Assessment of Antibacterial Activity of Periodontal Dressings By Agar Diffusion and Direct Contact Test

Periodontal Patların Antibakteriyel Aktivitesinin Agar Difüzyon ve Direkt Kontak Testiyle Değerlendirmesi

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Abstract

Objective: The aim of this *in vitro* study was to evaluate antibacterial activity of three periodontal dressings by agar diffusion test (ADT) and direct contact test (DCT).

Methods: Eighteen agar plates were inoculated with *E. faecalis, S. aureus, S. lutea*. Each dressing was placed into plates. The zones of inhibition on plates were measured. Data were statistically analyzed using Kruskall-Wallis test and post hoc comparisons were made with Mann-Whitney U test. For DCT, dressings were placed on the side wall of microtiter-plate wells. Bacterial suspension was placed on the samples. Bacterial growth was spectrophotometrically measured.

Results: According to ADT, Coe-Pak showed inhibition to *S. lutea* and Voco pac showed inhibition to *S. aureus* and *S. lutea*. In DCT, Coe-Pak was more potent against all test microorganisms than the others after 24 h contact test.

Conclusions: Using DCT together with ADT as standard methods has an importance to assess the periodontal dressings as well as other dental materials, but the antimicrobial effect can be influenced by the experimental methods and exposure time.

Keywords: Periodontal dressings, agar diffusion test, direct contact test

Özet

Amaç: *Bu in vitro çalışmanın amacı, üç periodontal patın antimikrobiyal aktivitesini agar difüzyon testi (ADT) ve direkt kontak testi (DKT) ile değerlendirmektir.*

Yöntem: *On sekiz petri E. faecalis, S. aureus, S. lutea ile inokule edildi. Her pat, petrilere yerleştirildi. Petriler üzerindeki inhibisyon alanları ölçüldü. Veriler Kruskall-Wallis test ile ikili karşılaştırmalar ise Mann-Whitney U test ile analiz edildi. DKT için, patlar mikrotitre plak çukurlarının yan duvarları üzerine yerleştirildi. Bakteriyel süspansiyon test edilen örnekler üzerine yerleştirildi. Bakteriyel gelişim spektrofotometrik olarak ölçüldü.*

Bulgular: *ADT sonuçlarına göre Coe-Pak S. lutea'ya, Voco pac S. aureus ve S. lutea'ya karşı inhibisyon oluşturdu. DKT'e göre 24 saat sonunda Coe-Pak diğer patlara göre test mikroorganizmalarının tümüne karşı daha etkiliydi.*

Sonuçlar: *DKT ile birlikte ADT'nin standart yöntemler olarak kullanılması diğer dental materyallerin yanı sıra periodontal patların değerlendirilmesinde de önemlidir, ancak antimikrobiyal etki deney yöntemleri ve uygulama süresinden etkilenebilir.*

Anahtar sözcükler: *Periodontal patlar, agar difüzyon testi, direkt kontak testi*

Introduction

In most cases, after the surgical periodontal procedures are completed, the area is covered with a surgical periodontal dressing. In general, dressings have no curative properties; they assist healing by protecting the tissue rather than by providing healing factors.[1]

The relationship between the build-up of bacterial plaque at the dento-gingival junction and the development of gingivitis is well established. The

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susceptibility of patients requiring periodontal surgery to the pathogenic potential of plaque would seem to necessitate the protection of the surgically treated area by a periodontal dressing until adequate plaque control by the patient is possible. Most commercial periodontal dressings claiming antibacterial activity lose this activity shortly after application.[1]

Numerous studies have been done to assess the antimicrobial efficiency of different periodontal dressings.[2-6] Antimicrobial activity of periodontal dressings can be evaluated either *in vitro* or *in vivo*. Although agar diffusion tests (ADT) have limitations such as lack of standardization of inoculum density, adequate culture medium, agar viscosity, plate storage conditions, size and number of specimens per plate, and time and temperature of incubation,[7] it is still the most widely used *in vitro* method of evaluation of antimicrobial activity.[8-10] To overcome some of the disadvantages of ADT, Weiss et. al.[11] described a direct contact test (DCT) assay which measures effect of direct and close contact between the test microorganism and the tested material on microbial viability, regardless of the solubility and diffusibility of the antimicrobial components.

The initial bacteria colonizing the pellicle-coated tooth surface are predominantly gram-positive microorganisms.[1] While *Enterecoccus faecalis* (formerly *Streptococcus faecalis*) and *Staphylococcus aureus* are gram-positive facultative anaerobe, *Sarcinia lutea* is gram-positive aerobe microorganisms.

