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*Original Article*

# Evaluation of antimicrobial effect of the Neo Pill device on fixed orthodontic appliances: in vitro pilot study

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## Abstract

Introduction: Oral hygiene in patients with fixed orthodontic appliances is not satisfactory in most cases. Namely, rough and irregular edges on fixed orthodontic appliances can facilitate attachment of microorganisms, which causes periodontal diseases and caries. The purpose of this *in vitro* study was to evaluate the reduction of microbes between the wires of fixed orthodontic appliances after the action of the Neo Pill electrolysis device.

Methodology: The study was carried out in the laboratory of the Faculty of Stomatology in Pančevo, where serial dilutions were prepared in buffered peptone water, and together with the most appropriate dilution rate for the desired colony-forming units in a given volume (80-120 colony-forming units per plate) for bacterial strains (*Streptococcus mutans* ATCC 25175, *Lactobacillus acidophilus* ATCC 4356, *Actinomyces odontolyticus* ATCC 17929) as well as for *Candida albicans* (ATCC 10231). The microbial assessment was done for the detection of colony-forming units from the samples before and after using the Neo Pill electrolysis device. All data were statistically analyzed with the Wilcoxon Two-Sample Test.

Results: Results of analyses revealed that the number of microbes in all Neo Pill groups was significantly lower compared to the Control group ($p < 0.05$).

Conclusions: The Neo Pill electrolysis device is efficient in the removal of oral bacteria and fungi and could be an addition to the oral hygiene for patients with fixed orthodontic appliances.

**Key words:** Toothbrushing; dental plaque; orthodontic appliance; Neo Pill electrolysis device.

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## Introduction

In everyday life, oral health is an essential part of general health and quality of life. Periodontal disease and dental caries, which are related to dental plaque, are among the most common diseases worldwide [1]. Dental plaque is a structurally and functionally organised biofilm. Plaque forms in an orderly manner and has a diverse microbial composition that remains relatively stable over time in a healthy state (microbial homeostasis). They are structurally and functionally complex polymicrobial communities that are embedded in an extracellular matrix and adhere to the dental hard tissue, but also to restorative materials, dentures, and dental implants, as well as to the soft tissues of the oral mucosa. It has long been known that the presence of dental plaque leads to gingivitis and periodontitis as well as dental caries [2]. In the past and even now, the manual toothbrush has been the most widely accepted method of oral hygiene for removing dental plaque and enjoys a high level of social acceptance [3,4]. However,

practical data show that manual toothbrushing, as practised by the majority of people, is unsatisfactory unless targeted plaque control is performed. In an effort to achieve better plaque removal, many electronic toothbrushes have been developed [5,6].

In orthodontic therapy, the insertion of fixed orthodontic appliances (FOA) for patients with misaligned teeth and bite problems is a basic principle. This therapy usually takes about 2 years or longer. One of the biggest challenges for these patients is maintaining proper oral hygiene, which is crucial for the health of the teeth and the overall health of the patient [7,8]. Rough and irregular edges on FOA can facilitate adhesion of microorganisms that can worsen oral hygiene conditions and cause gingivitis and further periodontitis [8]. Oral hygiene in patients with FOA is unsatisfactory in most cases. The frequency of gingivitis and the amount of dental plaque is significantly higher in patients with FOA, and studies show that the risk is increased by 50% to 100% [9,8].

The effectiveness of the oral hygiene techniques currently used (tooth brushing, rinsing the oral cavity with 0.12% chlorhexidine, dental floss, interdental brushes) is insufficient. In addition, up to 78% of patients do not follow the instructions of the current methods, which require discipline and effort [10]. Proper cleaning is time-consuming, especially since 75% of orthodontic patients are under 18 years old, who often do not have enough time and may have problems with their self-confidence for aesthetic reasons [11]. Of utmost importance are methods that motivate children to engage in regular and effective oral hygiene practices that improve the effectiveness of the tooth brushing technique alone [6].

In general, manual and electric toothbrushes are similarly effective in removing bacterial plaque, but for the reasons mentioned above, there was a great need for a simple medical device to ensure oral hygiene in an efficient, safe, and easy-to-use manner. Recently, an innovation was introduced in this field when the Neo Pill electrolysis device was developed, which removes bacteria from the FOA and eliminates them quickly. Although the device is authorized for sale and use in humans in Serbia, its effectiveness and safety must first be proven *in vitro*, which is precisely the aim of this research. Whilst this device cannot replace a toothbrush, it has a major advantage over all other supplementary devices as it can reach hidden bacteria on inaccessible surfaces, utilizes electricity, helping to maintain the health of teeth and gums in a patient with FOA.

The aim of this *in vitro* study was to evaluate the efficacy of the Neo Pill electrolysis device in reducing oral bacteria and fungi between arch wires of fixed orthodontic appliances.

