10573.83 Da. A similarity search against FDX identified in *U. pinnatifida* displayed that it is similar to the protein structure with the accession number of 3ab5. Primary structure prediction showed that FDX had 6 positively charged residues (Arg + Lys) and 15 negatively charged residues (Asp + Glu). As shown in Fig. 1, modeled spatial configuration of the FDX protein has two chains with Fe2/S2 cluster. According to the results, FDX was a stable protein with a grand average of hydropathicity value (GRAVY) -0.159. The very high aliphatic index was 85.46 and instability index (II) was computed to be 34.91, that provides the estimate of the stability of the protein. FDX theoretical pI was 4.17 and the extinction coefficient at 280 nm measured in water was 7825 $M^{-1} cm^{-1}$. This ensured that the quality of model was good. As well as, analysis of the local per-residue in different regions within the model and the estimation of the global quality of the entire model were the performance by QMEAN (Fig. 2). In this study, the QMEAN score and Z-score were 1.13 and 0.71 for FDX protein of *U. pinnatifida* (Fig. 2[a–c]). The results displayed that the model is valid.

3.2. Disc agar diffusion test analysis

Based on the results in Fig. 3, there was no growth inhibition zone around acrylic resin discs containing *U. pinnatifida* in any group.

3.3. Biofilm inhibition test analysis

As shown in Fig. 4, a considerable dose-dependent microbiocidal effect against biofilms of multispecies cariogenic biofilm-producing microbiota was observed with a reduction in viable microbial cells according to the percentages of *U. pinnatifida* incorporated into the acrylic resin. The results revealed that acrylic resins doped with *U. pinnatifida* following photo-activation significantly reduced *S. mutans* colony count to 79%, 97%, and 99% in concentrations of 0.5%, 1%, and 2%, respectively compared to the control group ($P < 0.05$; Fig. 4). On the other hand, only 1% and 2% concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation could considerably reduce the biofilm forms of *S. sanguinis*, L. *acidophilus* and, *C. albicans* in compared with the control group ($P < 0.05$). Based on the results, 2% concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation could inhibit the microbial biofilm form in all microorganisms to 99%.

3.4. Eluted component test analysis

Comparing the colony count in groups with 0.5%, 1% and 2% concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation showed that colony count of cariogenic microorganisms decreased with an increase in the concentration of *U. pinnatifida*; however, the differences were not statistically in several groups (Table 1).

As well as, as shown in Table 1, evaluation of microbial proliferation at four time points by statistical analysis revealed that there was a remarkable reduction in count of *S. mutans*, *S. sanguinis*, *L. acidophilus*, and *C. albicans* in 1% and 2% concentrations of acrylic resins doped with *U. pinnatifida* following photo-activation compared to the control group after 3 days ($P < 0.05$). However, in contrast to *L. acidophilus* and *C. albicans*, the 0.5% concentration of acrylic resins doped with *U. pinnatifida* following photo-activation did not reduce significantly the colony count of *S. mutans* and *S. sanguinis* ($P = 0.17$, $P = 0.08$, respectively) in comparison with the control group on the third day ($P > 0.05$). Based on the results of the current study, it was found that the CFUs/mL of all microorganisms exposed to different concentration of acrylic resins doped with *U. pinnatifida* following photo-activation were reduced significantly on day 7, 15 and 30 days ($P < 0.05$).

4. Discussion

Our findings based on the several bioinformatics tools and computer simulation molecular modeling data are consistent with the recent studies that show FDX comprise a ubiquitous and highly diverse group of soluble small, 6–13 kDa, proteins containing iron and sulfur atoms. These biological "capacitors" can discharge electrons produced from photo-excited Chl-a in *U. pinnatifida* and transfers them to FDX–$NADP^+$ reductase for $NADP^+$ reduction in photosystem I, which is essential in the Calvin cycle for carbon assimilation. FDX acts as an electron transfer agent in the redox reactions [20,26]. Several studies reported that, chlorophyll-a as a photosensitizer with light of appropriate wavelength in PDT generate ROS which are toxic to microbial cells. It has been shown that FDX transcripts have been observed to decrease under environment stresses including light, resulting in increased the ROS level in FDXs-limiting conditions. ROS including hydrogen peroxide

(H_2O_2) are further increased by the cells suffering from stress due to the light [20–22].

In the present study, we evaluated the antimicrobial potential of acrylic resin doped with different concentrations of *U. pinnatifida* following photo-activation using blue light during a 30-day time course of rinsing. As a common procedure that used to assess the activity of antimicrobial agents, antimicrobial activity was evaluated under two different experimental conditions, on multispecies cariogenic biofilm-producing microbiota including *S. mutans*, *S. sanguinis*, L. *acidophilus*, and *C. albicans* and pure cultures of these strains in disc agar diffusion assay.

