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### Electrochemical Synthesis, Characterization and Antibacterial Properties of Silver Nanobranch on Orthodontic Braces

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#### Highlights:

- Orthodontic brackets coated with Ag nano branch
- Affects the adhesion of bacteria on Ag-orthodontic braces

#### Keywords:

- Electrochemical synthesis
- Orthodontic braces
- Antibacterial effect
- Silver nanoparticles

#### ABSTRACT:

Silver nanodal was synthesized on orthodontic braces using silver nitrate salts as an electrochemical method. The synthesized Ag nanobranch were characterized by UV-visible spectroscopy (UV-vis), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and X-ray diffraction (XRD). Antibacterial microbial performance of *Lactobacillus salivarius* analyzed with electrochemically synthesized silver nanobranch on orthodontic brackets. The specific antibacterial properties of all the composites showed specific antibacterial effects or antiadherence, independently of the amount of silver nanobranch deposited, likely due to the differences in the bacterial cell wall structures. As a result, the brackets, which are very important for orthodontics, showed a good antibacterial performance with the nanostructures obtained by electrochemically coated with Ag, which is known to have antibacterial properties.

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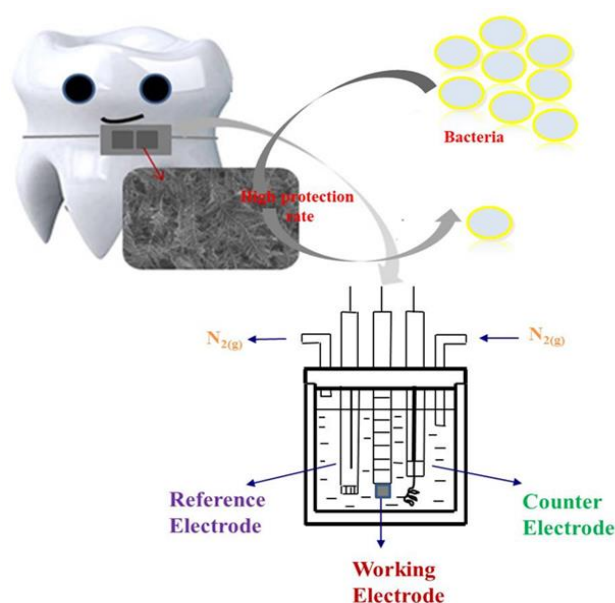
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## INTRODUCTION

Bacterial plaque is a soft, sticky substance that accumulates on the surface of the tooth, consisting entirely of bacteria and bacterial products. While some microorganisms in the oral environment have the ability to adhere to the tooth surface and mucosa (such as streptococci), microorganisms that do not have the ability to adhere are immediately removed by salivary flow and swallowing reflex. Streptococci mutans and Lactobacilli are microorganisms that can produce high amounts of acid (acidogenic) and can tolerate an acidic environment (aciduric). Because of these features, they are among the main organisms associated with caries in humans (Roberson et al. 2010). Orthodontic bands and brackets attached to the teeth during fixed orthodontic treatment cause an increase in the number of bacterial plaque retention areas and difficulties in providing proper oral care. Bacterial plaque accumulating on teeth and around orthodontic brackets causes demineralization due to mineral loss in enamel. This differentiation in the enamel structure turns into clinically visible white spot lesions (WSL) (Bishara and Ostby 2008). According to the studies; white spot lesions are one of the common side effects of orthodontic treatments and it is seen that the number and severity of lesions increase during the treatment in patients with poor oral hygiene (Lucchese and Gherlone, 2003; Tufekci et al. 2019).

