

# Effect of novel ora-aid intraoral dressing on salivary healing markers in smokers with tooth extraction: A randomized controlled clinical study

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**Original Article:**

**Effect of novel ora-aid intraoral dressing on salivary healing markers in smokers with tooth extraction: A randomized controlled clinical study**

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**Abstract**

**Background and Objectives:** Socket healing following tooth extraction is considered a big challenge among dentists. However, <sup>7</sup> there is no previous research testing the effects of commercial novel ora-aid dressing on the levels of salivary markers of wounds healing; Osteoprotegerin (OPG) and Hepatocyte growth factor (HGF) with relation to the smoking status among patients undergone tooth extraction. **Material and Methods:** The current study represents the first randomized, controlled, and clinical research conducted in Iraq for a period from February until May 2022. <sup>14</sup> Forty male outpatients (aged ranged from 20-60 years) visited the surgery clinic at the College of dentistry/University of Babylon/Iraq had tooth extraction. <sup>16</sup> The patients were randomly allocated into four groups; smoker patients with ora-aid applied following extraction (N=11), non-smoker patients with ora-aid applied following extraction (N=9), smoker patients without ora-aid applied (gauze only; N=11), and non-smoker patients without ora-aid applied (gauze only; N=9). Levels of preoperative and postoperative (2 weeks after extraction) salivary HGF and OPG were measured. **Results:** The results indicated that the levels of postoperative HGF were significantly greater in patients with ora-aid dressing compared to those without. Levels

of postoperative OPG were significantly reduced compared to their preoperative levels in non-smokers without ora-aid dressing. Smoking had significantly negative impacts on the levels of preoperative HGF. **Conclusions:** The study concludes that commercial ora-aid attachable dressing can be safely applied on the fresh socket after tooth extraction and it can accelerate the healing of intraoral wounds by significantly elevation healing salivary markers (HGFI) and maintaining the levels of other healing marker (OPG).

**Keywords:** Healing, ora-aid dressing, tooth extraction, salivary OPG, HGF.

<b>Abbreviations:</b>	<b>Full names</b>	<b>Abbreviations:</b>	<b>Full names</b>
OPG	Osteoprotegerin	SE	standard errors
HGF	Hepatocyte growth factor	SD	standard deviation
GLM	General linear model	RANK	nuclear factor receptor activator

## Introduction

Extraction of tooth is one of the prevalent actions made by maxillofacial surgeon and the healing of wounds following extraction is given special interests among dentists [1]. Wound healing refers to the physiological changes that aiming to restore the functions and integrity of both soft and hard tissues following extraction [2]. Similar to other parts of body wounds, Healing of oral socket includes sort of cellular changes starting with vascular alterations, inflammatory responses and endings with the restoration of the mislaid tissues [3]. In this regard, the interaction among secreted cytokines and growth factors at the site of healing is crucial for optimum cure [4].

Repairing of bone following extraction is initiated by activation of different cellular molecules and precursor cells for bone formation. It has been found that the integration between osteoprotegerin (OPG) and the ligand of the nuclear factor receptor activator B (RANKL) is a corner stone for osteoclast bone resorption and osteoblast bone formation [5]. OPG is a protein produced by osteoblastic cells and competes with RANK for RANKL binding. The physiological impacts of OPG on bone cells are shown by

<sup>8</sup> inhibition the terminal stages of osteoclast differentiation, activation of matrix osteoclasts suppression and accelerating osteoclast apoptosis [6]. It has been found that the level of OPG is an important for modeling and remodeling of the alveolar socket after extraction [2, 7, 8].

<sup>6</sup> Hepatocyte growth factor (HGF) is a post translational protein produced by mesenchymal cells. It is mainly involved in the healing process by regulating different physiological processes like vascular permeability, cell migration, re-epithelialization and tissue development [9, 10, 11].

Smoking is well recognized to be a risk factor for different sorts of systematic diseases such as cardiovascular diseases and cancer [12, 13]. In addition, it has also negative impacts on oral health status [14] in which could delay the healing processes [15]. Delaying the wounds healing by smoking is traced back to the effects of nicotine in reducing the blood flow at the site of wounds and hence impairing the healing process. In addition, nicotine could also interfere with platelet aggregation, raising the occurrence of ischemia in the wound tissues. Moreover, smoking could retard many of physiological mechanisms that are vital for tissue healing such as angiogenesis, re-epithelialization and osteogenesis, fibroblast proliferation and adhesion and collagen synthesis [15].

