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# Evaluation of Clinical and Antimicrobial Efficacy of Silver Nanoparticles and Tetracycline Films in the Treatment of Periodontal Pockets

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***Abstract:*** *Periodontitis is a multifactorial infection associated with a variable bacterial pattern. The treatment focuses mainly on the reduction of the total bacterial count. Local delivery of antimicrobials has been investigated as an adjunct to conventional therapy. Tetracycline was proved to inhibit collagenases and was thus proposed to be useful in treating diseases. In recent years, silver nanoparticles have attracted considerable attention for medical applications due to their antibacterial activity. This study aims to evaluate the clinical and the microbiological findings following intrasulcular applications of tetracycline films and silver nanoparticles in periodontal pockets. A total of 48 periodontal pockets were studied. Group (A) received scaling and root planing with tetracycline film application, Group (B): scaling and root planing with silver nanoparticles application and Group (C): scaling and root planing only. The drugs were applied once weekly for three weeks. Clinical parameters were taken at baseline, after one and three months. Samples of gingival crevicular fluid were obtained at baseline and after one month for microbiological analysis. Groups A and B showed a significant decrease in probing depth and clinical attachment level as well as the reduction in the bacterial count compared to Group C. Thus, local application of tetracycline films and silver nanoparticles were effective in improving the clinical outcome and elimination of bacterial infection in periodontal pockets.*
***Key words****: chronic periodontitis, silver nanoparticles, tetracycline, antibacterial activity.*

## I. Introduction

Periodontitis is an inflammatory disease of the gingiva and the adjacent attachment apparatus and is characterized by the loss of both, connective tissue attachment and alveolar bone. Chronic periodontitis, the most common form, is characterized by pocket formation and gingival recession.[1] The disease is initiated by polymicrobial infection which is composed of more than 300 different species of bacteria. The bacteria of supragingival plaque are predominantly Gram-positive cocci, whereas periodontal pathogens in subgingival plaque are dominated by Gram-negative anaerobic organisms.[2,3]

Predisposing factors include heredity, systemic disorders as well as environmental factors as smoking. These risk factors play a crucial role in determining the progression and severity of the disease.[4] Although strict anaerobic periodontal pathogenic microorganisms are directly involved in the onset and progression of chronic periodontitis, it is also important to mention that for several years, facultative anaerobe Gram-negative *Enterobacteriaceae* have also been found in the gingival sulcus of patients with chronic periodontitis.[5–8]

The normal immune response to bacterial invasion is the activation of inflammatory cells. The host inflammatory response is responsible for the majority of the hard- and soft-tissue breakdown that takes place in periodontitis.[9, 10] Thus periodontal destruction is caused either directly by the action of bacteria on the invaded tissues, or indirectly through the release of biologic mediators in the form of enzymes and chemical mediators from the host tissue cells that lead to host tissue destruction.[11] Interleukin I and tumor necrosis factor-alpha induce the release of other mediators that aggravate the inflammatory response, such as prostaglandins.[12-14] These host-derived mediators have the potential to stimulate bone resorption and activate or inhibit other host immune cells.[15]

The great challenge for successful periodontal therapy is to eliminate pathogenic organisms present in the dental plaque. Different treatment modalities were attempted including surgical intervention, non-surgical procedures, mechanical therapy and the use of pharmacological agents.[1,16] Mechanical therapy that comprises scaling and root planing (SRP) has become the "gold standard" nonsurgical treatment for periodontitis.[17] Several

studies have indicated that the reduction of the microbial level can effectively improve clinical parameters like Papillary Bleeding Index (PBI), Gingival Index (GI), Probing Depth (PD) and Clinical Attachment Loss (CAL).[18,19] However, in case of deeper pocket sites, manual SRP alone, is not sufficient to completely eradicate pathogenic bacteria. Subsequently, power-driven ultrasonic mechanical instruments were developed to enhance the capability of the operator enabling it to reach through the depth of the pocket more conveniently.[20,21] On the other hand, various studies have reported that the microbiological and clinical effects attained by ultrasonic debridement are similar to those of manual SRP.[22,23]

Therefore, the adjunct use of antimicrobial agents with mechanical debridement could be more effective.[24] Nevertheless, the concentration of systemic antibiotics is usually less in the gingival crevicular fluid (GCF) compared to its concentration in the blood stream. The systemic use of antibiotics causes different side effects as hypersensitivity and gastrointestinal intolerance, in addition to the bacterial resistance that may arise.[25,26] Thus, currently systemic antibiotics are prescribed only for the treatment of aggressive or refractory periodontitis.[27] The shortcomings of systemic antimicrobial treatment led to the development of local drug delivery systems.[28]