Silver nanoparticles have strong antibacterial effects and due to their size, they are used in different areas of life, especially in the health field, thanks to their ability to stick to surfaces. Nowadays, in dentistry, interest in the synthesis and characterization of Ag nanoparticles is increasing due to their antibacterial properties. (Jasso-Ruiz et al. 2019) In dentistry, antibacterial properties of silver are used in the treatment of active caries and periodontal diseases. The effects of silver nanoparticles against dental caries and bacteria that cause tooth decay (such as Lactobacilli and Streptococci) are supported by studies (Bromberg et al., 2000; Butron et al., 2000; Espihosa-Cristobal et al., 2015; Espinosa-Cristobal et al., 2019). There are studies using antibacterial silver nanoparticles in brackets, bands, wires, elastomeric ligatures and orthodontic adhesive materials used during orthodontic treatment to prevent the formation of caries and white spot lesions. The materials used in these studies are chemically coated with silver nanoparticles (Metin Bürsoy et al., 2007; Arash et al., 2016; Hernandez-Gomora et al., 2017; Espinosa-Cristobal et al., 2018; Jasso-Ruiz et al., 2019).



**Figure 1.** Schematic image indicating the antibacterial properties of Ag nanobraces deposited electrochemically on orthodontic brackets

This scope of work, of a different accession to the growth Ag nano-structures based on electrochemical synthesis to indium tin oxide (ITO) or orthodontic brackets electrodes is presented. The Ag nano-structures obtained were recognized by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), X-ray diffraction XRD. It is indicated by the test results that, by adjusting the deposition time, control of the morphology and size of Ag nanostructures can be possible.

As shown in Figure 1, the general experimental strategy used in this study involved first using electrochemical time deposition to create Ag nanobranch structures on orthodontic brackets of controlled sizes, and then evaluating the antibacterial performance of the Ag nanobranch formed on orthodontic brackets.

## MATERIALS AND METHODS

### Electrochemical Deposition of Ag Nanobranches on Orthodontic Brackets

The electrochemical studies were conducted using a BAS 100B/W electrochemical workstation coupled to a three-electrode cell (BAS C3 Cell Stand). A Rigaku powder X-ray diffractometer with a CuKX-ray source ( $\lambda = 1.5406$ ) was used to record the powder X-ray diffractograms of the deposited films. EDS used a ZEISS system connected to a scanning electron microscope to perform morphological analysis and elemental composition determination on Ag nanostructures. At room temperature, absorption measurements were taken with a Shimadzu UV-3101 UV-vis spectrometer. In all cases, the reference electrode was an Ag/AgCl (3 M NaCl) electrode, while the counter electrode was a platinum wire. For the electrochemical tests, a polycrystalline Au electrode was utilized as the working electrode, whereas for the UV-vis measurements, ITO-coated quartz was employed. All of the electrolyte solutions used in this investigation were made using deionized water from a Milli-system and were deoxygenated for 10 minutes before the oxygen-free experiments. A 0.1 M  $\text{KNO}_3$  solution was used to electrodeposit Ag nano branch electrodes at ambient temperature, containing 0.2 mM sodium citrate and 0.05 mM  $\text{AgNO}_3$  (Bayrakçeken Nişancı, 2020).

