

1-1-2014

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ÖZYILDIZ, FİGEN; UZEL, ATAÇ; HAZAR, AYŞE SERPİL; GÜDEN, MUSTAFA; ÖLMEZ, SULTAN; ARAS, IŞIL; and KARABOZ, İSMAİL (2014) "Photocatalytic antimicrobial effect of TiO₂ anatase thin-film-coated orthodontic arch wires on 3 oral pathogens," *Turkish Journal of Biology*. Vol. 38: No. 2, Article 15.

<https://doi.org/10.3906/biy-1308-43>

Available at: <https://journals.tubitak.gov.tr/biology/vol38/iss2/15>

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Photocatalytic antimicrobial effect of TiO₂ anatase thin-film-coated orthodontic arch wires on 3 oral pathogens

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Received: 18.08.2013 • Accepted: 16.12.2013 • Published Online: 28.03.2014 • Printed: 28.04.2014

Abstract: The aim of this study was to introduce antimicrobial activity to stainless steel orthodontic arch wires by coating them with TiO₂ in anatase form. Stainless steel (0.016 × 0.022 inch), D-rect (0.016 × 0.022 inch), and multistranded hammered retainer wires (0.014 × 0.018 inch) were coated with TiO₂ anatase by the sol-gel dip coating method. The wires were assessed for their photocatalytic antimicrobial activity against *Streptococcus mutans*, *Candida albicans*, and *Enterococcus faecalis*. After illumination under UVA (315–400 nm) at 1.0 mW/cm² for 1 h, the reduction efficiencies of the anatase-coated arch wires were calculated by using colony-forming unit counts. All anatase-coated arch wires showed remarkable inhibitor effects against the test microorganisms under UVA. The most efficient wire on *S. mutans* was the stainless steel wire, with a 99.99% reduction in growth, but multistranded hammered retainer wire was the most active against both *C. albicans* and *E. faecalis*, with 98.0% and 91.68% reduction rates, respectively. TiO₂-coated arch wires exposed to UVA illumination showed significant antimicrobial activity when compared with uncoated samples and coated, but not UVA-exposed, samples. Our results suggest that the antimicrobial effect of TiO₂-coated arch wires in long-lasting orthodontic treatments would be beneficial for the prophylaxis of caries.

Key words: Antimicrobial activity, *Candida albicans*, *Enterococcus faecalis*, orthodontic arch wires, *Streptococcus mutans*, TiO₂

1. Introduction

Microorganisms in dental plaque metabolize starch and sugar, producing acids and eventually resulting in enamel decalcification (Choi et al., 2007). Therefore, enamel demineralization is considered a bacterial infectious disease. Streptococci bacteria are the earliest colonizers of the teeth (Blake et al., 1999), and it is generally accepted that *Streptococcus mutans* is the primary causative agent of dental caries. Mutans streptococci produce glucosyltransferase, an enzyme that plays a role in the catalysis of sucrose (Arslan et al., 2012). The byproducts of sucrose catalysis are glucans, which enable adherence and accumulation of other cariogenic microorganisms to the tooth, forming dental plaque and eventually the cariogenic acidic habitat (Schilling and Bowen, 1992).

Candida spp. are opportunistic pathogens present in about 50%–60% of the healthy human population (Hibino et al., 2009; Özyildiz et al., 2010). The most common *Candida* species isolated in orthodontic patients is *C. albicans* (Siqueira and Sen, 2004). They have 2 roles in the oral environment: coaggregating with oral bacteria,

and producing biofilms on dental surfaces (Özyildiz et al., 2010). Although no healthy individuals develop *Candida* infection from orthodontic appliances, non-*Candida* carriers can become *Candida* carriers via orthodontic therapy (Hibino et al., 2009).

It is claimed that *Enterococcus faecalis* exists in patients with periodontitis in subgingival regions and in the root canal of endodontic patients, with the emphasis that *E. faecalis* is normally not found in the oral flora of individuals with good oral hygiene (Al-Ahmad et al., 2009). Isolates of this bacterium are encountered in individuals with poor oral hygiene and in 20% of orthodontic patients who do not pay enough attention to oral hygiene procedures (Al-Ahmad et al., 2009; Özyildiz et al., 2010).

