A NEW CHEMICAL DEVICE BASED ON SILVER AND CATIONIC SURFACTENTS AS AN ADJUNCT OF DOMESTIC ORAL HYGIENE. A SINGLE BLIND STUDY ON PERIODONTAL PATIENTS.


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Abstract

The indication for using chemical devices as an adjunct of domestic oral hygiene has become increasingly used in last decades, usually following mechanical instrumentation. The efficacy of chemical devices for oral biofilm control is proven by evidence from clinical studies. The purpose of this study was to assess the effect of a new oral gel named ADC to reduce oral bacterial loading investigated by means of Polymerase Chain Reaction (PCR).

Materials and methods

A total of 10 patients with a diagnosis of chronic periodontitis in the age group >25 years, were selected. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Four sites in separate quadrants were selected in each patient for testing the efficacy of the new medical device. Microbial analysis (MA) was performed at baseline and at day 15. Paired T-Test was used to detect statistical significant reduction of total bacterial loading and specific bacteria. The results showed statistically significant reduction of the overall bacterial loading from baseline to day 15.

Results

Specimens of subgingival plaque from a total of patients were investigated for the presence of “red complex” bacterial species by quantitative PCR. The total amount of bacteria detected a statistically significant difference was detected between total bacterial loading pre and post treatment. Total bacteria loading was significant statistically reduced of about 87%, (p=0.029).

Conclusion

In this study, a strong reduction of total bacterial loading using ADC gel as an adjunct to oral home care was observed.

Key words: chronic periodontitis, bacterial load, oral biofilm, red complex.
Introduction

Prevention of dental caries and periodontal disease (two of the most prevalent diseases in the world population) should include oral hygiene protocols. With the widespread of prevention protocols, treatment of dental diseases required several methods. The indication for using chemical devices as an adjunct of domestic oral hygiene has become routine method in the last decades, usually following mechanical instrumentation. The efficacy of chemical devices for oral biofilm control is proven by evidence from clinical studies. The purpose of this paper was to assess the effect of a new oral gel named ADC on oral bacterial loading using Polymerase Chain Reaction (PCR).

Oral biofilm

Oral biofilm (OB) is a bacteria-structured aggregate that is formed on teeth, in the presence of saliva\(^1\). OB formation occurs on both teeth and implants surfaces, and is composed of bacterial micro colonies, which are considered independent communities that communicate dynamically via water channels that allow the passage of nutrients and other chemicals\(^2\). The presence of OB on tooth surfaces initiates an immune-inflammatory response in local tissue that may lead to an inflammatory process in gums (gingivitis) or periodontal tissues (periodontitis)\(^3\)-\(^4\). The presence of OB over time may promote periodontal disease progression, with consequent destruction of supporting structures and loss of teeth if the disease remains untreated. \(^5\).

OB elimination is considered the gold standard for reducing dental caries and periodontal diseases. Preventive protocols promote higher tooth longevity, and home oral care hygiene procedures are widely known to be essential to maintaining oral health. Tooth brushing has not proved effective enough to control OB. Mechanical devices such as interproximal cleaning brushes and tongue cleaners allow OB control, however, patient adherence to this routine seems to be the most difficult; in fact studies show that only 10% of the population use dental floss/tape and interdental brushes regularly\(^6\). Furthermore, proper oral care hygiene is the key to maintaining oral health and bacterial loading is the main cause of both gingivitis and periodontitis.

The prevention of OB formation and its elimination from the tooth surface is the first step to treat periodontitis. Periodontal therapy is based on scaling and root planning, whether
associated to antimicrobial agents\textsuperscript{7-10} or not, including chlorhexidine and essential oils. Among the antimicrobials, chlorhexidine and essential oils are proved most effective in reducing OB. Essential oil and different chemical devices were statistically significant in reducing OB also, offering few additional benefits respect to mechanical treatment\textsuperscript{11-14}.

**Aim**

The aim of this study was to assess the effect of a new oral gel named ADC on oral bacterial loading using Polymerase Chain Reaction (PCR).