An appropriate antimicrobial agent for addition to the acrylic resin in orthodontic adhesive must be able to diffuse into the environment. The data acquired from this study showed no growth inhibition zone around discs in any group.

The results from this study contradicted the results of Phull et al., [27]. They evaluated the inhibition zones of *U. pinnatifida* against Gram-positive bacteria (*Micrococcus luteus* and *S. aureus*), Gram-negative bacteria (*S. typhimurium*), and fungal strains (*Aspergillus flavus*, *A. fumigatus*, and Mucor species). Their data suggested that maximum zone of inhibition of the bacterial growth against *S. aureus* (15.67 ± 0.76 mm) and fungal growth against *A. fumigatus* (11.83 ± 1.01) among other microorganisms. The results of the current study indicate insolubility and poor diffusion of *U. pinnatifida* following photo-activation in the agar based medium around acrylic resin discs. Thus, unlike Phull et al., [27] study, *U. pinnatifida* following photo-activation do not have noncontact antimicrobial activity.

On the other hand, the current results showed that acrylic resins doped with *U. pinnatifida* following photo-activation caused a considerable reduction in the count of colonies and biofilms of test microorganisms over the test period. The analysis of biofilm inhibition demonstrated that 2% concentration of acrylic resins doped with *U. pinnatifida* following photo-activation inhibited the biofilms formation of all test microorganisms by 99%. Relevant to this research was a study was done by Ghorbanzadeh et al., [2] which generated silver nanoparticles *in situ* in PMMA. Their results demonstrated that the average levels of test cariogenic microorganisms decreased about 30.9–98.4% compared with the control group. In addition, biofilm inhibition analysis showed that silver nanoparticles in acrylic resin inhibited the biofilms of all test microorganisms by 20.1–79.9% compared to the control group.

The eluted component test presented the antimicrobial activity of acrylic resins doped with *U. pinnatifida* following photo-activation over time and demonstrated the substantively of antimicrobial activity. The results of the eluted component test in this study revealed a significant reduction in colony counts of all four microorganisms.

5. Conclusion

Overall, the results mentioned above, support *in vitro* significant antimicrobial activity of brown seaweed microalga *U. pinnatifida* following photo-activation against multispecies cariogenic biofilm-producing microbiota.

Acknowledgement

This research has been supported by Tehran University of Medical Sciences & health Services grant No. 97-02-30-38784.