Several compounds exist that are capable of photocatalytic degradation of microorganisms, although TiO₂ has gained the most popularity amongst the other photocatalysts for its chemical stability as well as its property of not being hazardous to health (Chun et al., 2007; Akhavan, 2009). Although it has 3 crystal structures that can be produced by different oxidation procedures,

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the anatase form has the highest reactivity (Horiuchi et al., 2007; Akhavan, 2009; Choi et al., 2009; Sato et al., 2009). When TiO₂ anatase film is irradiated with wavelengths smaller than 385 nm of light, hydroxyl radicals are formed (Choi et al., 2007, 2009). Hydroxyl radicals are highly reactive when in contact with organic compounds (Choi et al., 2007). Therefore, these radicals react with the cell walls of the microorganisms, causing them to disintegrate. The microbial population is suppressed in the photocatalytic antimicrobial manner described.

Based on the introductory information, the aim of this study was to coat 3 different types of stainless steel orthodontic arch wires with TiO₂ anatase film by using the sol-gel dipping method, and evaluate the antimicrobial activity of coated arch wires against 3 common oral pathogens, namely *S. mutans*, *C. albicans*, and *E. faecalis*.

2. Materials and methods

2.1. Sol-gel preparation and dip coating of orthodontics wires

In this study, we used 3 types of stainless steel orthodontic wire: 0.016 × 0.022 inch stainless steel wire (first wire), 0.016 × 0.022 inch D-rect wire (second wire), and 0.014 × 0.018 inch multistranded hammered retainer wire (third wire). The wires were coated with TiO₂ using the sol-gel dip-coating method. The solution of TiO₂ consisted of 12 mL of titanium(IV) isopropoxide (97%, Aldrich), 170 mL of 2-propanol, and 0.4 mL of hydrochloric acid (2 M). To decrease the surface roughness, polyethylene glycol (PEG, Mw = 600, Aldrich) at 3 wt.% was added to the TiO₂ solution (Sonawane et al., 2004). After adding PEG to the solution, it was stirred up with a magnetic stirrer at room temperature for 3 h. The stirred sol was aged for 24 h at 4 °C. The orthodontic wires were then cleaned in an ultrasonic bath before being coated with acetone and distilled water (Özyildiz et al., 2010).

In order to obtain an even coating, each type of wire was cut into 1-cm-long pieces with a wire cutter and then dipped and removed from the sol at a constant speed of 76.2 mm/min. Substrates were dried at room temperature prior to 1 h of furnace drying at 120 °C. This procedure was repeated 3 times to increase the thickness of the thin film. To obtain the anatase crystal structure, substrates were calcined for 1 h at 500 °C with a heating and cooling rate of 2 °C/min. The heat-treated orthodontic wires were kept in a desiccator until they were used in the microbial activity tests. The surface morphology of the TiO₂ thin film was investigated with a scanning electron microscope (SEM) (Philips XL 30-SFEG), and the crystal structure was analyzed by grazing incidence X-ray diffraction (GIXRD) (Phillips X'Pert Pro).

2.2. Microorganisms and antimicrobial activity assay

S. mutans ATCC 10449 and *E. faecalis* ATCC 29212 were cultured in brain heart infusion broth (Difco) (Helderman et al., 2004) and *C. albicans* ATCC 60193 was cultured in Sabouraud dextrose broth (Difco) at 37 °C overnight (Falloy et al., 1996). The initial concentration of microorganisms was adjusted to McFarland 0.5 after centrifugation by using sterile saline solution (NCCLS, 2003). The initial microbial concentration (IMC) of test microorganisms was determined by serial dilution and the spread plate technique onto Mueller–Hinton agar (MHA, Oxoid).

In order to evaluate the photocatalytic antimicrobial activity, the 1-cm anatase-coated arch wires were placed in 1 mL of microbial suspensions in such a way that the suspension completely covered the wires, within juxtaposed position on sterile plates (coated + UVA group). The samples were then exposed to UVA (315–400 nm) at 1.0 mW/cm² for 1 h. The UVA was irradiated perpendicularly, 10 cm away from the horizontally positioned arch wires. After the illumination period, 100 µL of culture liquid from each sample was serially diluted and inoculated on MHA. After incubation at 37 °C for 24 h, final microbial counts (FMCs) were calculated. The same procedure was repeated for the uncoated control group (uncoated + UVA group). Additionally, another group of TiO₂-anatase-coated wires was kept in complete darkness to compare the effect of photocatalysis (coated + dark group). All experiments were done in triplicate and the significance was set at P < 0.05.