**Materials and methods**

The ADC gel is a new formulation based on the complex silver-2-mercaptobenzoate, chlorhexidine digluconate and didecyldimethylammonium chloride as active ingredients. The silver complex, prepared according to the procedure described in the patent application IT 102018000000576, was mixed with the two organic surfactants in distilled water and the solution was then gelified with hydroxypolycellulose and polyvinilpyrrolidone. The gel contains didecyldimethylammonium at 0.5\%, chlorhexidine digluconate at 0.1\% and Ag\textsuperscript{+} ions at 0.001\%.

**Experimental design and patient selection**

A total of 10 patients with a diagnosis of chronic periodontitis, >25 years age, were selected. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Four non-adjacent sites in separate quadrants were selected in each patient for monitoring, based on criteria that the sites will localize chronic periodontitis. Microbial analysis (MA) was performed at baseline and after day 15.

During the initial phase, all the subjects were trained about proper home-care techniques and monitored during the study period, receiving full mouth scaling and root planning (SRP). All subjects received microbiological and clinical monitoring at baseline and after 15 days respectively. The research objectives were explained to the patients, who then signed an informed consent form. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee 06.09.2013 prot. n. 29579 University Study of L'Aquila.
Subject population and inclusion/exclusion criteria

A total of 10 subjects with untreated chronic periodontitis were selected by one of the authors. Detailed medical, periodontal and dental history was obtained. All the eligible subjects were informed of the nature, potential risks and benefits of the study. The inclusion criteria were as follows: age >25 years; probing depth of 3 mm or more; dentate with >20 natural teeth. The exclusion criteria were medically compromised patients, patients who had been administered antibiotics or antimicrobial in the past 6 months, smokers, pregnant and lactating mother. All the participants were evaluated clinically. The same examiner assessed the clinical and periodontal parameters. Probing depth measured to the nearest millimetre from the gingival margin to the bottom of the pocket was noted using calibrated William’s periodontal probe for all measurements.

Microbiological test

The antimicrobial activity of ADC gel was at first tested in vitro against Gram-positive and Gram-negative bacteria and Candida albicans. 50μl of ADC gel were placed in the centre of a PETRI capsula containing TSA (Tripton Soia Agar), previously contaminated at the surface with 100μl of a microbic pool containing Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 10536, Pseudomonas aeruginosa ATCC 15442, Enterococcus hirae ATCC 10541 and Candida albicans ATCC 1023 in concentration of the order of 1.5 - 5 x 10\(^{10}\) ufc/ml (Diagnostic International Distribution S.p.A). Figure 1 shows the remarkable inhibition of the microbial growth observed after 24 h of incubation at 36°C.
**Figure 1.** Inhibition ring produced by the ADC gel in presence of Gram-positive and Gram-negative bacteria and Candida albicans at concentration levels of the order of 1-5 x10⁹ ufc/ml.

LABtest® (LAB SRL®, Ferrara, Italy) on the selected patients was then used¹⁴. It detects Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus and Total bacteria loading.

**Real-Time Polymerase Chain Reaction**

Primers and probes oligonucleotides were designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservate spots. Three real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantifies the total amount of bacteria using two degenerate primers and a single probe matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detects and quantifies the three red complex bacteria, i.e. P. gingivalis, T. forsythia and T. denticola, in a multiplex PCR. The third reaction detects and quantifies Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum and Campylobacter rectus. These reactions include six primers each and three probes each that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile were initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including nontemplate controls to exclude reagents contamination.

**Agents (S1) 125**

(Eurofin MWG Operon, Ebersberg Germany) were standardly used for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction by using a mix of the same amount of plasmids in serial dilutions ranging from 10¹ to 10⁷ copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy
number over the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations were estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of specific bacterial species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

**Statistical analysis**

Descriptive statistics were performed using Microsoft Excel spreadsheets. Paired T-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

**Results**

Specimens of subgingival plaque from a total of patients were investigated for the presence of “red complex” bacterial species by quantitative PCR. There was a statistically significant difference between total bacterial loading detected pre and post treatment (see Table I). Total bacteria loading was significant statistically reduced of about 87%, (p= 0.029) (see Table II).

