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# Clinical and Radiographic Assessment of the Adjunctive Intra-Pocket Application of Triple-Layer Mucoadhesive Metformin Film in Non-Surgical Management of Chronic Periodontitis

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***Abstract:***
***Background :*** *Recent studies suggest that metformin (MF) is osteogenic.*
***Aim:*** *The assessment of the effect of a muco-adhesive, multiple layer film of MF in intra-pocket application in non-surgical management of moderate –severe chronic periodontitis.*
***Materials and Methods****: The study included 20 patients with moderate –severe chronic periodontitis. Scaling and root planing (SRP) were performed in all patients. Selected sites were randomly assigned to different treatment modalities. Group I: 10 sites managed by SRP and placebo, (control site) Group II: 10 sites managed by SRP plus metformin film (test site). Clinical parameters including site specific bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline, 3 and 6 months after treatment. Radiographic intrabony defect depth (IBD) and bone density (BD) were evaluated at baseline and 6 months post treatment.*
***Results****: Mean PD reduction and CAL gain were found to be statistically higher in test group than placebo. Moreover, a significantly higher reduction of mean IBD depth and increase of BD were observed in the MF group.*
***Conclusion:*** *The results suggest that local application of films loaded with MF is useful in non –surgical management of cases of moderate to severe chronic periodontitis.*
***Keywords:*** *chronic periodontitis, local delivery, and non-surgical periodontal therapy, Metformin.*

## I. Introduction

Chronic periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both [1].

Metformin MF (1,1-dimethylbiguanide HCl) is a drug that has been extensively used for the management of type 2 diabetes. The therapeutic effects of metformin are thought to be caused via the turning off of hepatic gluconeogenesis and may also entail improvements of insulin sensitivity in muscle and adipose tissues [2]. Reports suggested that metformin exerts other beneficial effects such as anti-endometriotic [3] antiatherogenic [4], antitumor [5], and antiobesity [6] effects. In vivo and in vitro studies suggested that MF has an osteogenic effect. MF was found to help in the development of osteoblast-like cell lines and had a direct osteogenic on osteoblasts in culture [7]. Histopathologic and micro-computed tomography findings suggested that MF may reduce inflammatory cell infiltration and alveolar bone loss in periodontal tissues [8]. MF 1% prepared as a biodegradable, controlled-release gel, as an adjunct to scaling and root planning showed encouraging results in the treatment of periodontal vertical defects in smokers [9]. In addition, a controlled clinical trial was conducted by Pradeep et al [10] to evaluate the efficacy of MF gel as local drug delivery in adjunct to scaling and root planning for the treatment of intrabony defects in patients with chronic periodontitis in comparison to placebo gel. Local delivery of MF into the periodontal pocket was found to stimulate a significant increase in probing depth reduction, clinical attachment level gain, and improved intrabony defects depth reduction compared with placebo in adjunct to scaling and root planning.

These previous studies have used carriers in gel form for the delivery of metformin. In order to overcome the drawbacks of using gels and to enhance the efficacy and duration of action, our group formulated a multiple layer muco-adhesive film of metformin. The in vitro characterization of the film and preliminary clinical evaluation were discussed in a previous paper [11].

In the present study, clinical and radiographic assessments of this mucoadhesive film in the non – surgical management of mild –severe chronic periodontitis are performed.

## II. Materials And Methods

### 2.1. Materials And Preperation Of Mucoahesive Film

Triple layer films were developed by double casting and compression methods .

Either 6% Carboxy methyl cellulose sodium (CMC) or sodium alginate (ALG) was used as the inner drug (0.6%) loaded layer. Thiolated sodium alginate (TSA; 2 or 4%) constituted the outer drug free layers to achieve a controlled drug release and enhanced muco adhesion. (Figure-1)[11-13].

### 2.1.1 Subjects

The study sample consisted of 20 subjects (9 males and 11 females), with an age range from 36-55, mean age 44.5 ± 5.7 years. They were recruited from the Department of Oral medicine, Periodontology, Oral diagnosis and Radiology, Alexandria University, Egypt. Clinical evaluation was carried out in accordance with World Medical Association Declaration of Helsinki (ethical principles for medical research involving human subjects) [14].

