Nano Vitamin C in healing of oral surgery wounds: a randomised, pilot placebo-controlled, double-blind clinical trial

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Abstract

The wound healing (WH) is a part of any surgical procedures. Oxidative stress plays important roles in WH. Oral cells are very sensitive to damage from free radicals. Antioxidants can be very important weapon in the fight against inflammation. Nanotechnology produced antioxidants can surpass the moist intraoral environment, enter the cells and support healing of oral surgery wounds. Nano-Vitamin C is 110 times more potent in the synthesis of collagen from regular vitamin C and on that is eliminated its toxicity. The aim of this pilot study was to compare the efficacy of newly developed nano-vitamin C infused gel (NIG) with a placebo gels (PG) in improving of the WH after surgical incisions in the oral cavity. The hypothesis was that no difference was present in WH between the both groups. The present study is designed according to the CONSORT rules. This randomized, double blinded study was performed in ninety patients in 2 years period on the University Clinic for Maxillofacial and Reconstructive Surgery in Skopje (Macedonia) which required the following types of treatment: surgical removal of benign tumor changes, preprosthetic surgery, extraction of impacted teeth and frenulectomy. Using MinimPy software for allocation through minimisation all patients after suturing were instructed to use either NIG or PG applying three times daily. Clinical outcomes measured were: assessment of WH on third and seventh day postoperatively with Modified index score of Landry and the index used by Galli (using a Likert scale). Reproducibility was assessed by evaluating agreement between two independent operators using the weighted Kappa statistic. The sample was equalized by: gender, age use of antibiotics. There were statistically significant differences for wound healing between the groups assessed clinically by the blinded operators in the Landry index after the third day and in the index Galli after the third and seventh day. There was a substantial agreement between operators in the wound scoring. No complications or side effects encountered. NIG gel showed better healing effect on the oral wounds according to both indices.

Background and Aim

The wound healing (WH) is a part of any surgical procedures. Oxidative stress plays important roles in WH. Oral cells are very sensitive to damage from free radicals. Antioxidants can be very important weapon in the fight against inflammation. Nanotechnology produced antioxidants can surpass the moist intraoral environment, enter the cells and support healing of oral surgery wounds.

- The wound healing (WH) is a part of any surgical procedures. Oxidative stress plays important roles in WH.
- **Oxidative stress** represents a misbalance between oxidants and antioxidants in the body and, it is a result of the **increased production of free radicals**, including **REACTIVE OXIDATIVE SPECIES** (ROS). Oxidative stress **can be systematic** and to affect the whole body or to be localized, **for example:** in the **oral soft tissues**.
- **Oral cells are very sensitive** to damage from free radicals because the mucous membrane allow rapid absorption; the cells are uniquely susceptible to free radical damage because the mucus membranes allow rapid absorption of substances across their surfaces
- In the **oral tissues, infection can generate oxidative stress** in the same way as it may do nicotine, alcohol, hydrogen. The **increased levels** of free radicals **leads** to further cell and tissues **damage**.
- **Antioxidants can be very important weapon in the fight against inflammation.** Nano- technology produced antioxidants can surpass the moist intraoral environment, enter the cells and support healing of oral surgery wounds..
- **Antioxidants may have beneficial effects on regulating fibroblast proliferation during gingival healing or periodontal repair [1].**

The antioxidants reduces 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. The scientific and clinical studies show that the use of topical antioxidants **can reduce skin damage caused by free radicals**.

Our studies have demonstrated that Nano-Vitamin C is 110 times more potent in the synthesis of collagen from regular vitamin C and on that is eliminated its toxicity.

- The **Nano particles** surpass this problem due to their ultra-fine size.
- They are **much more efficient in rapidly penetrating the cells than their regular size counterparts**.
- We can expect:
 - Increased (rapid) absorption in the gingiva & intraoral soft tissues
 - Improved clinical potency.
 - Decreased toxicity (the powerful antioxidants are soluble and not bio accumulative).

- **Vitamin C** stimulates the **activation of phagocytes** and thus it play 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 [2].

a) normal vitamin c,

b) nano size vitamin C

"NANO-vitamin C" is 110 times more potent in the synthesis of collagen from regular vitamin C and on that is eliminated its toxicity

The aim of this pilot study was to compare the efficacy of newly developed nano-vitamin C infused gel (NIG) with a placebo gels (PG) which contain regular sized vitamin C and without Vit C in improving of the WH after surgical incisions in the oral cavity. The hypothesis was that no difference was present in WH between the both groups. The present study is designed according to the CONSORT rules.

