POLYMERASE CHAIN REACTION TO EVALUATE THE EFFICACY OF SILICA DIOXIDE COLLOIDAL SOLUTIONS IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CASE CONTROL STUDY

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The objective of this study was to compare the efficacy of supportive periodontal therapy [i.e. scaling and rooth planing (SRP)] alone versus a chemical silica dioxide (SiO2) colloidal solution (SDCS) device used in association with SRP in the treatment of chronic periodontitis in adult patients. A total of 20 patients with a diagnosis of chronic periodontitis (40 localized chronic periodontitis sites) in the age group of 35 to 55 were selected. None of these patients had previously received any surgical or non-surgical periodontal therapy and had radiographic evidence of moderate bone loss. Two non-adjacent sites in separate quadrants were selected in each patient to monitorize treatment efficacy (split mouth design). Clinical pocket depth (PD) and microbial analysis (MA) were analyzed at baseline and on 15th day. SPSS program and paired simple statistic *t*-test were used to detect significant differences. Total bacteria loading, *Tannerella forsitia* and *Treponema denticola* loading were statistically reduced when SiO2 was locally delivered. SDCS gel is an adjuvant therapy which should be added to SRP in the management of moderate-to-severe chronic periodontitis.

Periodontal disease is one of the prevalent illnesses in the adult population. It is characterized by a triad of symptoms: tooth mobility, foetor ex ore, gingival bleeding. If left untreated, the disease can lead to tooth loss. Pathogenesis of periodontal disease is multifactorial and bacteria have a prominent role (1). The main pathogens implicated in periodontal disease are anaerobic gram-negative bacteria of which the most aggressive were identified in the "red complex" group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* (2).

The aim of periodontal treatment is to eliminate oral infection, and prevent the progression of the disease (3). Many studies have widely demonstrated that the non-surgical therapy including scaling and root planing (SRP) associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for correct maintenance of oral health (4, 5).

The aim of the present study is to evaluate the

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effectiveness of a new chemical device (i.e. silica dioxide colloidal solution, SDCS) used in association with SRP in the treatment of chronic periodontitis in adult patients.

Silica dioxide colloidal solutions

Silver ion is an effective antimicrobial agent and shows a rather broad spectrum of bactericidal activity (6) consistent with different mechanisms of action, which depend on the binding site. For instance, when binding occurs at the bacterial cell wall, ruptures can occur (7). When bound to proteins involved in respiration and nutrition of the organism, silver can block these processes with consequent elimination of the bacteria (8). When binding DNA, silver can affect the replication and division of the organism (9).

Preparation of SDCS

The product is composed of an aqueous solution containing the anionic silver complex, where silver ions are present at 0.57% and of chlorhexidine digluconate at 0.11%. The product can be prepared by direct dissolution of the different components in sterile water (10).

MATERIALS AND METHODS

A total of 20 patients with a diagnosis of chronic periodontitis were randomly selected. Patients qualified for the study if they had two non-adjacent sites located in separate quadrants which required periodontal treatment, in the age group of 35-55 years. The subjects had not previously received any surgical or non-surgical periodontal therapy. Subjects were excluded from the study if they met any of the following criteria: (1) pregnancy; (2) a history of taking antibiotics or using antibacterial mouth rinses during the previous 6 months; (3) teeth with furcation involvement; (4) smoking, or drug or alcohol abuse.

This study was approved by the ethics Committee of the hospital. Subjects participating in the study volunteered to follow a detailed verbal description of the procedure and by signing consent forms.

Clinical methods

A total of 20 patients (i.e. 40 sites) were selected and grouped into two categories: control and test (split mouth design). The control group (20 sites) was treated with SRP without using SDCS (control site). The test group (20 sites) was treated by SRP plus SDCS (test site).

All patients underwnt SRP at the baseline measurement. Prior to SRP, clinical pocket depth and microbial analysis were performed in each selected site. Then SRP was carried out at both sites using ultra sonic scaler. After SRP, SDCS was adjunct in the test site of each enrolled patient. After 2 weeks, microbiological samples were again collected from from both sites in each patient.

For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgengival samples and were immediately transferred to the microbiological laboratory for processing. *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* and total bacterial loading were evaluated.

Real-time polymerase chain reaction:

Oligonucleotide probes 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 homology of sequence. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified 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 detected and quantified the three red complex bacteria, i.e. *P. gingivalis, T. forsythia* and *T. denticola*, in a multiplex PCR. This reaction included a total of six primers and three probes 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 reagent contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard 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 a Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative

amounts of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

Statistical analyses

SPSS program and paired simple statistic *t*-test were used to detect significant differences.

RESULTS

A statistically significant difference was detected between (A) Total Bacterial Loading pre and post SDCS treatment, (B) *T. forsythia* pre- and post-SDCS treatment and (C) *T. Denticola* and post-SDCS treatment (see Table I). Noteworthy, no statistical significant difference was detected between control and test site as regard pre-treatment bacteria concentration as well as pre- *vs* post-treatment bacteria concentration in control site (i.e. only SPR). When p-value is less than 0.05 then the difference between the two compared bacterial loadings is statistically significant (see last column in Table I).