#### *2.1.1.1 Inclusion criteria*

All patients were diagnosed with moderate – severe chronic periodontitis based on the 1999 consensus classification of periodontal disease [15]. The nature and objectives of the study were carefully explained to each participant, and all subjects gave their informed consent of the study and the work was approved by institutional ethical committee (Faculty of Dentistry, Alexandria University).

Patients had at least 20 remaining teeth in the oral cavity. All participants had at least 2-3 inter-proximal sites in each quadrant exhibiting probing pocket depths greater than or equal to 5 mm and no more than 7 mm of the (CAL) at these sites (CAL ≤ 7mm).

#### *2.1.1.2 Exclusion criteria*

Patients having a history of any antibiotic therapy within the past 6 months, those with a history of any immune compromised condition or chronic illness like diabetes, HIV infection, or receiving radiotherapy or chemotherapy. Pregnant, lactating females and those having any hormonal disturbances were also excluded. Patients with known allergies to the biguanide group and those receiving systemic MF were excluded as well.

**The study comprised two groups:**

**Group I:** (control group): Included 10 interproximal sites that were treated by SRP and placebo.

**Group II:** (study group): Included 10 interproximal sites that were treated by scaling and root planning (SRP) and the application of 1% Metformin multiple layer film.

Patients were blindly assigned to either group by computer generated random numbers.


**Figure-1a**:
Mucoadhesive film shaped as standardized round disc

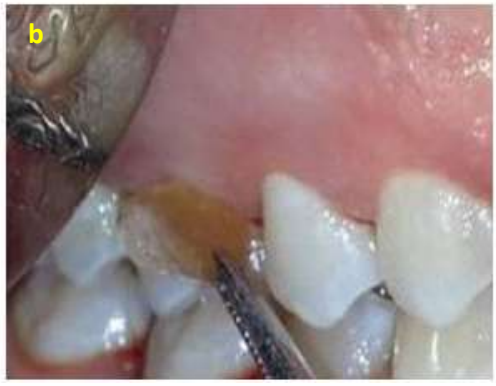


**Figure-1b**:
Placement of the metformin film in the periodontal pocket

### *2.1.2 Clinical evaluation*

Before therapy, clinical parameters were recorded. These included bleeding upon probing (BOP) [16], pocket depth (PD) [17] and clinical attachment level (CAL) [17]. A Michigan 'O' probe with William's calibrations (Hue-Friedy, Chicago, Illinois, USA) was used to take the latter measurements. Clinical parameters were measured at baseline, 3 and 6 months after therapy. Scaling and root planning were performed in all teeth. Selected sites were randomly assigned to different treatment modalities as control site (SRP and placebo only) or test site (SRP plus metformin film).

Standardized metformin films were introduced into the periodontal pockets and adhered to the adjacent root surfaces. (Figure-1b).

Patients were instructed to refrain from chewing hard or sticky foods, brushing near the treated areas, or using any interdental aids for 1 week [18]. Any adverse effects or complaints were noted at recall visits, and any supragingival deposits were removed.

### *2.1.3 Radiographic evaluation*

#### *2.1.3.1 Measurement of intrabony defect depth*

The alveolar bone was evaluated radiographically carefully for any sign of bone loss in the interproximal sites of the teeth. Standardized radiographic evaluation was performed at 6 months of treatment of MF and control group to assess change in the height at region of interest (ROI).

The vertical measurement of intrabony defect was performed from CEJ to the radiographic base of the defect (r-BOD). The depth of IBD was evaluated at baseline and 6 months [19].

The radiographic IBD depth was measured by a computer-aided software program by (image J 1.36) (Image J: National Institute of Health (http;// rsb.info.nih.gov/ij/). software as follows.

#### *2.1.3.2 Measurement of bone density*

Bone density [20,21] was measured and compared between groups at base line before treatment and 6month after treatment, by using specific image processing program (image J 1.36). The degree of blacking and whitening was expressed in number from 0-255 (gray scale level) where normal human bone reading ranged from 40 (less detectable bone) to 120 (very dense areas).

### 2.2 Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.Quantitative data were described using range (minimum and maximum), mean, standard deviation and median.

For normally distributed data, comparison between two independent populations were done using independent t-test, also paired t-test was used to analyze two paired data. Significance of the obtained results was judged at the 5% level.

