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Research Article

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In Vitro Investigation of the Effects of Resveratrol on Peri-Implant Biofilm: A Pilot Study

Resveratrolün Peri-İmplant Biyofilm Üzerindeki Etkilerinin In Vitro İncelenmesi: Bir Pilot Çalışma

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Abstract

Objective: The aim of this study was to investigate the in vitro efficacy of resveratrol (3,5,4′-trihydroxystilbene), known for its potent antimicrobial, anti-inflammatory, and anti-biofilm properties, as a natural and biocompatible alternative for the inhibition and control of peri-implant biofilm.

Materials and Methods: Resveratrol (Sigma-Aldrich, USA) was dissolved in dimethyl sulfoxide (DMSO) to obtain an 80 mg/mL stock solution and diluted with distilled water to 800 µg/mL (final DMSO ≤1%). Chlorhexidine gluconate (0.2%) and phosphate-buffered saline (PBS) were used as positive and negative controls, respectively. The antimicrobial activity was tested against *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Fusobacterium nucleatum* ATCC 25586 and *Porphyromonas gingivalis* ATCC 33277 using the microdilution method to determine the minimum inhibitory concentrations (MICs). For biofilm analysis, dual-species biofilms of *F. nucleatum* and *P. gingivalis* were formed on titanium discs and treated with the test materials. Biofilm inhibition and disruption were assessed using confocal laser scanning microscopy after live/dead staining. Biofilm biomass and viability were quantified using ImageJ software. Data are summarized as mean ± SD, median and range. Group differences were assessed using the Kruskal–Wallis test with Dunn's post hoc (Bonferroni correction), accepting $p < 0.05$ as statistically significant. Analyses were performed using Statistics Kingdom (Australia, https://www.statskingdom.com).

Results: Resveratrol showed antimicrobial activity against *P. gingivalis* and *F. nucleatum* with MICs of 400 µg/mL and 200 µg/mL, respectively. No antimicrobial effect was observed on *S. aureus* and *C. albicans* at the dilution used in the study. Although resveratrol showed some inhibitory and decontaminating effects on biofilm formation, these were not statistically significant ($p>0.05$). In contrast, chlorhexidine showed significant efficacy under both experimental conditions ($p<0.05$).

Öz

Amaç: Bu çalışmanın amacı, resveratrolün (3,5,4′-trihidroksistilben) peri-implant biyofilm oluşumu ve kontrolündeki antimikrobiyal ve anti-biyofilm etkinliğini araştırmaktır.

Gereç ve Yöntem: Resveratrol (Sigma-Aldrich, ABD) Dimetil Sülfoksit (DMSO)'te çözülerek 80 mg/mL stok çözeltisi hazırlanmış ve distile su ile seyreltilerek 800 µg/mL'ye (son DMSO ≤%1) ulaşılmıştır. Pozitif kontrol olarak %0.2 Klorheksidin glukonat, negatif kontrol olarak ise fosfat tamponlu salin (PBS) kullanıldı. Antimikrobiyal etkinlik; *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Fusobacterium nucleatum* ATCC 25586 ve *Porphyromonas gingivalis* ATCC 33277 türlerine karşı mikrodilüsyon yöntemi ile minimal inhibitör konsantrasyonlar (MİK) belirlenerek test edildi. Biyofilm analizinde, Ti diskler üzerinde oluşturulan *F. nucleatum* ve *P. gingivalis* ikili biyofilmleri deney materyalleriyle muamele edildi. Biyofilm inhibisyonu ve dekontaminasyon etkisi, Live/Dead boyama sonrası konfokal lazer taramalı mikroskop (CLSM) ile değerlendirildi. Biyofilm biyokütlesi ve canlılık oranları ImageJ yazılımı ile analiz edildi. Veriler ortalama ± SS, medyan ve aralık olarak özetlendi. Gruplar arasındaki farklar Kruskal–Wallis testi ve Bonferroni düzeltmeli Dunn post hoc testi ile değerlendirildi; $p<0,05$ anlamlı kabul edildi. Analizler Statistics Kingdom (Avustralya, https://www.statskingdom.com) programı kullanılarak yapıldı.