The decreases of the colony-forming unit (CFU) counts were calculated by using the following formula: reduction efficiency (RE) % = $(IMC - FMC) / IMC$. The differences between the IMC and FMC data of the 3 different groups of wires (coated + UVA, coated + darkness, uncoated + UVA) were analyzed using the least significant difference test in one-way ANOVA. Comparison of the differences in photocatalytic antimicrobial effects of TiO₂ among arch wires was analyzed using two-way ANOVA statistical analysis.

3. Results

It was observed, by GIXRD, that the crystal structure of the TiO₂ thin film was anatase, as shown in Figure 1. The diffraction lines at 2θ = 25.4°, 38°, and 48.1° confirm the anatase-coated layer. The TiO₂-coated wire surfaces are shown in Figure 2. The coating layer was reasonably continuous and harbored uniformly distributed surface microcracks.

The inhibitor effect of TiO₂-coated arch wires was shown against important oral pathogens such as *S. mutans*, *E. faecalis*, and *C. albicans* (Table). The one-way ANOVA statistics showed that the TiO₂-coated arch wires have

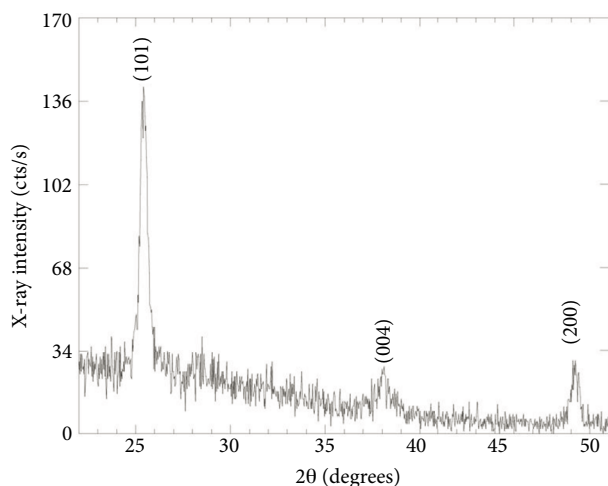


Figure 1. GIXRD spectra of TiO_2 -coated glass substrate after heat treatment at 500°C .

significant RE on all tested pathogens at different rates in the presence of UVA ($P < 0.05$).

The IMC, FMC, and RE values for *S. mutans*, *E. faecalis*, and *C. albicans* are recorded in the Table. Though the control groups (namely, the coated but kept-in-the-dark wires and uncoated but UVA-exposed wires) showed decreases in *S. mutans*, *E. faecalis*, and *C. albicans* counts, the coated and UVA-exposed arch wires showed the most drastic decreases. These results were found to be statistically significant in terms of intergroup comparisons ($P < 0.05$).

The most remarkable decreases in *S. mutans* concentrations were observed in the TiO_2 -coated and

UVA-exposed 0.016×0.022 inch stainless steel wire sample. However, multistranded hammered retainer wire showed the most potent activity against *E. faecalis* and *C. albicans*. Photocatalytic-effect-dependent cellular damage caused by anatase thin-film-coated arch wires on *S. mutans*, *E. faecalis*, and *C. albicans* is shown in Figure 3. It is clear that the cells broke down after UVA illumination.

4. Discussion

In medicine and dentistry, *E. faecalis* is known to cause nosocomial infections and is one of the persistent bacteria of endodontic problems, ending with chronic apical periodontitis. *E. faecalis* is commonly found in the oral habitat of individuals with poor hygiene, whereas healthy individuals with good oral hygiene only host this microorganism for transitional periods (Al-Ahmad et al., 2009). Moreover, *E. faecalis* is capable of bidirectional horizontal gene transfer for antibiotic resistance (Armitage, 1999; Sorum and Sunde, 2001; Al-Ahmad et al., 2009). The initial colonization of the acquired enamel pellicle starts with *Streptococcus sanguinis*, *S. mitis* (Babaahmady et al., 1998), and *S. mutans*, which are the members of the gram-positive cocci most frequently found in the oral cavity and have been implicated as the main causative organisms of human dental caries (Loesche, 1986). They also enable other microorganisms to adhere themselves to the tooth, when they would otherwise be incapable of cleaving to the hard tooth tissues. Finally, as the bacteria cohere to each other, the oral biofilm is formed, and it is the oral biofilm from which persistent infections are derived (Al-Ahmad et al., 2009).