### Antibacterial performance of Ag nanobranches on orthodontic brackets

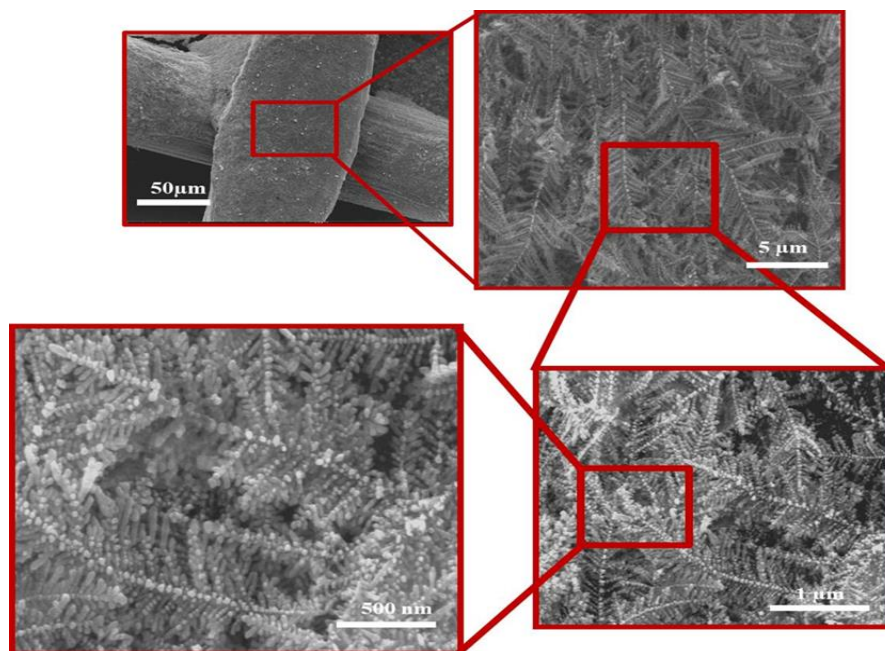
Silver Nanocoated Orthodontic Braces (NOBs) and untreated Orthodontic Braces (OBs) were first sterilized by autoclaving prior to trials. *Lactobacillus salivarius* bacteria was used for the study. For bacterial attachment on NOBs and OBs were incubated for 8 hours at 120 rpm 35 °C with 1 mL of 24-hour aforementioned bacterial culture [ $1-2 \times 10^8$  Colony Forming Units / mL (CFU / mL)] and an equal amount of sterile TSB (Tryptic Soy Broth). Then, the braces taken from the cultures in accordance with aseptic rules were gently washed with sterile isotonic solution (0.85 % NaCl w/v). Then, 5 mL of sterile TSB was added to the NOBs and OBs taken into sterile tubes in accordance with aseptic rules and incubated for 16 hours at 120 rpm 35 °C. At the end of the period, the absorbance of the cultures was measured with a spectrophotometer at a wavelength of 540 nm. It was checked for contamination at each step of the study. The negative control (NC) that were not treated with bacterial culture, and nanocoated positive controls (PCs) were studied under the same conditions in the study.

$$R = 100 (B - A) / B \quad (\text{Equation 1.})$$

(R: protection ratio, B: absorbance of positive controls, A: absorbance of treatments) (Gonçalves et al. 2010; Bae et al., 2021). The positive control was run in three parallels in the experiments (Equation 1). No washing process was performed for the three parallels (PC 3). Sterile medium was used as a blank.

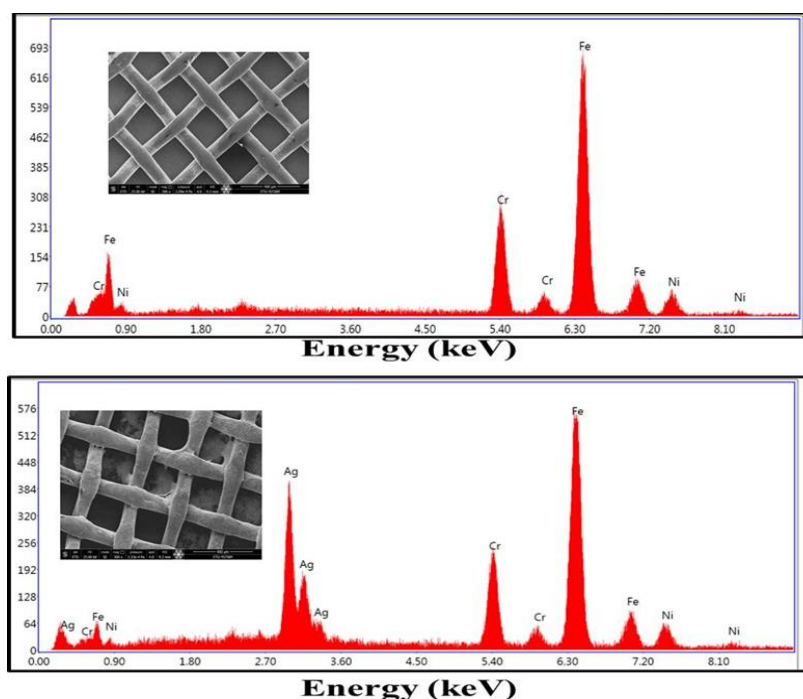
## RESULTS AND DISCUSSION

Figure 2 presents the SEM images of Ag on orthodontic brackets substrates after 60 min of electrodeposition at  $-200$  mV from the saturated solution containing a  $0.2$  mM sodium citrate,  $0.1$  M  $\text{KNO}_3$  and  $0.05$  mM  $\text{AgNO}_3$  at room temperature. It is clearly seen that Ag crystals in cubic structure come together to form nano branches.