Bulgular: Resveratrol, P. gingivalis ve F. nucleatum üzerinde sırasıyla 400 µg/mL ve 200 µg/mL MİK değerleri ile antimikrobiyal etki gösterdi. *S. aureus* ve *C. albicans* üzerinde antimikrobik etkisi saptanmadı. Resveratrolün biyofilm oluşumunu inhibe edici ve olgun biyofilmi dekontamine edici bazı etkileri gözlenmiş olsa da, bu etkiler istatistiksel olarak anlamlı bulunmadı ($p>0,05$). Klorheksidin ise her iki deneyde de anlamlı etki gösterdi ($p<0,05$).

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Conclusion: Resveratrol exhibited antimicrobial effects against key anaerobic pathogens involved in peri-implant diseases but did not significantly reduce the biofilm mass or viability under the tested conditions. Further studies are needed to explore its potential under varying doses, durations, and along with other agents.

Keywords Resveratrol · Antimicrobials · Anti-biofilm · Polyphenolic compounds · Peri-implant biofilm

Sonuç: Resveratrol, peri-implant hastalıklarla ilişkili başlıca anaerop patojenlere karşı antimikrobiyal etkin görünmektedir ancak uygulanan koşullar altında biyofilm kütlesi veya canlılığında anlamlı bir azalma sağlamamıştır. Farklı doz, uygulama süresi ve diğer ajanlarla kombinasyonları dikkate alınarak yapılacak ileri çalışmalara ihtiyaç duyulmaktadır

Anahtar Kelimeler Peri-implant biyofim · Resveratrol · Antimikrobiyaller · Anti-biyofilm · Polifenolik bileşikler

INTRODUCTION

Peri-implant disease is the most common biological complication and is characterized by inflammation and subsequent bone loss around dental implants (1). Inflammatory bone resorption around dental implants has a high rate of progression and can result in the loss of the implant. Although it seems similar to periodontal disease structurally and etiologically, it differs in some points in terms of the pathogenesis of inflammation. Peri-implant infection is mostly initiated by the peri-implant biofilm, as in periodontal disease. Recent studies have shown that some microorganisms such as *Porphyromonas gingivalis, Escherichia coli, Tannerella forsythia, Fusobacterium nucleatum, Staphylococcus aureus, Pseudomonas aeruginosa, Candida species* and viral species such as *cytomegalovirus* and *Epstein-Barr virus* dominate in peri-implant lesions (2-5). Although the primary goal of peri-implant disease therapy is to eliminate biofilm from the implant surface, the complexity of these biofilms presents a significant challenge in the effective management of peri-implant lesions, necessitating innovative therapeutic strategies.

Despite the differences between periodontal and peri-implant diseases, there are approaches similar to periodontal therapy for the treatment of peri-implant lesions within today's possibilities. In addition to the traditional concept of "Cumulative Interceptive Supportive Therapy" (C.I.S.T.) (6), approaches aiming to modify the implant surface, including mechanical treatment methods including hand instruments, titanium brushes and other similar tools, have been widely studied in the literature (7). However, studies have demonstrated the limited potential of these mechanical methods in challenging circumstances such as hard-to-reach deep and narrow areas, macro- and micro-roughness of implant surfaces, which may retain bacteria or deposits, or facilitation of bacterial accumulation in grooves and pits created during instrumentation (8-10).

In order to overcome these limitations, attempts have been made to add some antimicrobial agents to the mechanical therapy to decontaminate the implant surface chemically. Chemical preparations can penetrate and control the biofilm that accumulates in hard-to-access or deep areas, grooves, or threads in peri-implant surfaces. Studies have shown that the addition of chemotherapeutic agents to mechanical treatment yields superior results compared with mechanical treatment alone in managing peri-implant lesions (11-13). However, the potential toxicity and adverse effects of these chemical agents on the implant surface and adjacent biological environment (13-15) have shifted the research focus toward agents that offer both antimicrobial efficacy and enhanced biocompatibility.

As an alternative, natural compounds have recently gained attention for their antimicrobial and therapeutic properties in biofilm-associated inflammations (13). Resveratrol (RSV), a naturally occurring polyphenolic compound primarily found in grapes, red wine, peanuts, and various berries, has attracted considerable scientific interest due to its diverse biological activities, including antioxidant, anti-aging, anti-tumor, anti-inflammatory, and antimicrobial properties. In a study performed earlier, RSV was observed to exhibit antibacterial activity against gram-positive bacteria such as *S. aureus, Enterococcus faecalis,* and *Bacillus cereus* (16). Additionally, it inhibited the cariogenic virulence properties of *Streptococcus mutans* in a recent study (17). In a previous study, RSV demonstrated antibacterial activity against gram-positive bacteria, including *S. aureus*, *E. faecalis*, and *B. cereus* (16). Furthermore, it has recently been reported to inhibit the cariogenic virulence traits of *S. mutans* (17).