The intra-pocket application of antimicrobial therapy has evoked great interest as it overcomes the limitations of systemic antimicrobial therapy and is also considered as a local site-specific modality. The periodontal pocket acts as a natural reservoir for the application of a local delivery device. It is bathed by gingival crevicular fluid which provides a leaching medium for the release of a local delivery drug and facilitates its distribution throughout the pocket.[29,30] Different local drug delivery devices were approved for the treatment of periodontal pockets; such as those constituted from chlorhexidine gluconate,[31] doxycycline hyclate,[32] minocycline hydrochloride[33] and tetracycline fibers.[34]

Tetracyclines have been widely used in the field of periodontal therapy since the 1940s. They are semi-synthetic chemotherapeutic agents with a bacteriostatic action and hence are effective against rapidly multiplying bacteria.[35] Tetracycline and its derivatives have been used as local delivery drugs in the treatment of periodontal pockets in order to limit the drug to its target site with little or no systemic uptake, which in turn will avoid most of the side effects associated with systemic therapy.[34] They have been incorporated into various non-resorbable or bio-resorbable delivery systems for their insertion into periodontal pockets.[35,36] These systems include hollow fibers,[37]ethyl cellulose fibers,[38] acrylic strips,[39]ethylene vinyl acetate copolymer fibers,[40] collagen preparations,[41,42] and poly (D, L-lactic-co-glycolic acid) microspheres.[43] Freisen *et al*. (2002) demonstrated that local delivery of tetracycline with multiple polymer strips was effective in reducing papillary bleeding index, and that it was superior to root planing alone in reducing probing depth.[44]

As a result of development of microbial resistance to multiple antibiotics, the antibiotic-free delivery systems for treatment of periodontal infections have been tried. Recent advances in nanotechnology introduce new therapeutic materials for periodontal regeneration. Nanoparticles are clusters of atoms in the size range of 1-100 nm.[45] Inorganic nanoparticles and their nano-composites are good antibacterial agents. Focus has been made upon the medical and chemical applications of silver nanoparticles (Ag-NPs) due to their unique properties; including antibacterial activity, high resistance to oxidation, and high thermal conductivity.[46]

The exact mechanism by which Ag-NPs employ an antimicrobial effect is not clearly known and is a debatable topic. There are however different theories concerning their antibacterial activity. Ag-NPs have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane affecting its permeability and hence death of the cell.[47]Amro *et al*. (2000) reported that metal depletion may lead to formation of irregularly shaped pits in the outer membrane due to the progressive release of lipopolysaccharide molecules and membrane proteins, which in turn, increases the membrane's permeability.[48] Hence, 'pits' are formed and nanoparticles are accumulated on the cell surface.[49] The formation of free radicals by the Ag-NPs may be considered as another mechanism by which the cells die. Some studies suggested that free radicals are formed when the Ag-NPs come in contact with bacteria, and these free radicals have the ability to damage the cell membrane and rendering it porous, thus can ultimately lead to cell death .[50,51] It has also been proposed that there can be release of silver ions by the nanoparticles,[52] and these ions can interact with the thiol groups of many vital enzymes and inactivate them.[53]

Nowadays, Ag-NPs display different applications in the field of dentistry owing to their antimicrobial effect. They are used in restorative dentistry, where Ag-NPs were incorporated into nano-composites of quaternary ammonium dimethacrylate and calcium phosphate.[54,55] Results showed that these nano-composites are promising as they possess the double benefits of remineralization and antibacterial capabilities to inhibit dental caries. Ag-NPs were also incorporated into tissue conditioners for patients using dental prosthesis. It was demonstrated that a dose of 0.1% Ag-NPs combined to tissue conditioners displayed a minimal bactericidal effect against *Staphylococcus aureus* and *Streptococcus mutans* strains, whereas 0.5% was lethal for fungal strains.[56] In addition, Ag-NPS have been used with endodontic retrofill cements; where the combination of Ag-NPs to Angelus white mineral trioxide aggregate enhanced its antimicrobial activity against *Enterococcus faecalis*, *Candida albicans* and *Pseudomonas aeruginosa*.[57] In the field of implantology, Ag-NPs were

incorporated in the coating of titanium implants. Results revealed that titania nanotubes incorporated with Ag-NPs possess acceptable osteoconductivity, a relatively long-term antibacterial effect and good tissue integration.[58,59]

The present study was carried out to evaluate and compare the clinical improvement and antimicrobial efficacy of tetracycline films and Ag-NPs as an adjunct to SRP in persistent periodontal pockets.