## III. Results

The study sample consisted of 20 subjects (9 males and 11 females), with an age range from 36-55 mean age 44.5±5.7 years. The study included 20 inter-proximal sites that were randomly assigned to serve either as control, or test group. The control sites were managed by SRP and local sub-gingival delivery of a placebo film. The test sites were similarly managed, but 1% metformin films were delivered subgingivally in the interproximal pockets.

### 3.1 Bleeding on probing (BOP)

Prior to treatment the placebo and study groups had 75% and 80% of their allocated sites exhibiting BOP, respectively. These values were reduced after treatment to 20 and 15% in both groups. (Table-1)

### 3.2 Probing depth (PD)

Initially at baseline, the mean probing pocket depth was 6.2 ± 1.03mm in the control group and 6.4±1.07 mm in the test group.

At baseline, there was no significant difference in the mean PD between both groups, t value of test was 0.4243 and P = 0.8764.

In the control sites, a decline in the mean probing pocket depth was observed at three and six months, and the mean values were 5.00±0.82 mm, 4.40±0.52 mm, respectively.

In the MF group, at baseline examination, the average probing pocket depth was 6.4±1.07mm which decreased through out the follow up period to 4 ±1.05mm and 3.20 ± 0.92mm, respectively.

When comparing the two study groups to each other at three and six months, the MF group showed a statistically higher level of PD reduction when compared to the placebo group. Both values were significantly reduced when compared to the means of their control counter group values (P1= 0.0291*,t1 =2.3717 and P2 = 0.0020**, t2=3.600, respectively (P1 <0.001)..001). (Table-1)

### 3.3 Clinical attachment loss (CAL)

In the control group, the mean CAL was 5.50±1.07 mm at baseline, 4.40±1.07 which was further changed to 3.50 ±0.97 mm at three and six months after treatment, respectively. In the test group, the mean values of CAL declined at the different, time points. There was a decrease in mean CAL from baseline 5.40±0.95 mm to 3.50±0.97 at three months, then to 2.70±0.67 mm at six months after treatment with MF gel.

On comparing between the two treated sites , though there was no statistical significant difference between both groups at the baseline level p=1.00 and t=0.00,yet , the test group showed superiority over the control one after treatment at six months post treatment (P2= 0.0465,t2=2.1361.

At three months, however, these differences were not significant, P1=0.0652. (Table-1)

**Table-1:** Pd, Cal And Bop in control and test group at baseline, 3 and 6 months

| Parameter | Time | Control group | Test group | P value (t test) |
|---|---|---|---|---|
| PD (mm) | Baseline<br>3months<br>6months | 6.2±1.03<br>5 ±1.03<br>4.40±0.52 | 6.4 ±1.07<br>4±1.05<br>3.20±0.92 | P = 0.8764 NS<br>$P_1$ = 0.0291*<br>$P_2$ = 0.0020** |
| CAL (mm) | Baseline<br>3months<br>6months | 5.40±1.07<br>4.40±1.07<br>3.50±0.97 | 5.40± 0.97<br>3.50±0.97<br>2.70±0.67 | P = 1.00 NS<br>$P_1$ = 0.0652 NS<br>$P_2$ = 0.0465* |
| BOP % | Baseline<br>3months<br>6months | 75%<br>20 %<br>15 % | 80 %<br>25 %<br>15 % | |

NS = not statistically significant at 5 % level of significance
* = Statistically significant at 5 % level of significance
** = Highly statistically significant at 5 % level of significance

### 3.4 Radiographic results

#### *3.4.1 Intrabony defect depth (IBD)*

In the control group, the mean bone loss was 4.80±1.14 mm at baseline before treatment and 4.40 ±1.17 mm, 6 months after treatment.

Although there was some reduction of the mean IBD, this reduction was not statistically significant (p=0.4486, t=0.7746)

On the other hand, in the test group, statistical analysis showed that there was a significant decrease in mean bone loss from baseline values of 4.40±0.92 mm to 3.20-±0.92 mm, six months after treatment with MF P= 0.0107.