Clinically, management of wounds healing following extraction has been improved by applying commercial dressing materials on the site of wounds. These dressing materials are usually adhesive membrane that could protect the site of wounds from being infected, contaminated by smoking exposure, and hence could accelerate healing [16]. Ora-aid surgical dressing is one of those novel commercial materials being used for potentially minimizing pain and infection and hence improving wound healing. It is manufactured in Republic of Korea and composed from adhesive outer layer that is hydrophilic and inner layer that contacts to the wound surface and thought to maintain the moisture of oral cavity [17]. There are very paucity studies regarding to the testing the effects of ora-aid attachable dressing on wound healing following extraction. These studies conducted on both human and animal models have provided equivocal outcomes with two studies done on human indicated there were no significant effects of using this attachable dressing on pain scores, bleeding, and healing of socket [2, 18]. While only one recent human study indicated that post-operative bleeding was significantly reduced among patients with ora-

aid dressing paralleled with significantly greater levels of healing index [19]. Another study using Rabbit's model suggested that applying of ora-aid enhanced the closure of oral wounds in Rabbits exposed to the mechanical and chemical oral burn [20]. However, these limited studies have only focused on the morphological parameters with relation to the wounds healing. To our knowledge, there is no previous study concerning the assessment of ora-aid's effects on pre and postoperative levels of salivary wounds healing markers (OPG and HGF) following tooth extraction among smoker and non-smoker patients.

### **Patients and Methods:**

**Site and participants of the study:** This randomized, controlled, and clinical study was conducted at the Department of Oral and Maxillofacial Surgery/College of Babylon dentistry/Iraq for a period from February until May 2022. Forty male outpatients (aged ranged from 20-60 years) who needed a removal of their tooth, were randomly allocated into four groups; smoker patients with ora-aid applied following extraction (N=11), non-smoker patients with ora-aid applied following extraction (N=9), smoker patients without ora-aid applied (gauze only; N=11), and non-smoker patients without ora-aid applied (gauze only; N=9). The procedure of this study was explained to the participants and formal consent was firstly obtained from all enrolled participants. Females and patients with systematic and bleeding diseases were excluded from this study. All procedures used in the current study were performed according to the regulations issued by the Helsinki declaration and followed by approval from the ethical committee of the College of Dentistry /University of Babylon.

**Applying ora-aid dressing:** The applied commercial ora-aid attachable dressing was purchased from TBM Corporation, Republic of Korea. Based on the instructions mentioned by the manufacturer, ora-aid comprises from two layers; Outer layer with lipophilic properties that prevent the inner layer from being quickly dissolved and inner layer with hydrophilic properties that react with mucosa layer of oral cavity and converting it into a gelly state and hence absorbing wound exudate and micro bleeding. Once the ora-aid applied on the fresh socket, the inner layer reacted with oral mucosa and the outer



layer automatically eliminated within six to eight hours. Tooth extraction and ora-aid applying were done by a specialist maxillofacial surgeon (Figure 1-2).

**Measurements of salivary Hepatocyte growth factor and Osteoprotegerin:** Two samples of unstimulated saliva (2 ml) were collected from each patient. The first sample was collected at the day of extraction (Preoperative sample) and the other sample was collected 2 weeks following extraction (Postoperative sample). The saliva collection was performed between 10:00-12:00 am. The collected saliva was centrifuged at 3000 RPM for five minutes and the supernatant was then kept in -20°C for analysis of healing markers. Levels of salivary healing marker HGF were evaluated by ELISA kit purchased from (BT LAB, Shanghai Crystal Day Biotech, Shanghai, China). Double-antibody Sandwich technique was used to evaluate the levels of salivary HGF and we followed the procedures previously mentioned [21].

Levels of salivary healing marker OPG were evaluated using ELISA kit purchased from (Elabscience. Houston, Texas, 77079, USA). This kit uses the Sandwich technique for measuring the levels of salivary OPG as previously described [22].

**Follow up Instructions:** Patients were prescribed analgesics (only three days) and chlorhexidine mouthwash (2 weeks) and advised to have only cold and soft foods coupled with maintaining oral hygiene.

**Statistical Analysis:** Prior to analyze the collected data, normality test was applied to check for normal distributed data. Paired t-test was used to explore the significant differences in each variable for pre- and post-operative levels. General linear model (GLM) was also applied for testing the impacts of ora-aid dressing, and smoking on the pre and post-operative levels of measured variables, separately. Results were illustrated as mean±, standard errors (SE) and standard deviation (SD). P value with  $\leq 0.05$  was indicated significant. Minitab (Version 17; Minitab Inc., State College, PA, USA) software was performed to analyze the data.