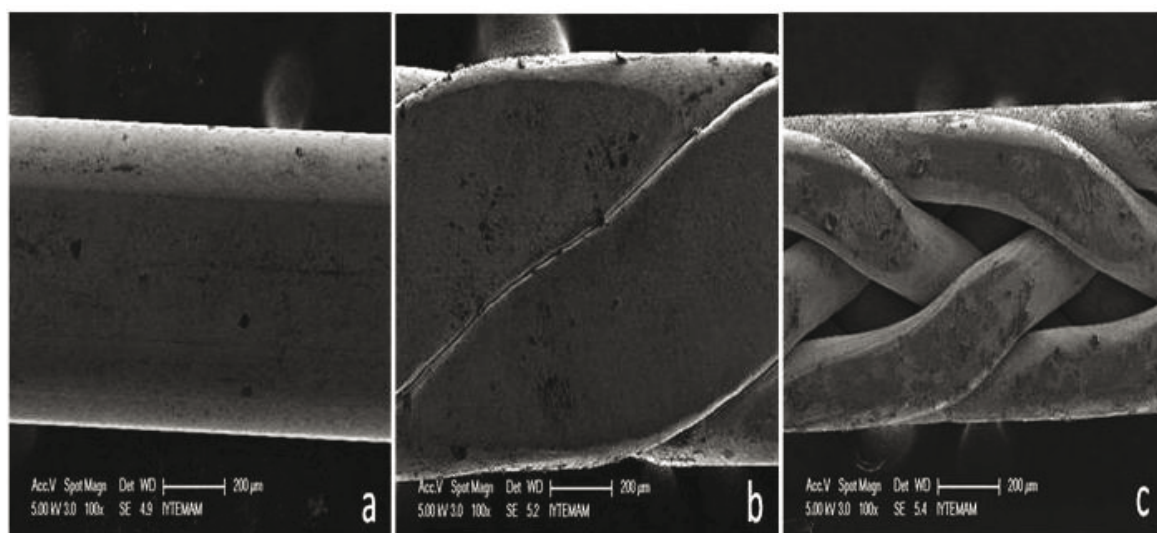


Figure 2. SEM micrographs of TiO_2 anatase film on orthodontic arch wire surfaces: a) 0.016×0.022 inch stainless steel wire, b) 0.016×0.022 inch D-rect wire, c) multistranded hammered retainer wire.

Table. IMC, FMC, and RE values for *S. mutans*, *E. faecalis*, and *C. albicans* for 3 kinds of orthodontic wires.