Regarding gram-negative pathogenic bacteria, several studies have reported that RSV inhibits various strains, virulence factors, viability, collagen destruction, and biofilm formation of *P. gingivalis*, which is particularly important in peri-implant inflammation and is defined as a key pathogen in periodontal disease (18). There are also studies reporting the inhibiting effect of RSV on *F. nucleatum*, which plays a critical role in periodontal and peri-implant inflammation, and on other pathogenic species including *E. coli, Pseudomonas aeruginosa, Vibrio cholerae and Candida species* (19-21).

Peri-implant lesions are very difficult to treat because of the difficulties mentioned above during mechanical approaches and because they are associated with diverse, resistant, and robust biofilms such as periodontal lesions. Chemotherapeutic agents should penetrate as deeply as possible and reduce or prevent biofilm formation on the titanium (Ti) surface to overcome the insufficiency of mechanical applications. While several studies have evaluated the effect of RSV against various planktonic bacteria, *only a few have investigated biofilm formation* (10, 22), *most of which use single-species biofilm models* (18, 20). In order to mimic the biofilm environment around the dental implants and to observe the effect of RSV on a microbial formation more similar to that around a dental implant, a dual-biofilm structure was selected.

Based on the hypothesis that resveratrol (RSV) application can significantly inhibit biofilm formation on titanium surfaces, reducing

both microbial viability and biofilm thickness, this study aimed to investigate the antimicrobial and anti-biofilm activities of RSV during the formation, maturation, and control of an artificial dual-species biofilm on sandblasted, large-grit, acid-etched (SLA) titanium surfaces, involving *P. gingivalis* and *F. nucleatum*, both of which play important roles in peri-implant lesions.

MATERIALS AND METHODS

Preparation of the Resveratrol Solution

Resveratrol (3,5,4'-trihidroksistilben) (Sigma-Aldrich, US) was dissolved in dimethyl sulfoxide (DMSO, Sigma- Aldrich, US) to a stock concentration of 80 mg/mL. The 800 µg/mL concentration used for the experiments was prepared by diluting the stock concentration with distilled water (the concentration of DMSO used was up to 1%) (17). Chlorhexidine gluconate (0.2% w/v) (Klorhex, Drogsan, TR) (positive) and phosphate-buffered saline (PBS) (negative) were used as controls.

Microbial Strains and Culture Conditions

The microorganisms selected for this study included *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, *F. nucleatum* ATCC 25586, and *P. gingivalis* ATCC 33277 strains. Cultures were maintained by 3 days subculture in Brucella agar (Merck KGaA, Germany) supplemented with 5 mg/mL hemin, 5 mg/mL menadione, and 5% defibrinated sheep blood incubated at 37 °C under anaerobic conditions (80% N_2, 10% CO_2, and 10% H_2) using an atmosphere generator (BD GasPak EZ, USA) and incubation jar for *P. gingivalis* and *F. nucleatum*, where overnight in Brain Heart Infusiin (BHI) agar (Merck) and Saboraoud Dextrose (SD) agar (Merck) at 37 °C under aerobic conditions for *S. aureus* and *C. albicans*, respectively. For preculture preparation, a single colony of each microorganism was inoculated into 10 mL of broth: BHI supplemented with hemin and menadione for *P. gingivalis* and *F. nucleatum*, plain BHI broth for *S. aureus*, and SD broth for *C. albicans*. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, corresponding to an approximate final concentration of 1.5×10^8 colony-forming units (CFU)/mL.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined using the broth dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2006), conducted in 96-well flat-bottom microliter plates. Briefly, serial twofold dilutions of the solutions were prepared in the organism-specific broth, with a final volume of 100µl per well. One well without solution was used as the control. Subsequently, 100 µL of the microbial suspension was inoculated into each well. The microplates were incubated at 37°C for 24 h under aerobic conditions for *S. aureus* and *C. albicans*, and then for 72 h under anaerobic conditions for *P. gingivalis* and *F. nucleatum*. The MIC was defined as the lowest concentration of the test material that visibly inhibited microbial growth. All experiments were performed in triplicate, and the median value from the three independent assays was reported as the MIC.