At present, well-documented studies about the antibacterial effects of periodontal dressings are lacking. Therefore the aim of this *in vitro* study was to evaluate antibacterial activity of three different periodontal dressings by both ADT and DCT.

Materials and Methods

The periodontal dressings used in this study were; Cavex (Cavex, Holland B.V. of Haarlem), Coe-Pak (GC, America Inc. U.S.A.) and Voco pac (Voco, Cuxhaven Germany). All materials were mixed according to manufacturers' instructions.

Test Microorganisms and Growth Conditions

Antibacterial activities of the periodontal dressings were evaluated against *E. faecalis* (RSKK 97008), *S. aureus* (ATCC 6538), *S. lutea* (ATCC 9341). All strains were obtained from Refik Saydam Health Institute, Ankara. ADT and DCT were used to evaluate the antibacterial activity.

Bacteria were grown aerobically to late logarithmic or early stationary phase from frozen stock cultures in brain heart infusion (BHI- Difco) broth at 37°C. In all cases, 24h cultures were used. Cells were harvested by centrifuging and resuspended in fresh medium. Inocula were prepared by the resuspension of the washed cells to predetermined optical densities relating to known concentrations. The test microorganisms were prepared by inoculating several colonies of each of the strains, 24 h on BHI agar plates into 20 ml of BHI broth in a 100 ml Erlenmeyer flask and incubated for 6 h at 37°C.

Agar Diffusion Test

Eighteen brain heart infusion agar plates were inoculated with three different microorganisms, *E. faecalis*, *S. aureus*, *S. lutea*. Five wells (5 mm diameter) were prepared in each plate. Each dressing was prepared according to the manufacturers' instructions and freshly mixed specimens from each tested material were placed into two plates which were inoculated with one of the test microorganisms. A total of 10 observation areas were used to evaluate each dressing. Three positive control plates were streaked with bacteria, but no dressing was used.

Plates were then incubated at 37°C. After varying periods of incubation (24 h, 48 h, 7 days and 10 days), the zones of inhibition of bacterial growth were observed.

Measurement of Inhibition Zone

The plates were examined under 2.5x magnification loupes and evaluated for growth inhibitory zones around each dressing as evidenced by lack of bacterial colonization (clearing of agar) adjacent to each dressing over 360 degrees. The most uniform diameter segment of the zone of inhibition was measured with an endodontic millimeter ruler in mm, with a diameter of 5 mm as the cut-off value.

Data were statistically analyzed using Kruskall-Wallis test and post-hoc comparisons were made with Mann-Whitney U test.

Direct Contact Test

The test was based on turbidometric determination bacterial growth in 96-well microtiter plates. The side walls of the wells was coated with freshly mixed Cavex, Coe-Pak, Voco pac by using a small size round ended dental instrument. A 10 µl bacterial suspension (ca. 10^5 bacteria) was placed on the test material. After incubation for 1 h (Group A) and 24 h (Group B) in a humid atmosphere at 37°C, evaporation of the suspension's liquid was observed to ensure direct contact between all bacteria and surfaces of the test materials. The kinetics of bacterial outgrowth was followed by densitometric measurements in each well and is monitored at 620 nm at 37°C and recorded using a temperature controlled microplate spectrophotometer (Anthos Labtec HT 2, Elisa Reader, Anthos Labtec Inst., Salzburg, Austria). Automixing prior to each reading ensured homogeneous bacterial cell suspension. Bacteria were allowed to contact directly to the dressings for 1 h and 24 h at 37°C. Bacterial growth was then spectrophotometrically measured through every hour for 15 h by using an Anthos Labtec HT 2. All experiments were repeated at least twice.

Results

According to ADT results none of the dressing materials showed any inhibition to *E. faecalis*. Voco pac (9.88±0.23) exhibited clinically relevant antimicrobial activity to *S. aureus* ($P<0.05$), but there were no antibacterial activities of Coe-Pak and Cavex to *S. aureus*. Cavex did not show any inhibition to *S. lutea*. There was statistically significant difference between antibacterial activity of Coe-Pak (10.25±0.31) and Voco pac (11.63±0.50) for *S. lutea* ($P<0.05$).