Pathogen	Stainless steel wire				D-rect wire				Multistranded hammered retainer wire			
	Mean (cfu/mL)	SD	RE (%)	log	Mean (cfu/mL)	SD	RE (%)	log	Mean (cfu/mL)	SD	RE (%)	log
<i>S. mutans</i>												
IMC	6.1×10^7	1.5×10^6		7.78	6.1×10^7	1.5×10^6		7.78	6.1×10^7	1.5×10^6		7.78
Coated + Dark FMC	$7.8 \times 10^{5*}$	3.2×10^4	98.72	5.89	5.8×10^7 NS	5.8×10^5	05.57	7.76	$6.7 \times 10^{6*}$	4.0×10^5	89.00	6.82
Uncoated + UVA FMC	$1.3 \times 10^{5*}$	2.9×10^3	99.78	5.11	$3.0 \times 10^7*$	4.7×10^6	51.47	7.47	$5.3 \times 10^{6*}$	3.6×10^5	91.31	6.72
Coated + UVA FMC	$<10^{3*}$	2.5×10^2	99.99	3.00	$2.0 \times 10^7*$	4.7×10^6	67.86	7.29	$1.1 \times 10^{6*}$	1.0×10^5	98.00	6.04
<i>C. albicans</i>												
IMC	3.0×10^5	5.6×10^4		5.47	3.0×10^5	5.6×10^4		5.47	3.0×10^5	5.6×10^4		5.47
Coated + Dark FMC	$1.3 \times 10^{5*}$	2.9×10^4	60.00	5.07	2.5×10^5 NS	5.0×10^4	16.66	5.39	$1.5 \times 10^{5*}$	5.0×10^4	50.00	5.17
Uncoated + UVA FMC	$9.0 \times 10^{5*}$	5.0×10^4	66.66	5.00	$1.9 \times 10^{5*}$	4.9×10^4	36.66	5.27	$5.2 \times 10^{4*}$	5.8×10^3	82.66	4.71
Coated + UVA FMC	$1.2 \times 10^{4*}$	1.5×10^3	96.00	4.07	$3.1 \times 10^{4*}$	4.0×10^3	89.66	4.49	$4.0 \times 10^{3*}$	2.6×10^3	98.66	3.60
<i>E. faecalis</i>												
IMC	8.3×10^7	5.1×10^6		7.91	8.3×10^7	5.1×10^6		7.91	8.3×10^7	5.1×10^6		7.91
Coated + Dark FMC	$5.7 \times 10^{7*}$	1.0×10^6	31.32	7.75	7.7×10^7 NS	6.0×10^6	07.22	7.88	$3.5 \times 10^{7*}$	2.6×10^6	57.83	7.54
Uncoated + UVA FMC	$5.3 \times 10^{7*}$	2.5×10^6	36.14	7.72	$4.4 \times 10^{7*}$	5.8×10^5	46.98	7.64	$1.1 \times 10^{7*}$	1.2×10^6	86.74	7.04
Coated + UVA FMC	$3.5 \times 10^{7*}$	2.0×10^6	57.83	7.54	$3.6 \times 10^{7*}$	1.7×10^6	56.62	7.55	$6.9 \times 10^{6*}$	2.2×10^6	91.68	6.83

IMC: Initial microbial count; FMC: final microbial count; RE: reduction efficiency. NS: not significant ($P \geq 0.05$); *: statistically significant reduction in microbial count ($P < 0.05$).

The relationship between orthodontic appliances and *Candida* has been studied in a few reports (Hägg et al., 2004; Casaccia et al., 2007; Hibino et al., 2009). It is a fact that the presence of prostheses or appliances increases the presence of *Candida* in the oral environment (Hibino et al., 2009). The prevalence of candidal recovery at some sites and candidal densities at all sites were significantly increased in fixed and removable appliance wearers (Al-Ahmad et al., 2009). Hägg et al. also suggested that being a fixed-appliance patient transiently initiated the carrier state (Hägg et al., 2004). Therefore, *E. faecalis*, *S. mutans*, and *C. albicans* are all found inherently in the oral cavity of orthodontic patients. Though the number of oral bacteria can be reduced by performing oral hygiene procedures using fluoride, mouthwash, and antibiotic administration (Choi et al., 2007), the most efficient way would be preventing the initial adherence and/or colonization by the bacterial population. There are some studies in the literature testing the antibacterial effect of TiO_2 against various microorganisms including *Escherichia coli*, *S. mutans*, *Porphyromonas gingivalis*, *Lactobacillus acidophilus*, *Aggregatibacter actinomycetemcomitans*, and

C. albicans (Yoshinari et al., 2001). However, *E. faecalis* was brought under the spotlight in the literature for the first time in terms of antibacterial activity pertaining to photocatalysis in our study. This study also demonstrates the antimicrobial properties given to the arch wires by a coating of TiO_2 anatase thin film.