Biofilm Assays

The inhibitory effect of RSV on biofilm formation, as well as its decomtaminating effect on the mature biofilm, was investigated with an in vitro *F. nucleatum-P. gingivalis* dual-species biofilm model generated on 5-mm-diameter titanium discs with SLA surfaces (Bilimplant, Proimtech, Istanbul, Turkiye). The discs were placed in 24-well polystyrene cell culture plates and coated with 500µL of saliva, prepared according to the protocol described by Baffone et al. (23). Following incubation at 37 °C for 1 h to allow for the formation of a salivary pellicle, the discs were gently rinsed with PBS and transferred into new wells. The samples were divided into two main groups, with six in each subgroup.

To assess the inhibition of biofilm formation, the discs were treated with 20 µL of the test solutions and then 2 ml of the precultured *F. nucleatum-P. gingivalis suspension* (prepared in equal volumes) was added to each well. The plates were incubated at 37°C under anaerobic conditions for 72 h. After culturing, the samples were gently rinsed with PBS to remove non-adherent bacteria.

To evaluate the decontaminating activity of the test solutions on the mature biofilm, Ti discs were first inoculated with the *F. nucleatum-P. gingivalis* preculture and incubated under anaerobic conditions at 37°C for 72 h, without the application of any test material. Then, the discs were rinsed with PBS to remove non-adherent bacteria. Subsequently, the test materials were applied directly to the biofilm layer for 2 min, followed by another PBS rinse.

All treated discs were transferred into 24-well plates and stained using a commercially available Bacterial Viability and Counting Kit (BacLight™ Invitrogen, BE) and kept for 15 min under light protection. For each disc, three random fields were selected and analyzed by confocal laser scanning microscopy (CLSM Leica TCS SPE; Leica Lasertechnik, DE) at excitation wavelengths of 488 nm and 532 nm, with x10/1.0 optical lenses. The images of the biofilm-covered surfaces were obtained for both qualitative and quantitative assessments. The acquired image stacks were analyzed using the ImageJ software to determine the number of viable and nonviable (dead) bacterial cells. In addition, the viable biomass ($\mu m^3/\mu m^2$) present on the disc surfaces was quantified using COMSTAT2 software (24).

Statistical Analysis

Descriptive statistics consisting of the mean, standard deviation (SD), median, and range (minimum–maximum) were calculated. Kruskal-Wallis test was used to determine any significant differences between the group parameters. The Post hoc Dunn's test using

Bonferroni correction was used for the pair-wise comparisons. Any probability level above %95 ($p<0.05$) was set as statistically significant. All statistical analyses were performed using a web-based program (Statistics Kingdom, AU Available from https://www.statskingdom.com).

RESULTS

Antibacterial Activity of RSV

Table 1. MIC values against the standard strains of the microorganisms.

MIC value µg/mL	Strains			
	S. aureus ATCC 25923	***C. albicans*** ATCC 10231	***P. gingivalis*** ATCC 33277	***F. nucleatum*** ATCC 25586
Resveratrol	>800	>800	400	200
Chlorhexidine	0.6	0.6	1	0.6

MIC: Minimum inhibitory concentration

The anti-microbial activity of RSV against *P. gingivalis, F. nucleatum, S. aureus, and C. albicans* was assessed using standard broth microdilution methods. The initial concentration of RSV used for the assays was 800 µg/mL. The MICs against the standard strains of the microorganisms are shown in Table 1. The MIC value of resveratrol for *P. gingivalis* was 400 µg/mL while it was 200 µg/mL for *F. nucleatum*. On the other hand, resveratrol did not show any antimicrobial effect on *S. aureus* and *C. albicans* at the dilution used in the study.

Biofilm Assays

The biofilm-forming activity of RSV on Ti surfaces

Table 2. Comparison results of CLSM analysis of the effects of the biofilm formation by groups. Kruskal– Wallis test, differences between groups marked with superscript uppercase letters and their lowercase counterparts in the same row are statistically significant, with a minimum difference of <0.05.