### Results:

Regardless of smoking status, levels of salivary postoperative HGF and POG were not significantly different in comparing with their preoperative levels among patients with ora-aid addressing (Table 1, 2).

Same picture was also found in terms of the levels of postoperative HGF and POG compared to the preoperative levels among smoker patients without ora-aid dressing (Table 3). However, non-smoker patients without ora-aid dressing have experienced significantly low levels of salivary postoperative OPG compared to those preoperative levels (Table 4).

On the other hand, patients with ora-aid dressing had greater levels of postoperative HGF compared to those patients without dressing and the difference was close to be significant ( $P=0.06$ ) (Table 5), while the levels of postoperative OPG were not significantly changed among groups (Table 6). Levels of preoperative HGF and OPG also were not significantly influenced by dressing status (Table 7, 8).

Testing the effect of smoking status on the postoperative levels of HGF and OPG indicated that the levels of both postoperative HGF and OPG were not significantly influenced by smoking status (Table 5, 6). However, smoker patients had significantly reduced their preoperative levels of salivary HGF compared to the findings in the non-smokers, while this difference was absent in the preoperative levels of OPG (Table 7, 8).

#### **Discussion:**

The current study is considered the first for examining the effects of commercial ora-aid attachable dressing on the levels of salivary tissue healing markers (OPG and HGF) with relation to the smoking status among patients who had tooth extraction.

Regardless of smoking status, applying ora-aid attachable dressing had no significant effects on the levels of salivary tissue healing (OPG and HGF) in the pre-operative levels compared to the post-operative levels ( $P>0.05$ ). However, levels of post-operative HGF (2 weeks after extraction) were greater among patients who used ora-aid dressing following tooth extraction compared to those who had no dressing and the  $p$  value was near to be significant ( $P=0.06$ ). This result could highlight the favor of using this attachable dressing after extraction in accelerating the healing of oral wounds. Despite the fact that there is no previous study done to compare our results with, greater level of post-operative salivary tissue healing (HGF) among patients who had ora-aid dressing following extraction could support the finding of a previous research on the role of this dressing in healing process. Applying ora-aid dressing in Iraqi patients who undergone the tooth

extraction had significantly reduced the incidence of dry socket (measured 4 days after extraction) compared to the incidence rate seen in the patients without ora-aid dressing [19]. The latter findings were explained due to the potential effects of dressing on the blood clot in the wound socket through the mucoadhesion properties of its inner layer and hence promote healing process [19, 23]. Therefore, current findings could suggest that the socket healing can be promoted by applying the ora-aid dressing through the elevation of salivary tissue healing marker (HGF). HGF is a cytokine that is mainly involved in different cellular functions like repairing of wounds and tissue regeneration [24, 25]. It is activated by varieties of host stimuli such as bacteria and inflammatory cytokines, HGF is thought to aid the healing of wounds through the mesenchymal epithelial contacts [26]. Another interesting outcome of current study is that non-smoker patients who never applied the ora-aid dressing had significantly reduced their salivary OPG postoperative levels compared to the preoperative levels, which in turn it could delay the healing process. This latter finding could indicate the benefit of using the ora-aid dressing following extraction for maintaining the levels of salivary postoperative OPG comparing to the preoperative levels. OPG is one of the osteoclastogenesis regulatory molecules that play a crucial role for bone remodeling [27].

In terms of smoking effects, smoker patients with (having ora-aid and not) had significantly reduced their salivary preoperative levels of HGF. This result was corroborated with previous research indicated that salivary HGF was significantly decreased among smokers [28]. This negative impact of smoking on the HGF could be resulted from its potential effects on the HGF secretion by mesenchymal cells [29]. Smoking is well documented as a risk factor for onset and progression in many of dental diseases as well as bone loss [30].

Nevertheless, the current study has some limitations in which having more sample size of patients may be essential to infer promising outcomes on the role of commercial ora-aid dressing on tissue healing markers (OPG, and HGF). In addition, the selection of smoker patients may have not been ideal as the degree of smoking severity could have changed the current outcomes.



**Conclusions:**

Commercial novel ora-aid attachable dressing can be safely applied on the fresh socket after tooth extraction. Patients with this dressing will accelerate the healing of intraoral wounds by significantly elevation the levels of salivary healing markers (HGFI) and maintaining the levels of other healing marker (OPG). Patients also did not complain from serious complications in using the Ora-aid dressing.