TiO_2 can be found in 3 different mineral forms in nature, namely rutile, anatase, and brookite. Among these, the most active form of TiO_2 is the anatase form (Fujishima et al., 2000). In our study, we obtained the anatase form by heating the substrates to 500 °C for 1 h and cooling at a rate of 2 °C/min. Figure 1 demonstrates the anatase form of TiO_2 . The anodic oxidation was utilized in this study to enable anatase crystal formation rather than rutile crystal formation. We thought that this method would be more suitable based on the conclusion of the studies by Choi et al. (2009). These compared the 2 methods and stated that greater and more rapid antibacterial activity was achieved by anodic oxidation, with stronger reduction in numbers in bacterial counts. However, the rutile form is more thermodynamically stable and resistant against fracture (Choi et al., 2007; Horiuchi et al., 2007; Choi et

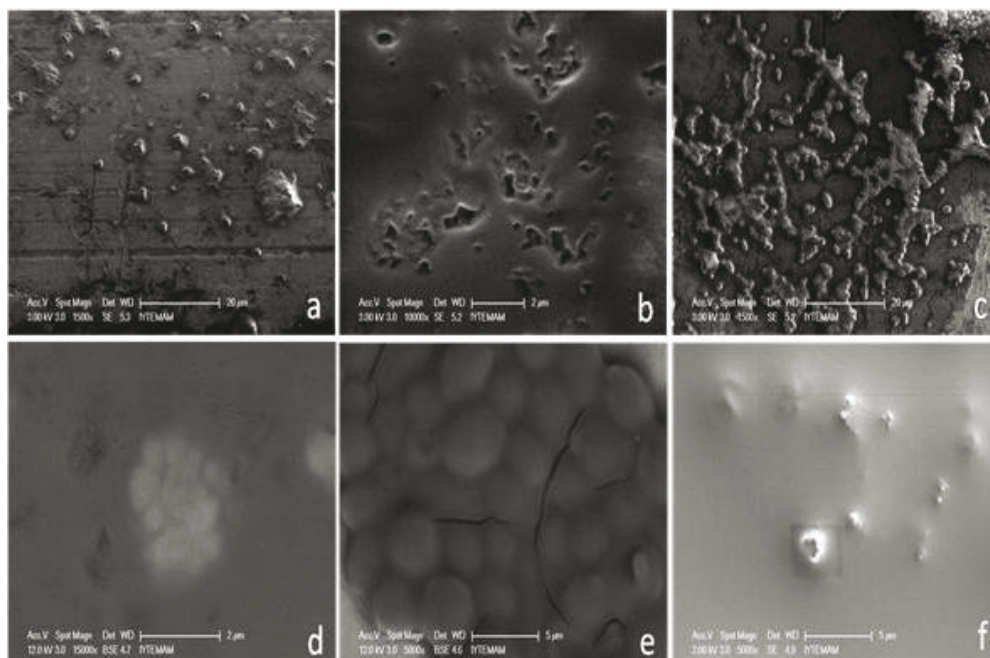


Figure 3. Photocatalytic effect of most potent anatase-film-coated orthodontic wires on *S. mutans*, *E. faecalis*, and *C. albicans* in SEM observations: *S. mutans* cells on TiO₂ anatase-film-coated stainless steel wire before (a) and after (b) UVA illumination. *E. faecalis* on multistranded hammered retainer wire before (c) and after (d) UVA illumination. *C. albicans* on multistranded hammered retainer wire before (e) and after (f) UVA illumination.

al., 2009). It is also commonly stated that anatase crystals have the highest photocatalytic activity among the crystal types of TiO₂ (Choi et al., 2007; Chun et al., 2007; Horiuchi et al., 2007; Choi et al., 2009; Özyildiz et al., 2010). Since traditional methods present problems with observing the crystal structure of the coated thin films, we used GIXRD and a separate glass slide, which was coated under the same conditions as the arch wires (Hamid, 2003).

The experimental data yielded decreases for all microorganism types in all types of wires that were coated and kept in the dark, but significant decreases were observed in bacterial counts for only 0.016 × 0.022 inch coated stainless steel wire and multistranded hammered retainer wire. This decrease may be attributable to limited nutritional resources or the build-up of waste, as the bacterial life cycle is dependent on these parameters, too.