**Figure 1.** Neo Pill device.

Simultaneous contact with the arch wires of fixed orthodontic appliance (soaked in buffered peptone solution in Petri dish) creates electric filed between the wires.

## Methodology

This study was conducted in the laboratory at the Faculty of Stomatology in Pančevo, from March to April 2024.

*General principles of the Neo Pill electrolysis device*

Neo Pill is an innovative device for maintaining oral hygiene in the oral cavity (CEO Neofunction d.o.o., Belgrade, Serbia, which was developed as a supplement to existing means of maintaining oral hygiene, especially for patients with FOA. Neo Pill utilizes a unique electrolytic process to remove bacteria from teeth, gums, and orthodontic wires. The Neo Pill is powered by two 1.5V batteries with a total voltage of 3V, making the treatment painless. The sensation is minimal as the entire process takes place well below the 9 V threshold. By attaching two electrodes to the upper and lower wire of the FOA, Neo Pill closes the circuit with the saliva as a conductor of electricity. This attracts and removes bacteria from the tooth surface and gums within 30 seconds, after which you simply rinse your mouth with a sip of water. Unlike other products that use mechanical and chemical methods, Neo Pill utilizes electricity to improve oral hygiene.

*In vitro study design with Neo Pill*

The process is performed by the negative surface charge of the bacterial cell membrane. This property of the bacteria enables their directional movement when they are in an electric field. During the simultaneous contact of the positive and negative electrodes of the Neo Pill with the upper and lower arch wires of the FOA, a slight electric field is formed between them (Figure 1). This leads to a directional movement of bacteria and other charged particles present in the contaminated buffered peptone solution. In this way, the circuit is closed, and a mild current flows through the liquid and the arch wires of the FOA along its entire length. The bacteria are attracted to the positive electrode or the entire arch wire of the FOA, to which the positively charged pill of the Neo Pill device is connected, due to its negative charge. Arch wires of the FOA were contaminated by bacterial or fungal suspension in buffered peptone solution. After 30 seconds of Neo Pill activity, a sterile swab was taken from the buffered peptone solution between the arch wires of FOA in a Petri dish. After that, different oral bacterial strains and *Candida albicans* were analyzed.

*Microorganisms used in research*

The bacterial strains investigated were: *Streptococcus mutans* ATCC 25175, *Lactobacillus acidophilus* ATCC 4356, *Actinomyces odontolyticus* ATCC 17929, and *Candida albicans* ATCC 10231.

For each of the bacteria listed above and for *C. albicans*, Control Plate 1 and Control Plate 2, as well as Neo Pill Plate 1 and Neo Pill Plate 2, were used.

*Preparation of the suspension (inoculum) for the test*

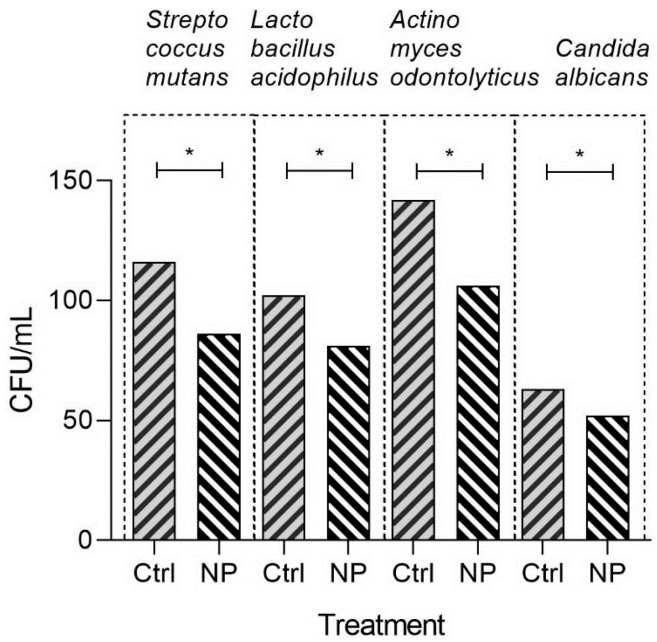
For this study, serial dilutions of the swabs were prepared in buffered peptone water ($10^0$, $10^{-1}$, $10^{-2}$). We used a dilution that yielded a count (CFU/mL) of 80 to 120. In our study, this count was obtained from a stock dilution ($10^0$) (ISSO 2014) [12].

The microbial assessment in this study was done for the detection of CFU from the samples before and after using the Neo Pill electrolysis device.

*Statistical Analysis*

In this study, the mean values of the dependent variable (number of bacterial colonies) were compared in two independent groups of samples (Control and Neo Pill treatment). Since the dependent variable is not normally distributed, the Wilcoxon-Mann-Whitney test was used to determine the statistical significance of the differences in the mean values. A $p$ value of 0.05 was set as the threshold for accepting/rejecting the null hypothesis.

**Figure 2.** Reduction of microbes after the treatment with Neo Pill electrolysis device.




Number of microbes by study arms (CFU/mL). *Statistically significant difference ($p < 0.05$). Ctrl: Control; NP: NeoPill.