## II. Materials And Methods

A randomized, controlled, clinical study was conducted, and the inclusion criteria for patient selection were females ranging from 30 to 40 years of age. Patients diagnosed with mild to moderate chronic periodontitis with at least three non-adjacent periodontal pockets with 4 to 6 mm pocket depth were included in the study. All selected females were ascertained to be in good general health with no history of systemic disorders (diabetes, osteoporosis, cardiovascular diseases, hyperthyroidism, glucose-6-phosphate-dehydrogenase deficiency or severe myasthenia) and with no history of antibiotic therapy, oral prophylaxis, or periodontal surgery during the last six months.[60,61] Pregnant, lactating females and smokers were excluded from the study.[62]

A total of 48 pockets from 16 patients were selected for the study. Three periodontal pockets in each patient were identified for the study in which SRP were performed followed by impressions for the fabrication of acrylic stents required for the standardized measurement of pocket depths and clinical attachment loss in the test and control groups throughout the study period[63] (Fig. 1). During the second visit baseline data were recorded. The selected pockets were assigned randomly to three groups. The three treatment groups were Test Group (A): SRP with tetracycline film application, Test Group (B): SRP with Ag-NPs application and Control Group (C): SRP alone.

### 2.1 Preparation and application of Tetracycline Film

A concentration of 1:10 tetracycline hydrochloride (TcHCl)[*] drug to Carboxymethyl cellulose sodium (Na CMC)[†] was prepared. Freshly prepared drug solution (100 mg TcHCl dissolved in 5 mL distilled water) was added to 15 ml of polymer solution, dropwise with continuous stirring using a glass rod. The mixture was left to stand until all air bubbles disappeared and was then casted in a horizontally leveled Teflon plate of 5 cm internal diameter. The polymeric drug solution was dried under ambient conditions with the aid of a fan in a dark place to avoid photo-decomposition of the drug. The dried films were carefully removed from the Teflon cups, checked for any imperfections or air bubbles[64] (Fig. 2). Tetracycline films were aseptically cut into portions that were smaller than the pocket dimensions. These portions were inserted into the pocket once weekly for three successive weeks (Fig.3).

### 2.2 Application of Silver Nanoparticles

100 nm Cytodiagnostic spherical Ag-NPs[‡] (20ml) of concentration 0.02 mg/ml were used in this study (Fig.4). 2 ml of the Ag-NPs solution were injected directly inside the periodontal pocket using an insulin syringe and were allowed to fill the pocket (Fig. 5). The intrasulcular application of Ag-NPs was repeated once weekly for three successive weeks. Periodontal dressing[§] was applied to achieve retention of the drug to the pocket for the required period (Fig. 6).

### 2.3 Post-procedure Instruction and Clinical Parameters

Patients were instructed to carry out normal oral hygiene procedures, without using dental floss, any mouth washes or oral irrigation devices. They were asked to report immediately if pain, swelling or any other problem occurred. Clinical measurements included, PBI,[65] GI,[66] PD and CAL.[67] All the clinical parameters were recorded at baseline, after one and three months.

### 2.4 Microbiological Analysis

Samples of GCF were obtained using sterile paper points, inserted into the pocket for 30 seconds until resistance was felt or the paper points had bent (Fig. 7).[68] They were immediately transferred to 5 ml screw capped test tubes containing brain heart infusion broth[**] that served as a transport and enrichment medium (Fig. 8). The GCF samples were taken at baseline and after one month. The test tubes were incubated under anaerobic conditions for 4 hours at $37^0$C; after which they were shaken by vortexing to ensure homogeneous mixture of the broth. Immediately 50µl were aseptically inoculated onto blood and MacConkey agar[††] plates and incubated

[*]Alexandria Pharmaceuticals Co., Alexandria, Egypt.
[†]Prolaboproducts Pour Laboratoires, Rhône-Poulenc, France.
[‡]Bio-Synthesis, Inc. Lewisville; Texas, Usa.
[§]Coe-Pak[tm] (Gc America Inc., Alsip, Il, Usa).
[**]Bd Bbl™ , Becton, Dickinson And Company, Usa.
[††]Oxoid Ltd, Waderoad,Basingstoke, Hampshire, Uk.

aerobically at $37^0$C for 48 hours. The resulting isolated colonies were counted to determine the bacterial load and were subjected to further identification by Gram stain and biochemical tests.[69, 70]

**2.5 Statistical analysis of the data**[71]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.[72] Quantitative data were described using range (minimum and maximum) mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For abnormally distributed data, comparison between the different studied groups were done using Kruskal Wallis test and pair wise comparison was assessed using Mann-Whitney test. To compare between the different periods Wilcoxon signed ranks test was used. Significance of the obtained results was judged at the 5% level.