When uncoated wires were irradiated with UVA light, all of the bacterial specimens displayed significant drops in numbers. This reduction in the control groups is an expected outcome, and is in correlation with the study by Choi et al. involving *S. mutans* (Choi et al., 2009). The decrease can be associated with the cell-damaging effect of UVA via oxidative stress caused by oxygen radicals or DNA. There are 3 wavelengths (185 nm, 254 nm, and 265 nm) of ultraviolet light that are best for killing bacteria. Ultraviolet light causes 2 thymine (an essential compound

of DNA) molecules that are next to each other on the bacteria's DNA strand to dimerize. The bacteria may not be killed right away; however, they lose the ability to replicate. We believe that UVA deteriorated the integral composition of the DNA molecules and caused the death of the bacteria, even though no coating was present. However, when titanium coating and UVA are used together, a more dramatic decrease in the microbial count is obtained. In addition, Kayano et al. (1998) showed that the photocatalytic effect of TiO₂ can also cause a high level of endotoxin degradation in *E. coli*. Although *S. mutans* and *E. faecalis* are gram-positive bacteria and do not possess endotoxins, many other pathogenic bacteria found in the oral cavity are gram-negative, and this phenomenon can further help to prevent overall health problems.

Drastic changes were observed in the coated and UVA-irradiated specimens, with some results yielding up to 200-fold decreases. Though the diminution differs among wire types and bacterial species, the important point is that all the reductions were statistically significant, and that statistically different changes were observed in the experimental group when compared with the dark-only and coated-only species. This result is in accordance with other antibacterial studies utilizing photocatalytic methods (Choi et al., 2007; Chun et al., 2007; Choi et al., 2009; Özyildiz et al., 2010).

From the results, it was observed that *C. albicans* is the most vulnerable microorganism to TiO₂ photocatalysis. *C. albicans* is followed by *S. mutans*, and lastly *E. faecalis*. *E. faecalis* is a microorganism known for persistent infections and antibacterial resistance. Thus, it would be logical to assume that we have observed its inherent resistance capability in this study. It is also reported that susceptibility to oxidative damage varies by structural components of the cell wall, cell-wall thickness, and microorganism type. However, our results of *C. albicans* showing higher sensitivity than *S. mutans* are not in accordance with the results of Özyildiz et al. (2010), who coated ceramic brackets and investigated antibacterial activity using the same methods (Özyildiz et al., 2010). The reason may be a result of the differences between the surface areas of the test materials.

In this study, 3 different orthodontic arch wires (stainless steel wire, D-rect wire and multistranded hammered retainer wire) were also compared against test microorganisms. According to our results, multistranded hammered retainer wire was the most effective against *E. faecalis* and *C. albicans*. Stainless steel wire seems to be the most potent wire against *S. mutans*. However, REs of the stainless steel wire and multistranded hammered retainer wire are 99.99% and 98.0%, respectively, showing a significant reduction for both wires. This means that the wire type can affect the photocatalytic activity. The reason for this phenomenon is not clear and should be further investigated.

The usefulness of TiO₂ coating alone still remains an important question, as repeated illumination with

UVA will be needed to maintain an acceptable level of antimicrobial action. However, long-term exposure to UVA can be harmful, although it has a lower impact on humans when compared to UVB or UVC. Development of visible light photocatalysts, or outdoor UV or UVA lamps at an intensity of 1 mW/cm², has been recommended in the literature (Choi et al., 2007). We think that nanocomposite layers can be incorporated onto the thin-film layer, as presented in Akhavan's study (Akhavan, 2009). His study showed that the durability of the antibacterial film increased at least 11 times with nanocomposite layers. Additionally, silver particles facilitated visible light activation of TiO₂ (Choi et al., 2007; Sato et al., 2009). The antibacterial effect acquired was also quicker. The addition of noble metals such as platinum and silver to the TiO₂ surface has also been reported in the literature (Choi et al., 2009). In recent years, using photocatalysis to reach an antibacterial stage has become a promising field of orthodontic treatment. However, there still are some issues to be worked on to improve the ease of the procedure for both patients and dentists.

In conclusion, the antimicrobial effects of TiO₂-coated arch wires were investigated against 3 oral pathogens: *S. mutans*, *E. faecalis*, and *C. albicans*. TiO₂-coated arch wires exposed to UVA illumination showed efficiently reduced microbial counts when compared with uncoated samples and coated but not UVA-exposed samples. The results of this preliminary study suggest that the antibacterial effect of TiO₂-coated arch wires in long-lasting orthodontic treatments would be beneficial for the prophylaxis of caries.

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