Nano-emulsion complex (propolis and vitamin C) promotes wound healing in the oral mucosa

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ABSTRACT

Introduction: NBF gingival gel (NBF GINGIVAL GEL*) is highly functional nano-bio fusion gel, created for the first time with a new technology called Nano-Bio fusion which is a combination of two cutting-edge technologies (on the one hand - nano-technology, and on the other side – medical Biotechnology).

Aim: To analyze and evaluate the effects of the local use of nano-bio fusion gel in healing process of soft tissue wounds in the oral cavity and to determine the reliability and correlation between the performance of two types of the biocompatible gel compared to the negative(placebo) group where is applied a local therapeutic agent.

Material and methods: A randomized, prospective double blinded study was performed in 90 patients (male 43 and female 47) in 2 years period (2015-2017) at the Private Dental Office in Skopje, Macedonia in a group of 19-65 years old. All patients were divided and analyzed into three groups and a sample size was not calculated. Thirty patients were included in each group. The anti-inflammatory activity and healing effects of this gel was evaluated by determination of wound healing parameters. We used the healing index score of Landry, Turnbull and Howley and index of Galli to describe the extent of patient healing after oral surgery. For all oral surgical procedures, the assessments were carried out in the same manner.

Results: It was shown that the propagation of the nano gel was dependent on the wound healing stages. The results showed significant differences in the score healing index and pain reduction when the nano gel treatment was compared to the placebo group. There were statistically significant differences for wound healing, assessed clinically by the two blinded operators or on photographs evaluated by a blinded and independent outcomes assessor.

Conclusions: Nano-emulsion containing nano vitamin C, E and propolis gives good effects to patient having oral surgical procedure.

Keywords: NBF gel, oral surgery, surgical wound, vitamin C, propolis.


INTRODUCTION

In the last 15 years the word Nanotechnology has gained prominence in almost every sphere of life¹². Increased production of free radicals, including reactive oxidative species (ROS), can lead to a misbalance between oxidants and antioxidants, which in turn leads to oxidative stress in the body¹³. Oxidative stress can be systematic and may affect the whole body or may be localized, for example: in the oral soft tissues⁴.

Oral cells are very sensitive to damage from free radicals because the mucous membrane allows rapid absorption across their surfaces⁵. In the oral tissues, infection can generate oxidative stress in the same way as it may do nicotine, alcohol, hydrogen. The increased level of free radicals leads to further cell and tissues damage. Application of topical antioxidants is an area of much interest in medical science today¹⁰.

Antioxidants may have beneficial effects on regulating fibroblast proliferation during gingival healing or periodontal repair. Nano Bio Fusion (NBF) gingival gel is the first product that contains nano-antioxidants and it is produced using nano-bio fusion technology. It is gel for topical use characterized with instantly absorption in the gums and all other oral tissues. It’s a gel type...
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The main ingredients of propolis are flavonoids. Flavonoids possess antibacterial, antifungal, antiviral, anti-inflammatory and potent antioxidant properties. It is known that they possess very strong antioxidant properties. Vitamin C stimulates the activation of phagocytes and thus it plays a direct role in the body’s defense. Vitamin C is a cofactor at least eight enzymatic reactions that are involved in collagen synthesis and thus directly participate in wounds healing and prevent bleeding from capillaries. Vitamin E is a naturally occurring proven powerful antioxidant which works in synergy with vitamin C in reepithelialization of the mucosa. It plays an important role in maintaining the integrity of the cell membrane and shortens the process of epitelialization.

We can explain with the mode of action when propolis (adhesiveness) and nano-emulsion (sodium ascorbyl phosphate and magnesium ascorbyl phosphate) create a nano-bioactive protective film. The antioxidants reduce free radicals, which in addition to bacteria build up are contributing factors in gum inflammation. The use of the topical antioxidants today represents one of the main topics in a medical science. Scientific research and clinical trials has shown that topical application of antioxidants can reduce the skin damage caused by free radicals.

The nano particles surpass this problem due to their ultra-fine size. They are much more efficient in penetrating the cells rapidly than their regular sized counterparts. This way the gel allows nourishment, rejuvenation, soothing and protection of the mouth tissue by increased (rapid) absorption in the gingiva and intraoral soft tissues (thanks to the nanotechnology, these substances enter the cells more easily and rapidly, and their effects are therefore exerted more rapidly).

**Aim:**
To analyze and evaluate the effects of the local use of nano-bio fusion (nbf) gel in healing process of soft tissue wounds in surgical procedures.

**Material and methods:**
A randomized, prospective double blinded study was performed in 90 patients (male 43 and female 47) in 2 years period (2015-2017) at the Private Dental Office in Skopje, Macedonia in a group of 19-65 years old. All patients were divided and analyzed into three groups and a sample size was not calculated. Thirty patients were included in each group.