The results of the DCT are shown in Fig. 1-6. In this test, all dressings showed similar results after 1 h contact test. They gave similar antibacterial effects against *E. faecalis* in the first 3 h (Fig. 1), *S. aureus* in the first 4 h (Fig. 2) and *S. lutea* in the first 5 h (Fig. 3). Coe-Pak was more potent against *E. faecalis* and *S. lutea* than the other dressings after 24 h contact test (Fig. 4 and 5). But it was effective only for the first 10 h against *S. aureus* (Fig. 6).

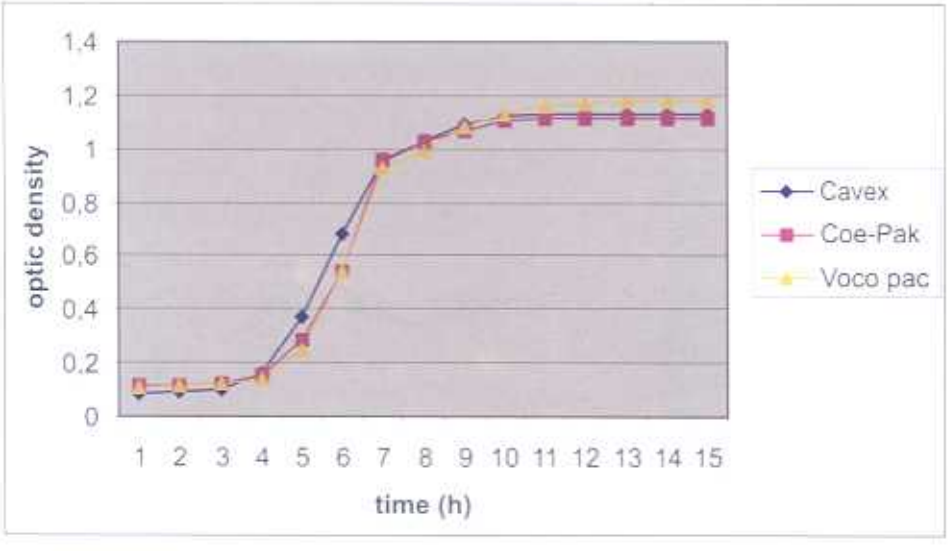

Fig. 1. Antibacterial effect of periodontal dressings against *E. faecalis* according to DCT after 1 h contact.

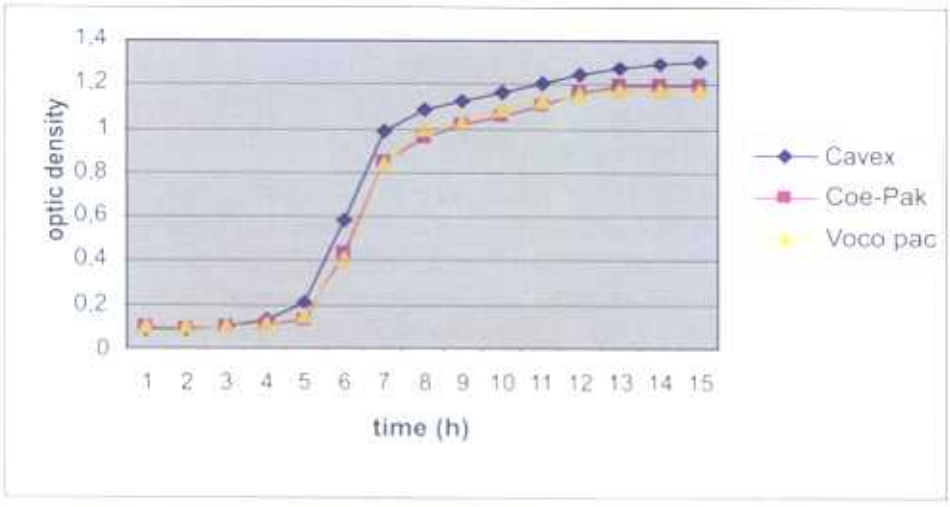

Fig. 2. Antibacterial effect of periodontal dressings against *S. aureus* according to DCT after 1 h contact.