**Figure 2.** SEM images of Ag nanobranched deposited on orthodontic brackets at  $-200$  mV for different sizes

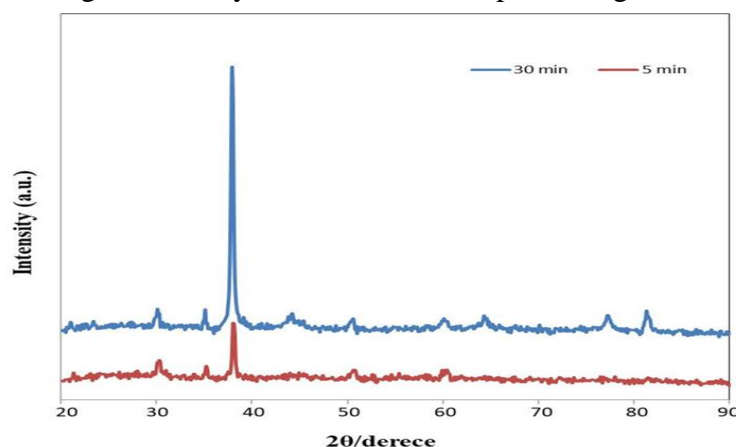
The elemental composition of pure orthodontic brackets and Ag nanobranched on orthodontic brackets has been determined using EDS technique (Figure 3). Figure 3a shows that it consists of elements such as Cr, Fe, Ni in the EDS spectra taken from pure orthodontic brackets.



**Figure 3.** EDS spectrum of pure orthodontic brackets (a) and EDS spectrum of the Ag nanobranched deposited on orthodontic brackets for 30 min (b)

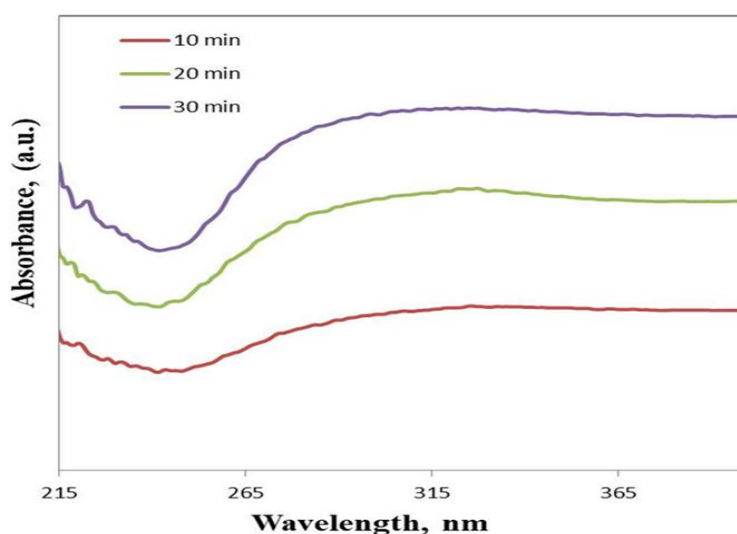
In Figure 3b, on the EDS taken on the Ag nanobranch on the orthodontic brackets, both the peaks of the brackets (Cr, Fe, Ni) and the Ag nanobranch coating on it are clearly seen. Indium, silicon, and oxygen signals associated with ITO with the substrate beneath the coatings are also present in the spectra.