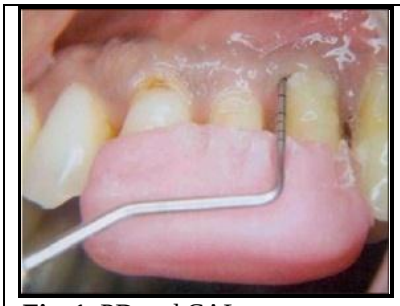

**Fig. 1**: PD and CAL measurements using Williams probe

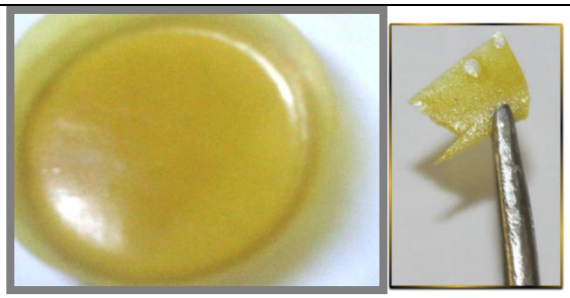

**Fig. 2**: Tetracycline films

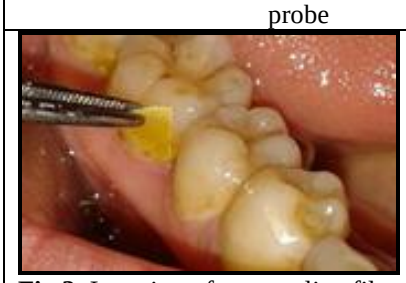

**Fig 3**: Insertion of tetracycline film portions into the periodontal pocket

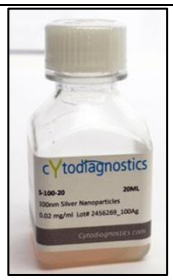


**Fig. 4**: Silver nanoparticles solution

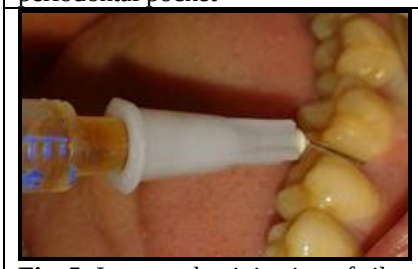

**Fig. 5**: Intra-pocket injection of silver nanoparticles

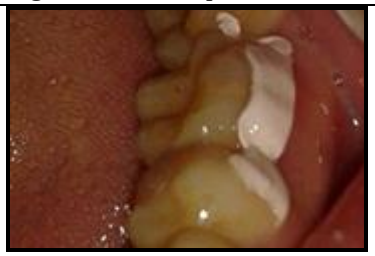

**Fig. 6**: Application of Coe-pack at the site of injection of silver nanoparticles

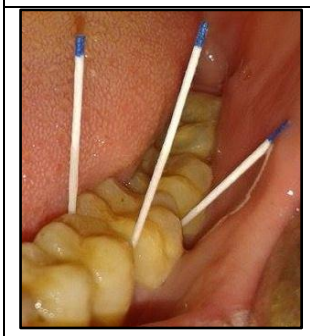

**Fig. 7**: Insertion of paper points into the pocket for GCF sampling

**Fig. 8**:Paper points in screw capped tubes containing brain heart infusion broth

## III. Results

The clinical findings at baseline, one month and three months postoperative are displayed in Tables (1 – 4) for the three treatment modalities; Group A: SRP with tetracycline film application, Group B: SRP with Ag-NPs application and Group C: SRP only (control group). At first, there was no significant difference between all treatment groups regarding the PBI, GI, PD, and CAL.

Table (1) shows the gradual reduction of PBI that occurred in all studied groups. This decrease was greater in Group A than in Group B. Group C showed the least decrease. The difference in PBI of all groups was insignificant. Similarly, a significant decrease in GI was observed in Group A after three months ($^{Z}p = 0.046$) followed by Group B, while the least amount of decrease was denoted in Group C; Table (2).

The PD of all groups showed a significant decrease after one and three months ($^{Z}p < 0.001$); Table (3). This decrease was most prevalent in Group B followed by Group A. The least amount of reduction was reported in Group C. Accordingly; Table (4) revealed more gain of attachment in Group B compared to Groups A and C.

**Table (1): Comparison between the Papillary Bleeding Index in the 48 periodontal pockets before and after the application of three treatment modalities**