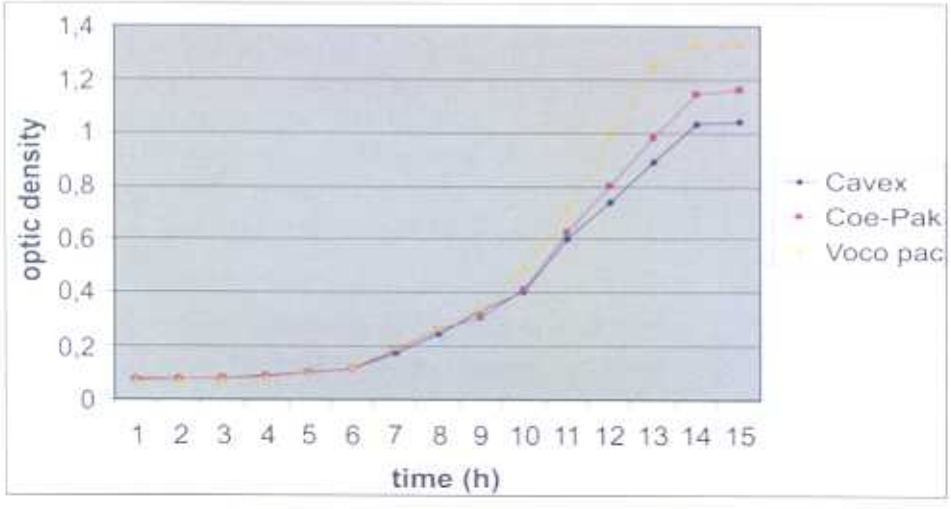

Fig. 3. Antibacterial effect of periodontal dressings against *S. lutea* according to DCT after 1 h contact.

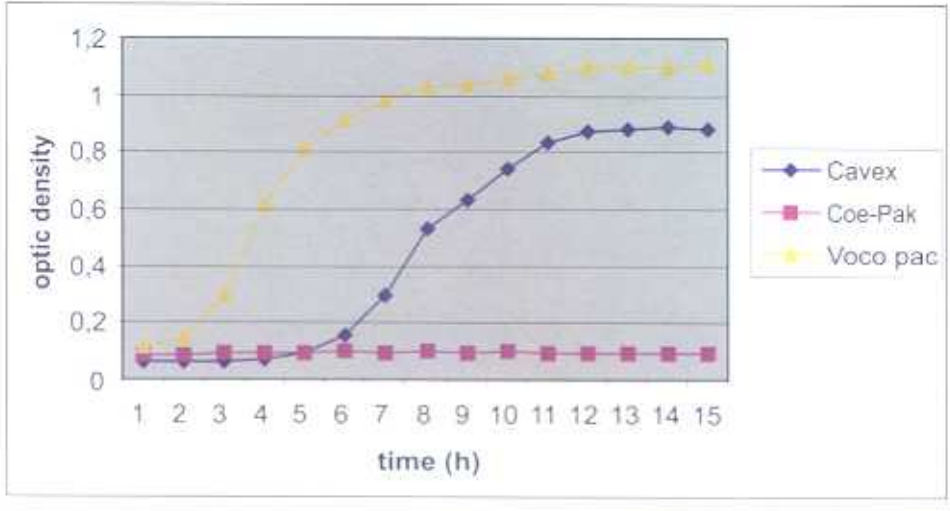

Fig. 4. Antibacterial effect of periodontal dressings against *E. faecalis* according to DCT after 24 h contact.

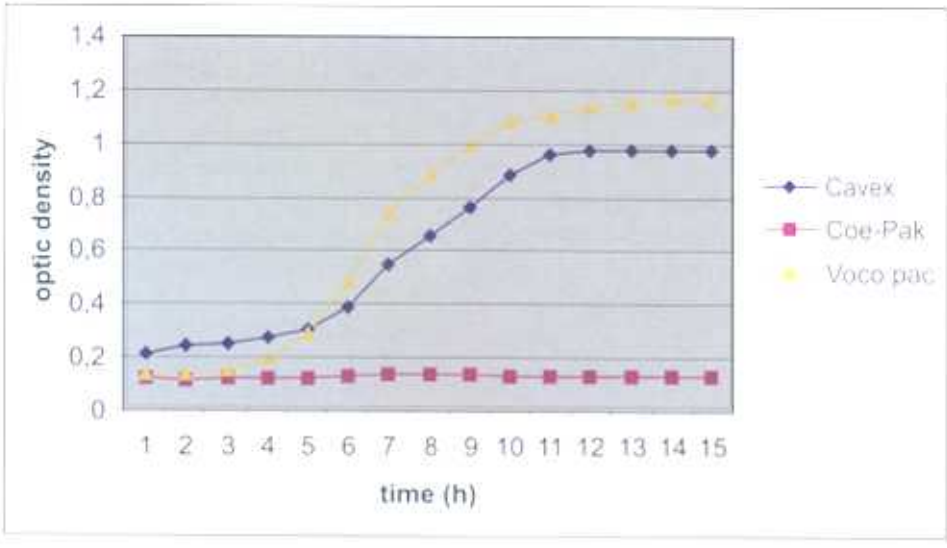

Fig. 5. Antibacterial effect of periodontal dressings against *S. lutea* according to DCT after 24 h contact.