The crystal structures of Ag electrodeposited at longer durations (30 and 5 min) were investigated using XRD measurements. Figure 4 shows the XRD pattern of silver nanoparticles deposited at  $-200$  mV and room temperature for 5 and 30 minutes. The XRD patterns of the produced silver nanoparticles obtained in the  $2\theta$  range of  $20^\circ$  to  $90^\circ$  are shown in Figure 4. The numbers for a face-centred cubic (fcc) crystal structure of silver agree with this data. The crystal planes (111), (200), (220), (311), and (222) correspond to the peaks at  $38.2^\circ$ ,  $44.4^\circ$ ,  $64.5^\circ$ ,  $77.5^\circ$ , and  $81.6^\circ$  (Fujii et al., 2005; Wu et al., 2017). There are no impurity diffraction peaks here. The intensity of Ag (200) increases when the deposition period is raised to 30 minutes, showing that the crystallites in the film prefer to grow in this direction.



**Figure 4.** XRD spectrum of the Ag nanobranch deposited on orthodontic brackets for 30 min and 5 min

In the 215-400 nm wavelength range, Figure 5 illustrates the typical absorption spectra of the Ag nanobranch synthesised on the ITO electrode by electrochemical deposition. The shoulder absorption band in this spectrum corresponds to a wide electronic transition at 300 nm. The transition in films occurs between the valence and conductivity bands and is fully dependent on the semiconductor's electrical structure. Figure 5 shows the UV-visible absorption spectra of Ag nanobranch on ITO-coated quartz substrates at various time intervals.



**Figure 5.** UV-visible spectra of Ag nanobranch deposited on ITO-coated quartz substrates at various times

The absorption edges (appearing as a shoulder) at 280 nm grow more apparent and move to lower energy with increasing deposition time, according to the absorption spectrum in the 250-400 nm spectral windows, which is fairly comparable to spectra reported for colloids and nanostructure of Ag. When compared to a deposition period of 30 minutes, there was a 25 nm red shift in its absorption edge at a deposition time of 10 minutes. In comparison to bare ITO glass, the sample at a constant potential of -200 mV for an electrodeposition period of 1800 s yielded a better result in terms of optical qualities.

As a result of the antimicrobial effect study, it is comprehensible that Ag nanobranches application affects the adhesion of bacteria on orthodontic braces (Table 1). Inhibition rates ranging from 90.00% to 3.08% were calculated. An effect that was independent of the growth time and consequently the coating thickness was observed. Remarkable effects were observed according to different coating models of silver nanoparticles on orthodontic wires (Gorçalış et al., 2020). In particular, the sample magnified for 60 minutes had a remarkable effect. The result of 5 min coating can be considered as important in terms of applicability.

**Table 1.** Quantification of bacterial attachment on silver nanocoating orthodontic braces.

Application	A <sub>540</sub>	Protection rate (%)
NC	0.001	-
PC (1)	0.908	-
PC (2)	0.920	-
PC (3)	1.150	Removed from evaluation
5 min	0.100	88.99-89.13
15 min	0.880	3.08-4.35
20 min	0.760	16.30-17.39
60 min	0.092	89.87-90.00
90 min	0.574	36.78-37.61

## CONCLUSION

This research clearly demonstrates that electrochemical deposition of Ag nanobranch in a solution including KNO<sub>3</sub>, sodium citrate, and AgNO<sub>3</sub> at -200 mV potentials is an experimentally straightforward approach for the fabrication of nanostructure materials. Furthermore, the findings of this study reveal that the amount of Ag nano branch deposited may be regulated by the deposition period. In a single procedure, we were able to create well-crystallized materials with acceptable optical characteristics. Increasing the duration for Ag electrodeposition results in an increase in both the quantity and the absorption wavelength of the material, causing a red shift in the band gap. The better quality of Ag nanobranch on orthodontic brackets created using this approach is confirmed by SEM and XRD data. The antibacterial effect against *Lactobacillus salivarius* was observed. Although the antibacterial properties of Ag have been studied a lot until now, they are important in terms of exhibiting high antibacterial performance, especially with Ag nanobranch structures formed electrochemically in brackets. As a results, orthodontic brackets coated with Ag nano branch by electrodeposition technique displayed to have a high potential for biomedical applications and in the field of health.

## ACKNOWLEDGEMENTS

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## Conflict of Interest

The article authors declare that there is no conflict of interest between them.

## Author's Contributions

The authors declare that they have contributed equally to the article.

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