| Papillary bleeding index | Group A (n = 16) | Group B (n = 16) | Group C (n = 16) | $^{KW}\chi^2$ | p |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Min. – Max. | 0.0 – 2.0 | 0.0 – 2.0 | 0.0 – 2.0 | 0.103 | 0.950 |
| Mean ± SD | 0.94 ± 0.68 | 0.94 ± 0.68 | 1.0 ± 0.63 | | |
| Median | 1.0 | 1.0 | 1.0 | | |
| **Sig. bet. groups** | $p_1 = 1.000$ , $p_2 = 0.781$ , $p_3 = 0.781$ | | | | |
| **After 1 month** | | | | | |
| Min. – Max. | 0.0 – 2.0 | 0.0 – 2.0 | 0.0 – 2.0 | 0.670 | 0.715 |
| Mean ± SD | 0.75 ± 0.68 | 0.81 ± 0.66 | 0.94 ± 0.68 | | |
| Median | 1.0 | 1.0 | 1.0 | | |
| **Sig. bet. groups** | $p_1 = 0.769$ , $p_2 = 0.428$, $p_3 = 0.598$ | | | | |
| **$^{Z}p$** | 0.405 | 0.564 | 0.317 | | |
| **After 3 months** | | | | | |
| Min. – Max. | 0.0 – 1.0 | 0.0 – 1.0 | 0.0 – 2.0 | 1.799 | 0.407 |
| Mean ± SD | 0.63 ± 0.50 | 0.63 ± 0.50 | 0.88 ± 0.62 | | |
| Median | 1.0 | 1.0 | 1.0 | | |
| **Sig. bet. groups** | $p_1 = 1.000$, $p_2 = 0.250$, $p_3 = 0.250$ | | | | |
| **$^{Z}p$** | 0.132 | 0.166 | 0.157 | | |

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

$p_1$: p value for Mann Whitney test for comparing between Group A and B

$p_2$: p value for Mann Whitney test for comparing between Group A and C

$p_3$: p value for Mann Whitney test for comparing between Group B and C

$^{Z}p$: p value for Wilcoxon signed ranks test for comparing between baseline with after 1 month and 3 months

**Table (2): Comparison between the Gingival Index in the 48 periodontal pockets before and after the application of three treatment modalities**

| Gingival index | Group A (n = 16) | Group B (n = 16) | Group C (n = 16) | $^{KW}\chi^2$ | p |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Min. – Max. | 0.0 – 1.0 | 0.0 – 1.0 | 0.0 – 1.0 | 0.164 | 0.921 |
| Mean ± SD | 0.44 ± 0.51 | 0.44 ± 0.51 | 0.50 ± 0.52 | | |
| Median | 0.0 | 0.0 | 0.50 | | |
| **Sig. bet. groups** | $p_1 = 1.000$, $p_2 = 0.727$ , $p_3 = 0.727$ | | | | |
| **After 1 month** | | | | | |
| Min. – Max. | 0.0 – 1.0 | 0.0 – 1.0 | 0.0 – 1.0 | 1.285 | 0.526 |
| Mean ± SD | 0.25 ± 0.45 | 0.31 ± 0.48 | 0.44 ± 0.51 | | |
| Median | 0.0 | 0.0 | 0.0 | | |
| **Sig. bet. groups** | $p_1 = 0.699$ , $p_2 = 0.272$ , $p_3 = 0.472$ | | | | |
| **$^{Z}p$** | 0.083 | 0.157 | 0.317 | | |
| **After 3 months** | | | | | |
| Min. – Max. | 0.0 – 1.0 | 0.0 – 1.0 | 0.0 – 1.0 | 1.446 | 0.485 |
| Mean ± SD | 0.19 ± 0.40 | 0.25 ± 0.45 | 0.38 ± 0.50 | | |
| Median | 0.0 | 0.0 | 0.0 | | |
| **Sig. bet. groups** | $p_1 = 0.674$, $p_2 = 0.246$, $p_3 = 0.453$ | | | | |
| **$^{Z}p$** | 0.046* | 0.083 | 0.157 | | |

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

$p_1$: p value for Mann Whitney test for comparing between Group A and B

$p_2$: p value for Mann Whitney test for comparing between Group A and C

$p_3$: p value for Mann Whitney test for comparing between Group B and C

$^{Z}p$: p value for Wilcoxon signed ranks test for comparing between baseline with after 1 month and 3 months

**Table (3): Comparison between the Probing Depth in the 48 periodontal pockets before and after the application of three treatment modalities**