The inclusion criteria are: surgical removal of benign tumor changes in oral cavity, preprosthetic surgery, extraction of impacted teeth, and frenulectomy. Smokers (under and above 10 cigarette per day) and non-smokers were also included in this study.

The exclusion criteria were immunocompromised patients, hematological malignancies or solid tumors, acute odontogenic infections, chronic neck lymph node swelling, dental of face trauma injury, orthognatic or cleft lip and palate surgery, cancer of oral cavity, chronic and aggressive local or generalized periodontitis, patients with dental implants, subject with chronic renal disease or myocardial infarction.

After performed surgery under local anesthesia (0.5 mL of 2% lidocaine containing 1:80,000 epinephrine in the upper and lower jawbone and 0.5 mL of 2% of epinephrine additive–free lidocain) and suturing, patients were randomized to receive the biocompatible gel Type 1 (original NBF gingival gel), biocompatible gel Type II (placebo gel), biocompatible gel Type III (without using the gel) in healing process of soft tissue wounds in surgical procedures.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>The use of antibiotics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - using the original gel</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
</tr>
<tr>
<td>%</td>
<td>40,0%</td>
<td>60,0%</td>
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<tr>
<td></td>
<td>100,0%</td>
<td></td>
</tr>
<tr>
<td>II - using the placebo gel</td>
<td>Yes</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
</tr>
<tr>
<td>%</td>
<td>43,3%</td>
<td>56,7%</td>
</tr>
<tr>
<td></td>
<td>100,0%</td>
<td></td>
</tr>
<tr>
<td>III - without using the gel</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
</tr>
<tr>
<td>%</td>
<td>36,7%</td>
<td>63,3%</td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
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<td>36</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>90</td>
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<tr>
<td>%</td>
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<tr>
<td></td>
<td>100,0%</td>
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</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Amount of cigarettes</th>
<th>DAY THREE Landry index</th>
<th>DAY THREE Index by Galli</th>
<th>DAY SEVEN Landry index</th>
<th>DAY SEVEN Index by Galli</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10</td>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>2,00</td>
<td>1,00</td>
<td>4,50</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>3,00</td>
<td>2,00</td>
<td>5,00</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td>2,0000</td>
<td>2,0000</td>
<td>5,0000</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>N</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>1,00</td>
<td>1,50</td>
<td>3,00</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>2,00</td>
<td>3,00</td>
<td>5,00</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td>2,0000</td>
<td>2,0000</td>
<td>4,0000</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>1,00</td>
<td>1,00</td>
<td>3,00</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>3,00</td>
<td>3,00</td>
<td>5,00</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td>2,0000</td>
<td>2,0000</td>
<td>4,0000</td>
</tr>
</tbody>
</table>

U=13,50 p=0,059 U=15,50 p=0,095 U=3,50 p=0,002 U=18,00 p=0,173
2 (Propolis and Vitamin C + E less) or placebo treatment (Figure 1 and 2). The nano-emulsion gel was applied 3 times daily in a thin film enough to cover the wound with a gentle massage for a period of 1 minute. Proper single-dose first-generation cephalosporins 1 hour before surgery was administered and postoperatively for 2 to 3 days max to prevent postoperative complication (Table 1). NBF gel not contains: antibiotics, corticosteroids, alcohol, benzocain or other anesthetics, chlorhexidine, sodium lauryl sulfate, dyes, colouring agents, artificial flavors, sugar (the gel contain stevia). All patients received thorough explanations and signed a written informed consent form prior to being enrolled in the trial.

Assessment of wound healing was done clinically - three times postoperatively using two indices: modified index score of Landry, Turnbull and Howley and the index used by Galli – (using a Likert scale) postoperatively. These outcomes were recorded on the 3rd and 7th day after surgical incision.

The measurement obtained was interpreted and subjected to statistical analysis. Reproducibility was assessed by evaluating agreement between two independent surgeons using the weighted Kappa statistic. The allocation of the participants were prepared from the independent person using the MinimPy Software for sequential allocation of subjects to treatment groups in clinical trials by using the method of minimization.

**Results:**

There was no statistically significant difference in the sex ratio ($\chi^2 = 0.623, df = 2, p = 0.732$) and age group ($\chi^2 = 2.32, df = 10, p = 0.993$) among the three groups, respectively. According to the age groups and healing process a statistically significant difference does not exist at any parameter because the statistical significance is above the threshold value of 0.05. There was no statistically significant difference in the use of antibiotics among the three groups of patients, respectively ($\chi^2 = 0.278, df = 2, p = 0.870$) (Table 1).