References

[1] H.A. Kiyak, Does orthodontic treatment affect patients' quality of life? J. Dent. Educ. 72 (2008) 886–894.
[2] R. Ghorbanzadeh, B. Pourakbari, A. Bahador, Effects of baseplates of orthodontic appliances with in situ generated silver nanoparticles on cariogenic bacteria: a randomized, double-blind cross-over clinical trial, J. Contemp. Dent. Pract. 16 (2015) 291–298.
[3] A. Sodagar, S. Khalil, M.Z. Kassaee, A.S. Shahroudi, B. Pourakbari, A. Bahador, Antimicrobial properties of poly (methyl methacrylate) acrylic resins incorporated with silicon dioxide and titanium dioxide nanoparticles on cariogenic bacteria, J. Orthod. Sci. 5 (2016) 7–13.
[4] M. Cierech, I. Osica, A. Kolenda, J. Wojnarowicz, D. Szmigiel, W. Łojkowski, et al., Mechanical and physicochemical properties of newly formed ZnO-PMMA nanocomposites for denture bases, Nanomaterials Basel (Basel) 8 (2018) e305.
[5] B.E. Costa Oliveira, J.A. Cury, A.P. Ricomini Filho, Biofilm extracellular polysaccharides degradation during starvation and enamel demineralization, PLoS One 12 (2017) e0181168.
[6] A.A. Ribeiro, Biological features of dental caries, JSM. Dent 4 (2016) 1065.
[7] D. Bourgeois, A. David, C. Inquimbert, P. Tramini, N. Molinari, F. Carrouel, Quantification of carious pathogens in the interdental microbiota of young caries-free adults, PLoS One 12 (2017) 185804.
[8] T.C. Schott, H. Meyer-Gutknecht, N. Mayer, J. Weber, K. Weimer, A comparison between indirect and objective wear-time assessment of removable orthodontic appliances, Eur. J. Orthod. 6 (2016) 65–69.
[9] M.M. Pithon, L.I.D. Santanna, F.C.S. Baiao, R. da Silva Coquerro, L.C. Maia, L.R. Paranhos, Effectiveness of different mechanical bacterial plaque removal methods in patients with fixed orthodontic appliance: a systematic review/meta-analysis, Biosci. Horiz. 33 (2017) 537–554.
[10] A.S. Altmann, F.M. Collares, F.A. Ogliari, S.M. Samuel, Effect of methacrylated-based antibacterial monomer on orthodontic adhesive system properties, Am. J. Orthod. Dentofacial Orthop. 147 (2015) 82–87.
[11] H.F. Saloom, H.S. Mohammed-Salih, S.F. Rasheed, The influence of different types of fixed orthodontic appliance on the growth and adherence of microorganisms (in vitro study), J. Clin. Exp. Dent. 5 (2013) 36–41.
[12] J. Patra, S. Lee, J. Park, K.H. Baek, Antioxidant and antibacterial properties of essential oil extracted from an edible seaweed *Undaria Pinnatifida*, J. Food Biochem. 41 (2016) e12278.
[13] H.J. Yang, J.H. Lee, K.Y. Lee, K. Bin Song, Antimicrobial effect of an *Undaria pinnatifida* composite film containing vanillin against *Escherichia coli* and its application in the packaging of smoked chicken breast, IFST 52 (2017) 398–403.
[14] J.M. Anderson, J. Barrett, Chlorophyll-protein complexes of brown algae: P700 reaction centre and light-harvesting complexes, Ciba Found. Symp. 9 (1978) 81–104.
[15] S.M. Kim, Y.F. Shang, B.H. Um, A preparative method for isolation of fucoxanthin from Eisenia bicyclis by centrifugal partition chromatography, Phytochem. Anal. 22 (2011) 322–329.
[16] B.H. Song, D.H. Lee, B.C. Kim, S.H. Ku, E.J. Park, I.H. Kwon, et al., Photodynamic therapy using chlorophyll-a in the treatment of acne vulgaris: a randomized, single-blind, split-face study, J. Am. Acad. Dermatol. 71 (2014) 764–771.
[17] V. Rosenbach-Belkin, L. Chen, L. Fiedor, I. Tregub, F. Pavlotsky, V. Brumfeld, et al., Serine conjugates of chlorophyll and bacteriochlorophyll: photocytotoxicity in vitro and tissue distribution in mice bearing melanoma tumors, Photochem. Photobiol. 64 (1996) 174–181.
[18] P. Semeraro, G. Chimienti, E. Altamura, P. Fini, V. Rizzi, P. Cosma, Chlorophyll a in cyclodextrin supramolecular complexes as a natural photosensitizer for photodynamic therapy (PDT) applications, Mater. Sci. Eng. C Mater. Biol. Appl. 85 (2018) 47–56.
[19] K. Ocakoglu, O. Er, G. Kiyak, F.Y. Lambrecht, C. Gunduz, C. Kayabasi, 131I-Zn-Chlorophyll derivative photosensitizer for tumor imaging and photodynamic therapy, Int. J. Pharm. 493 (2015) 96–101.
[20] L.F. Huang, J.Y. Lin, K.Y. Pan, C.K. Huang, Y.K. Chu, Overexpressing ferredoxins in *Chlamydomonas reinhardtii* increase starch and oil yields and enhance electric power production in a photo microbial fuel cell, Int. J. Mol. Sci. 16 (2015) 19308–19325.
[21] S. Takahashi, N. Murata, How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13 (2008) 178–182.
[22] N. Suzuki, S. Koussevitzky, R. Mittler, G. Miller, ROS and redox signalling in the response of plants to abiotic stress, Plant Cell Environ. 35 (2012) 259–270.
[23] G.A. Mosley, J.R. Gillis, Factors affecting tailing in ethylene oxide sterilization part 1: when tailing is an artifact and scientific deficiencies in ISO 11135 and EN 550, PDA J. Pharm. Sci. Technol. 58 (2004) 81–95.
[24] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—10th Ed. M07-A11, Clinical and Laboratory Standards Institute, Wayne, PA, 2018.
[25] A. Sodagar, A. Bahador, M. Pourhajibagher, B. Ahmadi, P. Baghaeian, Effect of addition of curcumin nanoparticles on antimicrobial property and shear bond strength of orthodontic composite to bovine enamel, J. Dent. Tehran (Tehran) 13 (2016) 373–382.
[26] D.I. Arnon, R.K. Chain, Regulation of ferredoxin-catalyzed photosynthetic phosphorylations, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 4961–4965.
[27] A. Phull, A. Ali, M. Ahmed, M. Zia, I. Haq, S.J. Kim, In vitro antileishmanial, antibacterial, antifungal and anticancer activity of fucoidan from Undaria pinnatifida, IJB 11 (2017) 219–227.