| Probing depth | Group A (n = 16) | Group B (n = 16) | Group C (n = 16) | $^{KW}\chi^2$ | p |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Min. – Max. | 4.0 – 6.0 | 4.0 – 5.0 | 4.0 – 5.0 | 1.390 | 0.499 |
| Mean ± SD | 5.0 ± 0.73 | 4.81 ± 0.40 | 4.75 ± 0.45 | | |
| Median | 5.0 | 5.0 | 5.0 | | |
| **Sig. bet. groups** | $p_1$ = 0.419 , $p_2$ = 0.293, $p_3$ = 0.674 | | | | |
| **After 1 month** | | | | | |
| Min. – Max. | 3.0 – 5.0 | 3.0 – 4.0 | 3.0 – 4.0 | 0.553 | 0.759 |
| Mean ± SD | 3.56 ± 0.73 | 3.50 ± 0.52 | 3.63 ± 0.50 | | |
| Median | 3.0 | 3.50 | 4.0 | | |
| **Sig. bet. groups** | $p_1$ = 1.000, $p_2$ = 0.554, $p_3$ = 0.483 | | | | |
| **$^{Z}p$** | <0.001* | <0.001* | <0.001* | | |
| **After 3 months** | | | | | |
| Min. – Max. | 2.0 – 3.0 | 1.0 – 3.0 | 2.0 – 4.0 | 18.573* | <0.001* |
| Mean ± SD | 2.25 ± 0.45 | 2.19 ± 0.66 | 3.25 ± 0.68 | | |
| Median | 2.0 | 2.0 | 3.0 | | |
| **Sig. bet. groups** | $p_1$ = 0.857, $p_2$<0.001*, $p_3$<0.001* | | | | |
| **$^{Z}p$** | <0.001* | <0.001* | <0.001* | | |

$^{KW}\chi^2$: Chi square for Kruskal Wallis test
$p_1$: p value for Mann Whitney test for comparing between Group A and B
$p_2$: p value for Mann Whitney test for comparing between Group A and C
$p_3$: p value for Mann Whitney test for comparing between Group B and C
$^{Z}p$: p value for Wilcoxon signed ranks test for comparing between baseline with after 1 month and 3 months
*: Statistically significant at $p \leq 0.05$

**Table (4): Comparison between the Clinical Attachment Loss in the 48 periodontal pockets before and after the application of three treatment modalities**

| Clinical attachment loss | Group A (n = 16) | Group B (n = 16) | Group C (n = 16) | $^{KW}\chi^2$ | p |
|---|---|---|---|---|---|
| **Baseline** | | | | | |
| Min. – Max. | 2.0 – 4.0 | 2.0 – 3.0 | 2.0 – 4.0 | 0.742 | 0.690 |
| Mean ± SD | 2.88 ± 0.72 | 2.75 ± 0.45 | 2.94 ± 0.57 | | |
| Median | 3.0 | 3.0 | 3.0 | | |
| **Sig. bet. groups** | $p_1$ = 0.660 , $p_2$ = 0.748, $p_3$ = 0.338 | | | | |
| **After 1 month** | | | | | |
| Min. – Max. | 2.0 - 3.0 | 2.0 – 3.0 | 2.0 – 4.0 | 3.776 | 0.151 |
| Mean ± SD | 2.56 ± 0.51 | 2.44 ± 0.51 | 2.81 ± 0.54 | | |
| Median | 3.0 | 2.0 | 3.0 | | |
| **Sig. bet. groups** | $p_1$ = 0.486, $p_2$ = 0.204, $p_3$ = 0.059 | | | | |
| **$^{Z}p$** | 0.025* | 0.025* | 0.157 | | |
| **After 3 months** | | | | | |
| Min. – Max. | 1.0 – 3.0 | 1.0 – 3.0 | 2.0 – 3.0 | 14.021* | 0.001* |
| Mean ± SD | 2.0 ± 0.63 | 1.88 ± 0.62 | 2.69 ± 0.48 | | |
| Median | 2.0 | 2.0 | 3.0 | | |
| **Sig. bet. groups** | $p_1$ = 0.570, $p_2$ = 0.003*, $p_3$ = 0.001* | | | | |
| **$^{Z}p$** | 0.001* | 0.001* | 0.046* | | |

$^{KW}\chi^2$: Chi square for Kruskal Wallis test
$p_1$: p value for Mann Whitney test for comparing between Group A and B
$p_2$: p value for Mann Whitney test for comparing between Group A and C
$p_3$: p value for Mann Whitney test for comparing between Group B and C
$^{Z}p$: p value for Wilcoxon signed ranks test for comparing between baseline with after 1 month and 3 months
*: Statistically significant at $p \leq 0.05$

Table (5) displays the type of bacteria in the total 48 periodontal pockets before and after the application of tetracycline films in Group A, Ag-NPs in Group B and in control Group C. It is clear from this table that the application of tetracycline films reduced the percentage of the total bacterial count in periodontal pockets revealing Gram positive bacteria from 43.8% to 6.3%, and pockets revealing Gram negative bacteria from 56.3% to 31.3%; while the pockets that revealed complete elimination of bacterial infection increased from 0.0% to 62.5% (highly significant, p = 0.006). Nearly similar results were recorded in the pockets that were treated by Ag-NPs, where the percentage of Gram positive pockets decreased from 43.8% to 12.5%, and Gram negative pockets from 50.0% to 31.3%; while the completely free pockets increased from 6.3% to 56.3% (significant, p = 0.041). On the other hand, the least percentage of reduction in periodontal pockets was

observed in Group C; where the percentage of Gram positive bacteria decreased only from 18.8% to 12.5% and Gram negative bacteria from 37.5% to 31.3% (insignificant, p = 0.631). Thus, it is evident that elimination of bacterial infection in the control pockets increased only from 43.8% to 56.3%.