**Discussion**

Domestic oral hygiene (DOH) has long been documented to preserve the natural dentition by achieving and maintaining a healthy periodontium. According to the current state of knowledge, species such as the red complex organisms have shown to play a major role in the pathogenesis of periodontal disease⁴.

The gold standard of periodontal therapy has been mechanical debridement during DOH; however, various adjunctive molecules like local drug delivery have been used in conjunction to DOH to improve the therapeutic results. The application of new chemical devices has gained increased attention in recent years¹¹-¹⁴.

In our study was observed a stronger reduction of total bacterial loading using the ADC gel during tooth brushing. This reduction of bacteria and presence of healing in the connective tissue subjacent to the junctional epithelium can be attributed to the antibactericide properties of the ADC gel.
LABtest® was used to monitor the effects of mechanical and/or chemical therapy on the sub-gingival microflora, as PCR assay has been shown to be highly sensitive and specific for the periodontal pathogens\(^{15}\). ADC gel has been shown to be effective in altering the flora and acting as an adjunct to DOH. As evident from this study, the results demonstrate clinical and microbiological improvements following the use of ADC gel.

Admittedly, the results obtained in regards to periodontal disease management can be directly affected by the introduction of ADC gel during therapy. Nevertheless, in the case of chronic pathologies, such as periodontal disease, the results provisionally demonstrate the most significant effect of using ADC gel as an adjuvant treatment is primarily related to its ability to reduce total bacterial loading. It may be speculated that ADC gel antibacterial activity, most likely contributes to an overall improvement in the DOH of the patient.

In the oral cavity the lack of DOH induces an inflammatory response with progressive destruction of the periodontal tissues and finally the loss of teeth.

In our opinion, the result of this clinical trial are very promising with regards to the benefits of using ADC gel as an adjuvant in the standard treatment of periodontal disease contributing to an overall improvement in oral health of the patient.

Further research with relatively large sample size, longer follow-up period, and use of advanced newer delivery systems are advocated to further study the efficacy of ADC gel as an effective local drug delivery agent in periodontal and peri-implantitis therapy.

**Conclusions**

Based on this study, ADC gel has proven to be effective in reducing total bacterial loading. ADC gel is an antiseptic with scientifically proven efficacy and with no side effects.
Tab I. Mean amounts of specific bacterial species before and after ADC gel treatment.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Species 1</th>
<th>Species 2</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Mean</th>
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<td>AA1</td>
<td>AA2</td>
<td>104,100</td>
<td>10</td>
<td>329,1931</td>
<td>104,000</td>
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<tr>
<td></td>
<td>CR1</td>
<td>CR2</td>
<td>123,500</td>
<td>10</td>
<td>172,4743</td>
<td>54,5412</td>
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<td>FN1</td>
<td>FN2</td>
<td>2601,9000</td>
<td>10</td>
<td>2895,5308</td>
<td>915,6472</td>
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<tr>
<td></td>
<td>TBL1</td>
<td>TBL2</td>
<td>505001,8</td>
<td>10</td>
<td>514769,3</td>
<td>162784,4</td>
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<tr>
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<td>TD1</td>
<td>TD2</td>
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<td>10</td>
<td>160,3638</td>
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<tr>
<td></td>
<td>TF1</td>
<td>TF2</td>
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Tab II. Output of paired samples T-test.

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<th>Pair</th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
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<td>763,1969</td>
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<td>744,8129</td>
<td>235,5305</td>
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<td>-.443</td>
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<td>.668</td>
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<td>Pair 3</td>
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<td>3570,2866</td>
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<td>9</td>
<td>.420</td>
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<tr>
<td>Pair 4</td>
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<td>534870,8</td>
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<tr>
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<td>-284,3026</td>
<td>.027</td>
<td>9</td>
<td>.979</td>
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</table>
References


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