**Conflicts of interest:**

There are no conflicts of interest

**Author contributions:** A. Al Jothery and A. Al Agele designed the study. A. Al Agele and H. Al-essa had done the tooth extraction and applied the ora-aid intraoral dressing following extraction. A. Al Jothery performed the lab work and analyzed the collected data. A. Al Jothery wrote the manuscript and it's revised and approved by all co-authors.

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**Tables:****Table 1: The statistical comparisons between postoperative levels of salivary (HGF and OPG) and their preoperative levels among non-smoker patients with ora-aid dressing**

Biomarker	Sample size	Mean	SE	ST dev.	T-test	P-value
Preoperative HGF	N=9	2019	465	1396	0.96	0.363
Postoperative HGF	N=9	1612	251	754		
Preoperative OPG	N=9	4.749	0.39	1.17	1.44	0.188
Postoperative OPG	N=9	3.969	0.557	1.67		

**Table 2: The statistical comparisons between postoperative levels of salivary (HGF and OPG) and their preoperative levels among smoker patients with ora-aid dressing**

Biomarker	Sample size	Mean	SE	ST dev.	T-test	P-value
Preoperative HGF	N=11	1048	159	528	-0.63	0.546
Postoperative HGF	N=11	1249	211	699		
Preoperative OPG	N=11	3.341	0.474	1.17	-0.03	0.977
Postoperative OPG	N=11	3.36	0.557	0.447		

**Table 3: The statistical comparisons between postoperative levels of salivary (HGF and OPG) and their preoperative levels among smoker patients without ora-aid dressing**

Biomarker	Sample size	Mean	SE	ST dev.	T-test	P-value
Preoperative HGF	N=11	941	209	661	-0.09	0.932
Postoperative HGF	N=11	959	149	472		
Preoperative OPG	N=11	3.097	0.518	1.637	-0.77	0.46
Postoperative OPG	N=11	3.519	0.475	1.503		



**Table 4: The statistical comparisons between postoperative levels of salivary (HGF and OPG) and their preoperative levels among non-smoker patients without ora-aid dressing**

Biomarker	Sample size	Mean	SE	ST dev.	T-test	P-value
Preoperative HGF	N=9	1239	185	556	1.24	0.251
Postoperative HGF	N=9	1121	219	658		
Preoperative OPG	N=9	3.804	0.692	2.077	2.46	0.039*
Postoperative OPG	N=9	3.015	0.66	1.98		

\*Significant

**Table 5: Effects of ora-aid dressing and smoking on the postoperative salivary HGF among groups**

Group	Mean±SE	Effects of smoking status	Effects of dressing
Non-smokers-with ora-aid	1612±251	<i>P</i> =0.321	<i>P</i> =0.06
Non-smokers-without ora aid	1121±219		
Smokers-with ora-aid	1249 ±211		
Smokers-without ora-aid	959 ±149		

**Table 6: Effects of ora-aid dressing and smoking on the postoperative salivary OPG among groups**

Group	Mean±SE	Effects of smoking status	Effects of dressing
Non-smokers-with ora-aid	3.969±0.557	<i>P</i> =0.288	<i>P</i> =0.396
Non-smokers-without ora-aid	3.015±0.660		
Smokers-with ora-aid	3.360±0.447		
Smokers-without ora-aid	3.519±0.475		

**Table 7: Effects of ora-aid dressing and smoking on the preoperative salivary HGF among groups**

Group	Mean±SE	Effects of smoking status	Effects of dressing
Non-smokers-with ora-aid	2019±465	<i>P</i> =0.05* sig.	<i>P</i> =0.140
Non-smokers-without ora-aid	1239±185		
Smokers-with ora-aid	1048±159		
Smokers-without ora-aid	941±209		

Table 8: Effects of ora-aid dressing and smoking on the preoperative salivary OPG among groups

Group	Mean±SE	Effects of smoking status	Effects of dressing
Non-smokers-with ora-aid	4.749±0.390	<i>P</i> =0.154	<i>P</i> =0.313
Non-smokers-without ora-aid	3.804±0.692		
Smokers-with ora-aid	3.341±0.474		
Smokers-without ora-aid	3.097±0.518		

## Figures



Figure 1: Commercial novel ora-aid attachable dressing manufactured by TBM Corporation, Republic of Korea



Figure 2: Applying the ora –aid attachable intraoral dressing on fresh socket after extraction