**Table 5: Comparison between the type of bacteria in the 48 periodontal pockets before and after the application of three treatment modalities**

| Type of bacteria | Group A (n = 16) | | Group B (n = 16) | | Group C (n = 16) | | $\chi^2$ | $^{MC}p$ |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | | |
| **Before** | | | | | | | | |
| Gram positive | 7 | 43.8 | 7 | 43.8 | 3 | 18.8 | 11.476* | 0.017* |
| Gram negative | 9 | 56.3 | 8 | 50.0 | 6 | 37.5 | | |
| Free | 0 | 0.0 | 1 | 6.3 | 7 | 43.8 | | |
| **After** | | | | | | | | |
| Gram positive | 1 | 6.3 | 2 | 12.5 | 2 | 12.5 | 0.761 | 1.000 |
| Gram negative | 5 | 31.3 | 5 | 31.3 | 5 | 31.3 | | |
| Free | 10 | 62.5 | 9 | 56.3 | 9 | 56.3 | | |
| $p_1$ | 0.006* | | 0.041* | | 0.631 | | | |

$\chi^2$: Value for Chi square
MC: Monte Carlo test
$p_1$: p value for Marginal Homogeneity test for comparing between before and after in each group
*: Statistically significant at $p \leq 0.05$

## IV. Discussion

Periodontitis is a chronic inflammation of the periodontium that results in periodontal tissue destruction and alveolar bone loss. Tissue destruction occurs as a consequence of the host's attempt to eliminate bacteria from the gingival sulcus by evoking an immuno-inflammatory response.[3,9] The main objective of periodontal therapy is to reduce the pathogenic bacterial count to the level at which the periodontal destruction is arrested.[19]

The nonsurgical periodontal treatment remains the gold standard for managing the patients with periodontitis. Matthews (2005)[73], Cobb (2008)[74] and Apatzidou *et al.* (2010)[75] postulated that the nonsurgical treatment can result in reduction of inflammation, decrease in pocket depth and gain of attachment. However, mechanical therapy may fail to eliminate the pathogenic bacteria because of their location within gingival tissues or in other areas inaccessible to periodontal instrumentation.[76] Although an additional clinical benefit of adjuvant systemic antibiotics has been described, it is only recommended in cases of refractory or aggressive periodontitis to prevent the development of antimicrobial resistance.[77] Previous studies reported that local delivery of adjunctive antimicrobial therapy is considered a safe and effective alternative to systemic administration.[78,30,28]

A nonsurgical approach using the repeated intrasulcular application of tetracycline films and Ag-NPs was used in this study. The main advantage of tetracycline films is its' ease of insertion inside the pocket and that the dimension of the films could be easily adjusted according to the size of periodontal pocket, causing no or only minimal discomfort to the patient.[79] However, the application of Ag-NPs may be an alternative modality for patients hypersensitive to tetracycline.

The PBI and GI were used to monitor the changes in gingival inflammation throughout the study. The PBI of all groups decreased throughout the study period. This decrease was greater in Group A [tetracycline film] by one month (mean of PBI = 0.75 ± 0.68), followed by Group B [Ag-NPs] (0.81 ± 0.66). After three months, both groups A and B showed similar results (0.63 ± 0.50) while in Group C [control] showed the least decrease (mean = 0.88 ± 0.62). Similar results were observed concerning the GI as it showed a decrease in all groups after one and three months. This decrease was greater in Group A by one month (mean= 0.25 ± 0.45), followed by Group B (0.31 ± 0.48). The least amount of decrease was denoted in Group C (0.44 ± 0.51). This decrease was insignificant in all groups. By three months, the decrease in Group A showed significant difference compared to baseline (mean = 0.19 ± 0.40, $^{Z}p = 0.046$). The decrease in Group B and C was insignificant 0.25 ± 0.45 and 0.38 ± 0.50 respectively.