A statistically significant difference exists in the Landry index after the third day and the index Galli after the third and seventh day (Table 2). Landry index after the third day is higher with first group (Me = 3) compared to the other group (2 = Me). Thus, according to this index, the healing was better of those who used the NBF gel. After the seventh day according to this index, there is no difference between the first and second groups. However, according to the index by Galli higher score after the third and seventh day has the placebo group (Me = 2, Me = 1) compared to the use of the gel (Me = 1, Me = 0). NBF gel showed better healing effect on the oral wounds.

In the framework of the second group, there is a statistically significant difference between smokers and non-smokers at all indices (Table 3). The Landry index is higher in non-smokers after the third and the seventh day in relation to the value of the index in smokers. Namely, this index was higher (Me = 5) with those that use ≤ 10 per day compared to those using > 10 cigarettes per day (Me = 4). The index by Galli is higher in smokers than in non-smokers after the third and the seventh day.

**Discussion:**

The unique environment and microbial commensals present in the oral cavity, makes the treatment of oral mucoperiosteal wounds difficult. Rapid dilution of drug, limits its retention in the oral cavity and thus complicates the treatment of oral mucosal diseases-25. To combat these problems only nanosize particle medicine proved to be effective in this characteristics.

To alleviate the post-operative symptoms usually analgesics and antibiotics are prescribed. These medications can be divided into oral and topical, according to their mode of administration. Common side effects of oral medications are gastrointestinal discomfort and topical medication hardly retains in oral cavity due
to highly humid intraoral environment. So the need of the hour was an effective local agent which will protect oral lesions and facilitate healing.

Nano biofusion gel containing Vitamin C, E and Propolis extract is a combination of two newer technologies (nano-technology and medical biotechnology) 20-25. Nano Vitamin C, E is already biocompatible and is known to act on immunity and antioxidants. Studies on Propolis have proved its antimicrobial and anti-inflammatory effects. Due to its nano-complex nature, nano emulsion containing multiple Vitamins and propolis extract directly gets absorbed into tissues and forms a bio-active protective film. This film protects the lesion from moist oral environment and absorbed gel contents facilitate healing because of antioxidant and anti-inflammatory effects. Positive favourable results of wound healing were obtained which is reported in this study.

There are a few studies for the usage of NBF gingival gel and propolis and its clinico-microbiological impact of wound healing. Debnath K, Chatterjee A, Priya VS24, in their study analyzed the effectiveness of locally delivered NBF technology gel as an adjunctive therapy in treatment of chronic periodontitis. They found that locally delivered NBF gel exhibited a significant improvement compared with SRP alone in chronic periodontitis.

Preliminary Report on the Effects of Propolis on Wound Healing in the Dental Pulp published by Bretz WA et al.23, since 1998 suggested that propolis maintaining a low inflammatory and microbial cell population as well as in stimulating the formation of reparative dentin.

Propolis is a natural resinous mixture produced by honeybees said Mohsin S et al.20 in their paper of anti-bacterial efficacy of a propolis based dentifrice on Mutans Streptococci colonizing the oral cavity of young patients. They concluded that Propolis dentifrice reduces in vivo microbial load in microenvironments especially against Mutans streptococci in the oral cavity of young patients.

S VK25 wrote that wax-cum-resin substance is responsible for many valuable properties in the study of propolis in dentistry and oral cancer management.

The anti-inflammatory action of propolis was seen in the manuscript of S Malhotra, V K Gupta20.

V Dodwad and BJ Kukreja26 published that propolis was found to be only marginally better than chlorhexidine in improving gingival scores.

The assessment of oral microflora exposed to 3% ethanolic extract of Brazilian green propolis was analyzed by T Morawiec et al.20. They concluded that optimal oral hygiene, supported by antiseptic topical measures (mouthwash, toothpaste, and gel), is fundamental in the prevention of alveolar wound infections. K.A. Ravi Varma Prasad21 found that NBF gel can be used as an adjunct for improving the gingival status of an individual.

Mc Lennan SV et al.21 proposed that anti-inflammatory agent Propolis improves wound healing in a rodent model of experimental diabetes and propolis can accelerate wound healing in diabetes.

On the basis of their findings, since propolis may induce significant change in healing wound parameters, it is claimed that long-term administration of propolis might have positive effect for oral cavity.

Conclusion:

These results suggested that local using of NBF gel placed over surgical procedures under local anesthesia in the oral cavity does appear to improve wound healing especially in the first three days after the surgical procedure. It gives good prevention against early complications from wound healing (like bleeding) with good healing and anti-inflammatory esthetic effects to patients having oral surgical procedure. Further trials are needed to better understand the potential role of NBF GINGIVAL GEL in dental applications including the antibacterial, anti-inflammatory effects and the reduction of pain after the surgery procedures in the oral and perioral area.

Acknowledgement:
The presented study has been carried with interdisciplinary assistance of all authors.

Ethical considerations:

In accordance with the ethical standards of experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at https://www.wma.net) initials, or hospital numbers, especially in illustrative material.

References:

857.