		Groups		
		Control	**Resveratrol**	**Chlorhexidine**
Biomass	**Mean±SD**	150.8 ± 22.1 A	142.12 ± 50.7 B	13.6 ± 5 ab
	Median (min-max)	151.3 (103.8-181.7)	165.2 (128.4-183.9)	13.8 (7.4-21.4)
Live/Dead	**Mean±SD**	1.27 ± 0.76 A	1.22 ± 0.1 B	1.72 ± 6.8 ab
	Median (min-max)	1.28 (1.05-1.54)	1.21 (1.08-1.45)	1.76 (0.6-3.1)

CLSM: confocal laser scanning microscopy, sd: standard deviation

The impact of RSV on biofilm formation was assessed when *P. gingivalis* and *F. nucleatum* were grown on Ti-discs after treatment with RSV. After 72 h of incubation, the mature biofilm was evaluated by CLSM analysis (Figures 1 and 2). The biomass and live/dead bacteria ratio of the biofilms for the test group and the negative (treated with only PBS) and positive (treated with CHX) control groups are shown in Table 2. The biofilm biomass for the RSV group was found to be lower than that for the negative control group, but this difference was not statistically significant ($p>0.05$). On the other hand, the CHX group exhibited significantly lower biofilm biomass than the other groups ($p<0.05$). Regarding the live/dead bacteria ratio, no significant difference was found between the groups ($p>0.05$).

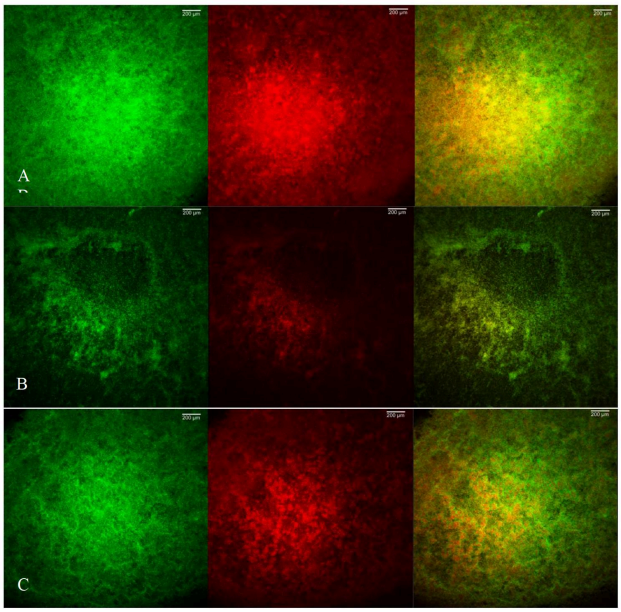


Figure 1. CLSM images (x4) of the dual-species biofilm model formed on the Ti surface pretreated with PBS (control), CHX, and RSV.
The green color indicates live bacterial cells. The red color defines nonviable cells. The images on the far right combine two situations from the same area. A: Control, B: CHX, C: RSV. CLSM: confocal laser scanning microscopy, PBS: phosphate buffer saline, CHX: chlorhexidine, RSV: resveratrol.

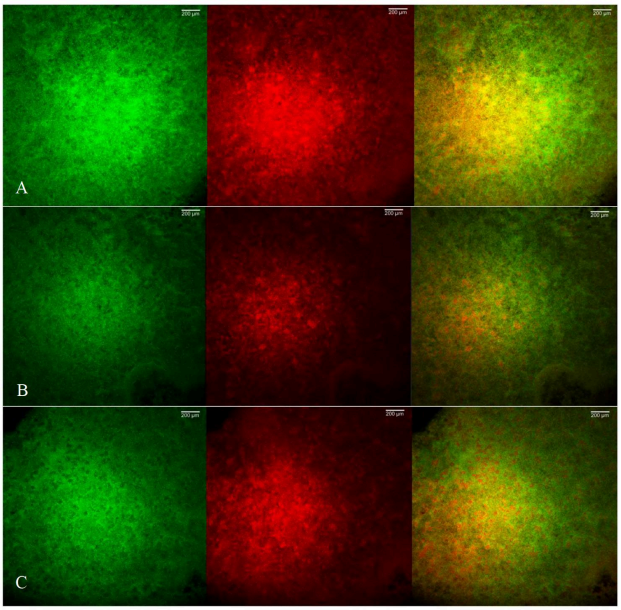


Figure 2. CLSM images (x4) of the dual-species biofilm model formed on the Ti surface after treatment with PBS (control), CHX and RSV.
The green color indicates live bacterial cells. The red color defines nonviable cells. The images on the far right combine two situations from the same area. A: Control, B: CHX, C: RSV. CLSM: confocal laser scanning microscopy, PBS: phosphate buffer saline, CHX: chlorhexidine, RSV: resveratrol