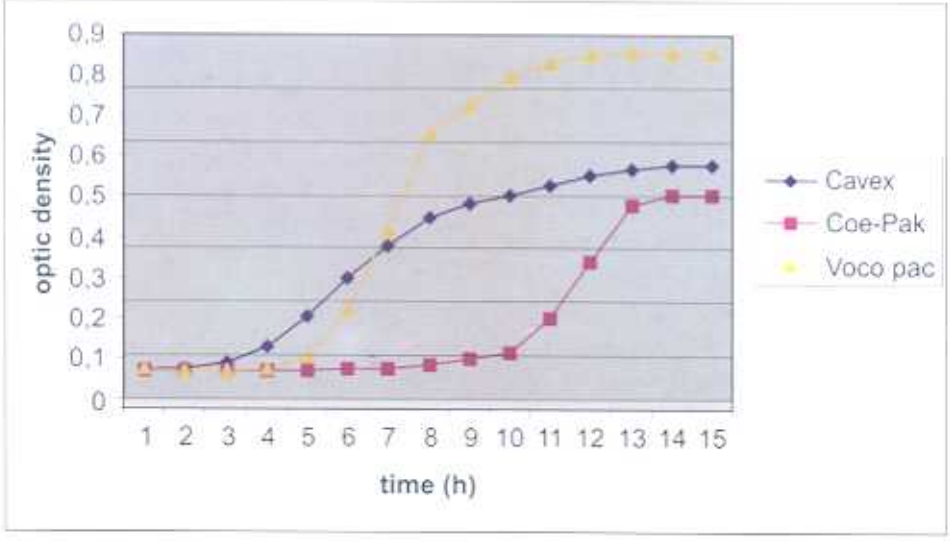

Fig. 6. Antibacterial effect of periodontal dressings against *S. aureus* according to DCT after 24 h contact.

Discussion

Most commercial periodontal dressings claiming antibacterial activity lose this activity shortly after application.[12] In this study, the antibacterial activity of three periodontal dressings was evaluated by two different methods.

One of the most often used methods to assess dental materials' antibacterial activity is the ADT.[8,10,11] However, this method has several limitations such as lack of standardization of inoculum density, adequate culture medium, agar viscosity, plate storage conditions, size and number of specimens per plate, and time and temperature of incubation.[8,11] Beside these disadvantages, it is also relatively insensitive and semiquantitative and does not distinguish between bacteriostatic or bactericidal properties of materials.[7] The results of ADT not depend on only the toxicity of the material for the particular microorganism, but are also highly influenced by the diffusibility of the material across the medium.[13] Therefore, only water soluble agents can be tested.[14] Larger zones of inhibition can

be seen if a material diffuses more easily. Thus, in addition to direct cytotoxicity, the different diffusion rates of the different dressings may also influence the results. However, it is still the most widely used *in vitro* method of evaluation of antimicrobial activity.[9,10]

In an assay such as DCT, bacteria are brought in direct contact with the tested samples for a controlled period of time, thus overcoming some of the disadvantages of the ADT. DCT provides significant advantages such as reproducibility, quantitative assay, simultaneous testing of 50 samples and continuous measurements of bacterial outgrowth with over 2400 measurements per plate.[11] In addition, this method is virtually independent of the diffusion properties of both the tested material and the media. Therefore, it is difficult to compare the results of ADT and DCT. It has been demonstrated that data obtained using ADT and DCT can not be compared.[15]

In the present study, the antibacterial properties of three periodontal dressings were evaluated by both methods. Based on the ADT, Voco pac was superior in its antibacterial activity compared with the other two dressings. The conflicting result was obtained when the DCT was applied to assess the antibacterial activity. Coe-Pak was better than the other two dressings. It may contain more potent antibacterial component which is either less soluble or less diffusible in the surrounding agar medium of the ADT. The results demonstrated that although both tests measure antibacterial activity, the results were highly affected by different material properties.

The incorporation of antimicrobial components into periodontal dressings may become an essential factor in preventing bacterial colonization. It has been reported that incorporation of antibacterial agents with high retention and slow release properties in the mouth in surgical dressings seems advantageous.[12]

Conclusion

As a conclusion, the present data showed the importance of using the DCT in conjunction with ADT to assess periodontal dressings.

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