Thus, the least amount of inflammation was denoted in Group A followed by Group B. The tetracycline films used in the present study provided a sustained release for the TcHCl which showed positive effects on reducing the inflammation. These results are in agreement with those of Sachdeva *et al*. (2011) who demonstrated a significant decrease in GI from baseline to one month (difference was 0.95 ± 0.33) and from baseline to three month (difference was 1.79 ± 0.35) in the test group treated by tetracycline films.[80] This could be attributed to the better substantivety and good binding and/or penetration into the root surfaces offered by tetracycline as a bacteriostatic antibiotic.[81] It interferes with bacterial protein synthesis and has a broad spectrum of activity inhibiting both Gram negative and Gram positive organisms.[82]

On the other hand, the reduction of inflammation in Group B could be attributed to the antibacterial activity of Ag-NPs which plays an important role in subsiding inflammation. This suggestion is in accordance with that reported by Nadworny *et al*. (2008) when they found that Ag-NPs had direct anti-inflammatory effects.[83]

Nanoparticles, owing to their small size, show high penetration capability to deep periodontal pockets which may be inaccessible to other delivery systems.[84] In addition, nanoparticles provide a uniform distribution of the active agent over an extended period of time, and thus the frequency of administration of these systems is reduced.[79]

Regarding the reduction in pocket depth and attachment gain, both PD and CAL showed significant decrease in all groups after one month ($^{Z}p < 0.001$). This could be attributed to a great extent to the resolution of inflammation. The degree of probe penetration is greatly influenced by the inflammation of the gingival tissue.[80] After three month significant difference existed between Group A and C and between Group B and C.

The high levels in attachment gain in the tetracycline group could be attributed to its bacteriostatic effect. Tetracyclines have long been considered useful adjuncts in periodontal therapy based on their antimicrobial efficacy against putative periodonto-pathogens.[42,43] Moreover, these drugs were found to inhibit collagenases and several other matrix metalloproteinases (MMPs) from cells such as neutrophils, macrophages, osteoblasts and chondrocytes in gingival tissue.[85] Tetracyclines inhibit MMPs directly by an interaction between the tetracycline molecule and metal ions within the MMP and also indirectly by inhibiting the expression of MMPs.[86]

Although no significant difference in PD or CAL existed between Group A and B; Group B revealed the lowest results (mean = 2.19 ± 0.66, 1.88 ± 0.62 for PD and CAL respectively), showing better improvement in attachment gain. The positive role of Ag-NPs in fibroblast maturation and proliferation was supported by previous studies.[87,88] Liu *et al*. (2010) stated that the presence of Ag-NPs may have a key role in the enhancement of fibroblast maturation and proliferation by providing an inflammatory free environment. [89]

In the present work, the bacteria revealed from the periodontal pockets were all facultative anaerobes that included mainly *Enterobacter cloacae*, *Escherichia coli*, followed by *Enterococcus faecalis*, *Streptococcus mutans* and *Streptococcus viridans*. In the human oral environment, *Enterobacteriaceae* have been isolated from mucosa and teeth in addition to the gingival sulcus.[90,91] Its presence in the oral cavity can be attributed to orofecal transmission, deficient oral hygiene, or contamination from food or drink.[92,93]*Enterobacteriaceae* are considered as key pathogens in some cases of refractory periodontitis.[94,95] This is due to the inadequate use of antibiotics leading to suppression of normal oral microbiota which may result in persistent colonization of the oral cavity by the opportunistic microorganisms.[96]

It is well known that Ag-NPs possess an antibacterial activity against both Gram positive and negative species.[97] According to Okafor *et al*. (2013), Ag-NPs at a concentration of 2 parts/million (2 ppm) are not cytotoxic for human healthy cells but inhibit bacterial growth.[98] In the current study the utilized concentration, 0.2 ppm of Ag-NPs was found to be lethal against most of the pathogenic bacteria revealed from the periodontal pockets.

Lansdown (2002)[99] and Castellano *et al*. (2007)[100] attributed the antibacterial activity of silver ions to its high reactivity. The bacterial cells in contact with silver take in silver ions, which thereby inhibit several functions in the cell and lead to its damage. The antimicrobial activity of silver depends upon its ions, which bind strongly to electron donor groups in biological molecules containing sulfur, oxygen or nitrogen.[101] It has also been postulated that the generation of reactive oxygen species, which are possibly produced through the inhibition of a respiratory enzyme by silver ions, may attack the cell itself.[102]

Another mode of action for silver ions is the interaction with the bacterial DNA which will prevent the cell reproduction.[103] DNA has sulfur and phosphorus as its major components. Nanoparticles can act on these soft bases and destroy DNA.[104] The interaction of the Ag-NPs with sulfur and phosphorus of DNA, can inhibit DNA replication of the bacteria and result in cell death. It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well-established fact that phosphorylation of protein substrates in bacteria influences bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of Gram-negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nanoparticles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the inhibition of growth.[105]

## V. Conclusion

Based on the results of the present study, it can be concluded that, intrasulcular injection of Ag-NPs resulted in a pronounced improvement in clinical parameters and reduction of microbial infection. Ag-NPs were as effective as local application of tetracycline films in treatment of periodontal pockets. However, more comprehensive and long-term studies that monitor clinical and microbiological activity together are also necessary.

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