DISCUSSION

It is well understood that most destructive types

Table I. *Results of microbiological analysis at baseline and after therapy.*

Variables	Bacteria	Mean	*P* Value
PAIR 1	CBT1-CBT2	-1.00E+06	0.671
PAIR 2	CBTS1-CBTS2	1974667	0.005
PAIR3	PG1-PG2	67807.5	0.134
PAIR4	PGS1-PGS2	146378	0.167
PAIR5	TD1-TD2	-62.684	0.996
PAIR 6	TDS1-TDS2	71305.1	0.033
PAIR 7	TF1-TF2	32294.7	0.110
PAIR 8	TFS1-TFS2	70521.1	0.047
PAIR 9	CBT1-CBTS1	-159089	0.900
PAIR 10	CBT2-CBTS2	314156	0.303
PAIR 11	PG1-PGS1	-124696	0.156
PAIR 12	PG2-PGS2	-46126	0.443
PAIR 13	TD1-TDS1	-77013	0.074
PAIR 14	TD2-TDS2	-5645.3	0.443
PAIR 15	TF1-TFS1	-33452	0.272
PAIR 16	TF2-TFS2	4774.53	0.794

CBT1: bacterial loading at baseline in control sites. CBT2: bacterial loading after therapy in control sites. CBTS1: bacterial loading at baseline in test sites. CBTS2: bacterial loading after therapy in test sites. PG1: Porphyromonas gingivalis loading at baseline in control sites PG2: Porphyromonas gingivalis loading after theraphy in control sites PGS1: Porphyromonas gingivalis loading at baseline in test sites PGS2: Porphyromonas gingivalis loading after theraphy in test sites. TF1: Tannerella forsythia loading at baseline in control sites. TF2: Tannerella forsythia loading after therapy in control sites. TFS1: Tannerella forsythia loading al baseline in test sites. TFS2: Tannerella forsythia loading after therapy in test sites. TD1: Treponema denticola loading at baseline in control sites. TD2: Treponema denticola loading after therapy in control sites. TDS1: Treponema denticola loading at baseline in test sites. TDS2: Treponema denticola loading after therapy in test sites.

of periodontal diseases occur due to the presence of pathogenic micro-organisms colonizing the subgingival area, and the suppression or eradication of these microbes result in improvement in periodontal health. Mechanical debridement is effective in both disturbing the biofilm and reducing the bacterial load. However, sometimes mechanical instrumentation may not be sufficient to control the disease due to tissue invasive pathogens, or other tooth-related anatomic factors. In such conditions, adjunctive use of a chemical device provides an additional benefit in controlling the disease.

Support periodontal therapy is widely used, but a greater effectiveness is demonstrated by the administration of topical antimocrobicals in association. The advantages of topical therapy involve the use of antimicrobical agents directly into the periodontal pocket, minimizing the adverse effects related to systemic therapy (5). The potential benefits of local drug delivery include improved patient compliance, an easier access to periodontal pocket and a lower dosage of antimicrobial agent. The most commonly used methods for local drug delivery are local gengival irrigations (1). The antimicrobial agents used as local drug delivery agents include tetracycline, ofloxacin, clindamycin, chlorhexidine, etc. (5). These local drug delivery devices have been used either alone or as adjunct with SRP. These local antimicrobials are administered directly into the periodontal pocket and the effectiveness of these chemical deviceS is related to their bactericidal activity and the subsequent reduction of gingival inflammation (11-14).

The topical use of a chemical device along with mechanotherapy dramatically improves clinical results, and at the same time is free from its inherent adverse effects and disadvantages. Local delivery of chemical devices into the pocket achieves a greater concentration of the drug locally, proving bactericidal for most periopathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body. Due to the high level of heterogeneity of the studies in literature, it is not possible to establish definitive conclusions regarding the use of adjunctive local drugs. However, the use of local antibiotics with SRP may be beneficial for periodontal pocket treatment. Standardized clinical disease diagnostic criteria and additional randomized controlled clinical trials are necessary to verify the effectiveness of the use of local antibiotics with SRP.

Our study evaluated the efficacy of SDCS gel in the management of moderate-to- severe chronic periodontitis. The results of this investigation demonstrated an overall improvement in most bacterial parameters. In this study, we explored the antimicrobial activity of aqueous solutions containing a thermally and photochemically stable anionic silver complex, where the silver ion is mixed with chlorhexidine digluconate. Previous studies have testified the existence of a synergic antimicrobial action between these components (10), responsible for a vitality reduction higher than 5 log for the following test organisms:

Pseudomonas aeruginosa	ATCC 15442
Staphylococcus aureus	ATCC 6538
Escherichia coli	ATCC 10536
Enterococcus hirae	ATCC 10541
Candida albicans	ATCC 10231

Microbiological testing was thought appropriate to evaluate the effect of SDCS ona subgingival microbial population, the primary etiological factor for periodontitis. Several methods have been used for microbiological testing in periodontitis (15), however, many techniques have not been fully accepted due to low sensitivity or specificity. Moreover, sometimes these methods are slow, expensive and laborious. Recently, a rapid, sensitive test (LAB SRL, Ferrara, Italy) was developed to detect and quantify the three bacterial species most involved in periodontitis: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* (16, 17).

It is well known that both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction (18). They appear closely 'linked' topologically in the developing biofilm, and show an *in vitro* ability to produce a number of outer membrane-associated proteinases. They are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover, both these and *T. forsythia,* show a higher prevalence in disease than in health, suggesting that these bacteria are associated with the local development of periodontitis. Based on this reported data, red complex triad was investigated and SDCS was able to significantly reduce their concentration.

SDCS is an adjuvant therapy which should be added to SRP in the management of moderate-to-severe chronic periodontitis.

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