On comparing between the two groups, there was no statistical significant difference between both groups at the baseline level, yet after 6 months of treatment, the test group showed a statistical superiority over the control one (p = 0.0203, t = 2.545) (Table-2)

**Table-2:** IBD depth and bone density in control and test group at baseline and six months.

| Parameter | Time | Control group | Test group | P-value |
|---|---|---|---|---|
| Intrabony defect depth (IBD) mm. | Baseline<br>6 months | 4.80 ± 1.14<br>4.40 ±1.17 | 4.40±0.97<br>3.20 ± 0.92 | P1=0.4079 NS<br>P2=0.0203 * |

#### *3.4.2 Radiographic bone density*

In the control group, at base line the bone density was 75.44 ±11.18. At 6 months, it changed to 80.09 ± 12.16, this difference was not significant.

In the test group, at base line the bone density reached 80.62 ±12.16, and then increased throughout the follow up period with statistically significant gain 106.14 ±10.09 at 6 months from application of MF.

On comparing between both groups at baseline there was no statistical significant difference, yet the test group showed superiority over the control one after treatment (p= 0.031*). Table-3

**Table-3:** Comparison between the two studied groups with respect to Bone density (BD) in pixels.

| **Bone density** | **Control group** | **Test group** | **T** | **P** |
|---|---|---|---|---|
| Baseline | 75.44 ± 11.18 | 80.62 ± 12.16 | 0.415 | 0.686 |
| 6 months | 80.09 ± 12.16 | 106.14 ± 10.09 | 3.122 | 0.031* |

**p:** p value for comparing between the two studied groups
**p1:** p value for comparing between the two before treatment at baseline and after treatment
**t:** Student t-test
***:** Statistically significant at p ≤ 0.05

## IV. Discussion

The primary goal of periodontal therapy is to arrest the inflammatory disease process. Treatment involves mechanical removal of the subgingival biofilm, and the establishment of a local environment and microflora compatible with periodontal health. Parameters including clinical attachment level (CAL) and probing pocket depth (PPD) measurements, and the presence of bleeding on probing (BOP) are commonly used to assess and monitor the periodontal status. To improve periodontal health, treatment aims to reduce probing pocket depths (PPD), maintain or improve clinical attachment levels (CAL) and reduce the incidence of BOP [22].

Intra-pocket drug delivery systems are highly attractive systems due to the following advantages: less unwanted side effects, superior worth and improved patient compliance [23]. Moreover, the charm of treating periodontal diseases by localized drug delivery systems is based on the fact that they provide higher accessibility to the oral cavity and deliver drugs concentration at the targeted sites for extended periods of time to generate the wanted clinical benefits [24].

Various chemotherapeutic agents were studied for localized intra pocket drug delivery for the treatment of periodontal diseases. These include antiseptics, antimicrobials, host response modulating agents such as anti-inflammatory agents, growth factors and enamel derivative matrices [24].

Periodontal pockets provide natural reservoirs, which are easily accessible for the insertion of a delivery device. At the site of periodontal disease, the flow rate of gingival crevicular fluid (GCF) becomes 40 times higher than in the normal conditions [25]. The GCF provides a leaching medium for the release of the drug from its delivery form, for distribution through -out the pocket. These features, together with the fact that periodontal diseases are localized to the immediate environment of the pocket, make the later an ideal site for treatment with local delivery systems [23].

Biodegradable and biocompatible polymers of natural, synthetic or semi-synthetic origin are used for development of intra-pocket drug delivery devices to meet the pharmacological and biological obligations [23].

In the current study, we chose a synthetic biodegradable carrier made of carboxymethylcellulose sodium (CMC) and sodium alginate (ALG). The advantages of these materials include availability, biocompatibility, ease of manipulation and compatibility with the active ingredient, in this case metformin.

Thiolated sodium alginate (TSA) has enhanced muco adhesive properties. In the current work it was noticed that MF film had the ability to solidify once introduced subgingivally and underwent slow dissolution, allowing the controlled release of metformin, providing a prolonged release, in situ. These properties are fortified by previous studies that indicated that 1 %MF when applied as an intra-pocket gel, peaked at 2 hours after application in the GCF. The drug was retained in the target compartment for a long period, suggesting a controlled release of the drug until 4 weeks [10].