Effect of RSV on the mature biofilm

The reduction rates of the experimental groups on matured biofilms were evaluated by CLSM analysis. Resveratrol reduced the matured biofilm biomass, but the reduction was not statistically significant ($p>0.05$), whereas it was significant for the CHX group ($p<0.05$). The CHX group also exhibited a significantly lower live/dead bacteria ratio than the other groups (Table 3).

Table 3. Comparison results of the effects of groups on mature biofilm using CLSM analysis Kruskal–Wallis test, differences between groups marked with superscript uppercase letters and their lowercase counterparts in the same row are statistically significant, with a minimum difference of <0.05.

		Groups		
		Control	Resveratrol	Chlorhexidine
Biomass	Mean±SD	150.8 ± 22.1 A	129.41 ± 43.9 B	24.94 ± 18.76 ab
	Median (min-max)	151.3 (103,8-181,7)	145.4 (58.1-181.3)	20.2 (12.0-46.5)
Live/Dead	Mean±SD	1.27 ± 0.76 A	1.42 ± 0.35 B	0.50 ± 0.37 ab
	Median (min-max)	1.2 (1.05-1.54)	1.33 (1.04-2.14)	0.58 (0.26-0.92)

CLSM: confocal laser scanning microscopy, sd: standard deviation

DISCUSSION

Peri-implant lesions are inflammatory conditions resulting from multispecies biofilms, predominantly consisting of anaerobic bacteria that gradually degrade the peri-implant supporting tissues (25, 26). These biofilms can exhibit significant resistance to antibiotic therapies due to their complex architecture and microbial diversity (27). To accurately evaluate the efficacy of antibacterial agents against such resilient biofilms and to elucidate the underlying mechanisms, advanced three-dimensional (3D) in vitro models that reproduce the complex triangular interactions between the host tissue, implant surface, and biofilm are essential. In recent years, dual-species biofilm models have gained increasing attention in periodontal research because of their simplicity and experimental reliability. These models offer a controlled and reliable platform for studying interspecies interactions and evaluating the initial efficacy of antimicrobial agents in environments that closely mimic the microbial conditions of pathological sites such as peri-implant lesions. The majority of previous studies have relied on single-species models, which might not accurately capture the synergistic interactions found in multispecies biofilms. This study aimed to provide a more clinically relevant evaluation of the antibiofilm activity of RSV using a dual-species biofilm model.

Chemical decontamination remains a fundamental approach in the management of peri-implant diseases. Despite increasing interest in natural compounds, the antimicrobial efficacy of plant-derived extracts on titanium (Ti) has not yet been sufficiently explored. Conventional chemical decontamination agents such as chlorhexidine, hydrogen peroxide, citric acid, and laser application have been widely studied; however, their use is associated with several drawbacks, including irritation, cytotoxicity, or limited efficacy (28-32). In the present study, resveratrol (RSV) exhibited MIC values of 400 µg/mL for *P. gingivalis* and 200 µg/mL for *F. nucleatum*, with the corresponding minimum bactericidal concentration (MBC) values aligning closely. Previous studies evaluating the antibacterial activity against gram-negative anaerobes have reported variable MIC values. He et al. (20) reported an MIC of 100 µg/ml for *F. nucleatum*, whereas Kugaji et al. (18) found MIC values ranging from 78 to 156 µg/mL against *P. gingivalis* and other clinical strains. O'Connor et al. (33) reported an MIC of 80 µg/mL for *P. gingivalis* but no effect on *F. nucleatum, S. aureus* and *C. albicans*. Furthermore, Paulo et al. (16) reported the potential antimicrobial activity of RSV against gram-positive bacteria with high sensitivity observed at 100 µg / mL, whereas the MIC values for gram-negative bacteria exceeded 400 µg/mL. On the contrary, no antimicrobial activity was detected against *S. aureus* and *C. albicans* in the present study, consistent with studies that found RSV to be ineffective against these strains or gram-positive bacteria (34, 35). Vestergaard and Ingmer (36) attribute discrepancies in the reported MIC values for *S. aureus* (ranging from 100 to >1000 µg/mL) to variations in the growth media (16, 37-39).