## Results

This study demonstrated the efficacy of the Neo Pill electrolysis device in reducing the number of *S. mutants* ATCC 25175, *L. acidophilus* ATCC 4356, *A. odontolyticus* ATCC 17929, and *C. albicans* ATCC 10231 *in vitro*, between the arches of FOA.

The results of the analyses showed that the Neo Pill treatment significantly reduced the number of tested bacteria and *C. albicans* compared to the control groups (Figure 2).

Distribution of the number of microorganisms is presented using a boxplot (Figure 2). In all four cases, $p$ is less than 0.05, so it can be concluded that the difference between the control and Neo Pill treatment is statistically significant.

## Discussion

Patients with fixed orthodontic appliances are having great difficulty maintaining their oral hygiene. Fixed orthodontic appliances are associated with an increased number of pathogenic bacteria and the formation of a biofilm that causes gingivitis and white spots on the enamel, a precursor to caries. Although mechanical means of maintaining oral hygiene are effective in preventing gingivitis and white spots when used regularly and appropriately, it is difficult to

achieve complete protection in everyday life, as patients do not fully comply.

Although manual toothbrushes are still mostly used in everyday life, the research of Chandra *et al.* (2019) [6] showed that plaque removal with an electric toothbrush is significantly more effective than with a manual toothbrush. A statistically significant reduction in the mean plaque index from day 0 to day 30 was found compared to the manual toothbrush. In addition, no soft tissue trauma occurred after using either type of toothbrush. Some other studies also observed that electric toothbrushes remove plaque more efficiently [13].

The clinical study by Ikawa *et al.* (2021) [14] has shown that the electric ionic toothbrush is a useful device for plaque removal and helps to prevent dental problems caused by plaque. Likewise, the clinical study by Peručić *et al.* (2020) [15] concluded that, in addition to mechanical cleaning, the ionic brush has an additional effect that helps to maintain oral hygiene. Although the results showed improvements in both measured components (the plaque index and the papilla bleeding index), further research is needed to prove clinical efficacy. The clinical study by Deshmukh *et al.* (2006) [2] concluded that both active and inactive ionic toothbrushes significantly reduced plaque index and the gingival bleeding index. Active ionic toothbrushes were more effective compared to inactive ionic toothbrushes. No soft tissue trauma occurred after using either type of toothbrush, indicating that ionic toothbrushes are equally safe with regular long-term use. These results are similar to previous studies by Singh *et al.* (2011) [13] as well as Van Swol *et al.* (1996) [4].

Despite regular use of the available products, they are often powerless in the fight against various bacteria located in inaccessible places: in narrow interdental spaces, on the adhesive that holds the prosthesis, and in small cracks on the tooth surface, and especially on orthodontic wires and brackets. The Neo Pill electrolysis device is a unique product for patients with fixed orthodontic appliances that complements their oral hygiene in an innovative way, helping to maintain oral health by effectively reducing the number of bacteria in hard-to-reach areas. The mechanism of antibacterial activity of the Neo Pill electrolytic device is similar to ionic electric tooth brushes [16]. The result of the present *in vitro* study showed a significant reduction of the most common oral bacteria and *C. albicans* after only 30 seconds of treatment with the Neo Pill electrolysis device. Namely, a relatively short time needed for the effectiveness of the Neo Pill electrolytic device could be clinically acceptable for the orthodontic patients. This is an important finding that may have positive clinical implications, especially in orthodontic patients who are under 18 years old, and who often do not have enough time.

The *in vitro* study of Zituni *et al.* [17] demonstrated a decrease in the viable count of *Staphylococcus aureus* treated with an electric current of 2.5V, which is consistent with the results of our study with oral bacteria. Microbial deterioration in our study could possibly be the result of lipid peroxidation and production of free oxygen species caused by the unique electrolytic activity of the Neo Pill device, similar to ionic electric toothbrushes and bioelectrical toothbrushes [18,16]. Additionally, it has been speculated that the electrolytic process removes bacteria from the orthodontic wires and brackets and allows their elimination. Further studies are needed to determine if chronic use of electrical current from the Neo Pill device can produce bacterial resistance.

## Conclusions

The results of this *in vitro* study showed that the Neo Pill electrolysis device significantly reduced the number of the most common oral bacteria and *C. albicans* between the arch wires of fixed orthodontic appliances. Although clinical studies are needed, the results of this *in vitro* study imply that the Neo Pill electrolysis device could also be successfully implemented for patients with fixed orthodontic appliances.

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### Authors' contributions

Zoran Tambur and Vladimir Biočanin designed the study. Jovanka Trifunović, Ema Aleksić, and Adam Malešević performed the experiments. Stevan Avramov analysed the data. Vladimir Biočanin wrote the paper with input from all authors.

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### Conflict of interest

No conflict of interest is declared.

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