The concentration of metformin used in the present study is similar to that applied previous ones. Furthermore, Pradeep et al 10 have shown this concentration to be superior to 0.5%, and 1.5% concentrations providing maximum clinical benefits.

All previous studies have used carriers in gel form for the delivery of metformin [9,10]. Although easy to apply, gels may prove to be more likely to be washed off from the pocket than slowly resorbable films, thereby reducing the accuracy of the delivered dose. The use of film form for drug delivery proved to be easier to use, more accurate and patient friendly. Once introduced into the pocket the device underwent quick adhesion to the tissues. Moreover, the round edges of the film added to the patients' comfort upon intra-pocket insertion.

The recruited patients were categorized into two main groups (control and test) and the clinical parameters in both groups were evaluated before and after treatment, following the scheduled protocol. Ethical considerations preclude the use of untreated control groups in clinical trials. As a result, the aim of current study was to compare the method under consideration with the standard treatment of mechanical infection control in the form of SRP with the test group where additional MF gel was delivered in the pockets.

Our findings indicated that non-surgical periodontal therapy (SRP) in combination with a MF gel, provided significant improvements in clinical parameters (BOP, PPD and CAL) for MF group throughout the follow up period.

Regarding PPD, there was a progressive decline in the values for probing pocket depths in both groups. The baseline means of PPD were 6.2±1.03 mm in the control group and 6.4±1.07 mm in the MF group. After six month follow-up, the values had decreased to 4.40±0.52 mm and 3.20±0.92 mm, respectively. These results indicated that PPD reduction was more in MF group. Furthermore, a mean of 3 mm is compatible with gingival and periodontal health and may be stabilized with proper maintenance and prophylactic care.

In addition, the mean CAL results showed the same trend to decrease as in PPD results. At baseline, the mean CAL was 5.40±1.07 mm in the control group and 5.40±0.97mm in the MF sites. After treatment, the final assessment revealed a gradual reduction in the mean CAL values to reach 3.50±0.97 mm in placebo sites and 2.70±0.67 mm in SRP+ MF sites. The difference between the mean values for both parameters were statistically significant in favour of the test group.

The improvement in both PPD and CAL of the test group over the control group may be attributed that MF partly ameliorates the inflammatory response by inhibiting the production of TNF in human monocytes [25].

These findings are in are in agreement with Rao et al [9]. They reported that the use of locally delivered 1% Metformin gel in the treatment of smokers with chronic periodontitis, demonstrated a statistically significant reduction of the PPD and a significant gain in CAL after 3 and 6 months. They are also in line with previous studies by Pradeep et al [10] and Abdel Maktoub et al [26] all of whom reported a decrease in PD and attachment loss after the local administration of MF.

Regarding BOP index, 75% and 80 % of control and test sites, respectively demonstrated bleeding on probing at the beginning of examination. The final reassessment of BOP at six months demonstrated great reduction in both groups. The BOP levels fell to 15% in both groups. These results are expected due to the continuous motivation and education in plaque control that was implemented at the start and throughout the study period. An additional aspect in the test group, may be attributed to the fact the metformin exhibits an anti-inflammatory effect and produces a good hemostasis [3,28].

The results of the present study showed that bone density increased at six months when compared to base line values in the MF group. This result, however, was not evident in the placebo group. Therefore, the addition of MF may have enhanced bone formation in the former group.

Regarding change in bone height, IBD measurements were made from a fixed point which was usually the radiographic CEJ to the radiographic base of the defect the difference between the pre - and post- treatment values were defined as the radiographic bone fill. This method, reported by Joly et al, [21] proved to be an acceptable and valid mean to measure bone fill and change in defect depth.

Previous experiments have been performed on biologic transport of MF in osteoblasts to verify the feasibility of local drug delivery in vitro and found that the osteoblasts can uptake MF [20]. MF was found to significantly decrease intracellular reactive oxygen species and apoptosis and also had a direct osteogenic effect on osteoblasts that could be partially mediated via promotion of Runx2 and insulin-like growth factor. Therefore it may have a high impact on bone formation and remodeling [28].

## V. Conclusion

The use of this novel MF carrier system shows great potential in the non -surgical management of moderate chronic periodontitis. It might be useful to compare this form to gel one to verify whether it provides superior results, or not.

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