Although RSV demonstrated notable antibacterial activity against *P. gingivalis* and *F. nucleatum* in the planktonic form, its inhibitory effect on dual-species biofilm formation and matured biofilm structure did not reach statistical significance. Previous studies generally evaluated its inhibitory effect on mono-species biofilm structures of *F. nucleatum, P. gingivalis, E. coli, S. aureus, C. albicans* and many other gram-positive and gram-negative bacteria with significant (16, 18, 20, 33) or non-significant (34, 35) results. Most of them evaluated biofilms accumulated different surfaces than the Ti surface. A recent study evaluated the same activity of RSV and naringin (NAR) on the biofilm structure accumulated on SLA Ti surfaces and compared it with conventional chemical agents using a mono-species model. They found that both of these molecules reduced the biofilm biomass of *S. aureus* and *P. gingivalis* on rough titanium surfaces (10).

Resveratrol was also found to be effective on the biomass and viability of titanium surfaces, but this effect did not reach statistical significance. Conversely, chlorhexidine (CHX) significantly reduced both the biofilm biomass and the live bacterial ratios. These findings contrast with those of Millhouse et al., who used a multispecies biofilm model composed of *S. mitis*, *F. nucleatum*, *P. gingivalis*, and *Aggregatibacter actinomycetemcomitans* co-cultured with human oral epithelial cells (22). While RSV in that study did not significantly reduce the bacterial viability of the mature biofilm, it notably downregulated host-derived IL-8 protein release, suggesting an anti-inflammatory, host-modulatory effect rather than a direct antimicrobial action. Taken together, these findings highlight a key distinction: RSV may exhibit limited antibiofilm efficacy on its own in

dual or multispecies biofilms but could exert a relevant modulatory effect on the host immune response within complex periodontal microenvironments.

In addition, in the present study, both the biofilm inhibition and mature biofilm decontamination effects of RSV were assessed under strict anaerobic conditions using CLSM and COMSTAT2-based quantitative analysis. This approach reflects a more clinically relevant setting by incorporating bacterial synergy and interspecies interactions, which are absent in single-species models. Therefore, while the findings of Zhou et al. are valuable in understanding individual bacterial responses to RSV, the dual-species model used herein provides deeper insight into its performance within a complex peri-implant-like biofilm structure (10).

The biofilm assay revealed a surprisingly higher viable cell ratio in the RSV-treated group, possibly because the antioxidant activity of RSV modulated the redox-sensitive bacterial viability under anaerobic conditions. Although this phenomenon may have influenced the in vitro results, its translational significance in the naturally hypoxic peri-implant microenvironment remains unclear and warrants further investigation.

This *in vitro* pilot study presents certain limitations that should be acknowledged when interpreting the results. First, the dual-species biofilm model employed—comprising *P. gingivalis* and *F. nucleatum*—does not fully replicate the complexity of multispecies peri-implant biofilms encountered in clinical conditions. The absence of host-derived factors, such as immune responses and salivary components, further limits the model's physiological relevance. Additionally, the short exposure time to resveratrol and the static incubation conditions may not accurately reflect the dynamic environment of the oral cavity. Finally, the findings are limited to a specific resveratrol concentration and bacterial combination; broader antimicrobial or antibiofilm potential may require testing across varying doses, time points, and microbial communities.

CONCLUSION

In conclusion, this pilot study presented findings that only partially supported our hypothesis that RSV inhibits biofilm formation and development on implant surfaces. Although the observed effects were modest and did not reach statistical significance in terms of biofilm inhibition and disruption, RSV demonstrated selective antimicrobial activity against key periodontal pathogens. Its biocompatibility and natural origin underscore its potential as a complementary agent in peri-implant disease management. However, further in vivo and translational studies are needed to optimize its delivery and validate its clinical effectiveness.

These findings highlight the promise of RSV as a safe, natural adjunct in peri-implant care while also underscoring the importance of optimizing its application strategies. The dual-species in vitro biofilm model used in this study provided a clinically relevant platform for evaluating antibiofilm agents; however, more complex multispecies models and in vivo studies will be essential to confirm the clinical potential of RSV in managing peri-implant infections.

Peer Review	Externally peer-reviewed.
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