He aim of this randomised controlled double blind clinical trial (RCT) was to compare the efficacy of application of GEL TYPE 1 with the GEL TYPES 2 (with Regular sized Vitamin C and without Vitamin C (control group) in improving of the wound healing after surgical incisions in the oral cavity. The null hypothesis was that no difference was present in wound healing, post-operative complications and adverse events between patients receiving gel, and the second group (those receiving the gel type 2 and the control group), against the alternative hypothesis that a difference could be found.

Methods and Materials

This **randomized, double blinded study** was performed in **ninety patients in 2 years period** on the University Clinic for Maxillofacial and Reconstructive Surgery in Skopje (Macedonia) which required the following types of treatment:

-surgical removal of benign tumor changes, preprosthetic surgery, extraction of impacted teeth and frenulectomy.

All **patients** received thorough **explanations** and signed a **written informed consent form** prior to being enrolled in the trial. Patients were recruited and treated from January 2014 to December 2015.

Patients were **divided and analyzed into three groups** . A sample size was not calculated. Thirty patients were included in each group.ONLY NON-SMOKING PATIENTS WERE ANALYZED.

After suturing patients were randomized to **receive** the biocompatible gel **Type 1**, or **Types 2** treatment.

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**.

Assessment of **wound healing clinically** - three times **postoperatively** using two indices:

1 - Modified index score of **Landry, Turnbull** and **Howley** and

2 - The index used by **Galli – (using a Likert scale)** postoperatively. These outcomes were recorded on the 3rd and 7th day after surgical incision.

Reproducibility was assessed by evaluating agreement between **two indipendent operators** 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.

- So, the randomization of this controlled study was created by minimization.

INDICES

HEALING INDEX OF LANDRY, TURNBULL AND HOWLEY	
Healing Index 1 – Very Poor: Has 2 or more of the following: (1) tissue color: == 50% of gingiva red (2) response to palpation: bleeding (3) granulation tissue: present (4) incision margin: not epithelialized, with loss of epithelium beyond incision margin (5) supuration present	Healing Index 4 – Very Good: (1) tissue color: = 25% of gingiva red (2) response to palpation: no bleeding (3) granulation tissue: none (4) incision margin: no connective tissue exposed Healing Index 5 – Excellent: (1) tissue color: all tissues pink (2) response to palpation: no bleeding (3) granulation tissue: none (4) incision margin: no connective tissue exposed
Healing Index 2 – Poor: (1) tissue color: == 50% of gingiva red (2) response to palpation: bleeding (3) granulation tissue: present (4) incision margin: not epithelialized, with connective tissue exposed	
Healing Index 3 – Good: (1) tissue color: == 25% and < 50% of gingiva red (2) response to palpation: no bleeding (3) granulation tissue: none (4) incision margin: no connective tissue exposed	

The index used by Galli (using a Likert scale)

- **0 Complete closure – no fibrin**
- **1 Complete closure – thin line of fibrin**
 - **2 Complete closure – fibrin**
- **3 Incomplete closure – dehiscence**
 - **4 Incomplete closure – necrosis**

TYPES OF USED GELS

Results

- The sample was equalized by gender structure ($\chi^2 = 0.623$, $df = 2$, $p = 0.732$). There was **no statistically significant** difference in the **sex ratio** among the three groups. **The number of men and women in all three groups included in this study was about the same.**
- The sample was equalized by age ($\chi^2 = 2.32$, $df = 10$, $p = 0.993$). There was **no statistically significant difference** in the **age structure** between the three groups.
- There was no statistically significant difference in the use of antibiotics among the three groups of patients ($\chi^2 = 0.278$, $df = 2$, $p = 0.870$).
- **Thus, examinees are uniform according to these criteria's.**
- The Kappa coefficient we used to examine the extent to which doctors who were evaluating the degree of wound healing in patients agree to assign grades –
- **The very high level of agreement between appraisers gives us the ability to count the degree of wound healing in patients to be treated like one number.**

THE DIFFERENCE IN HEALING BETWEEN THREE GROUPS

Group		DAY THREE, Landry index	DAY THREE, Index by Galli	DAY SEVEN, Landry index	DAY SEVEN, Index by Galli
		N	N	N	N
I group - using the type 1 gel	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	3,0000	1,0000	5,0000	,0000
	N	30	30	30	30
II group - using the type 2 gel	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	2,0000	2,0000	5,0000	1,0000
	N	30	30	30	30
III group - NEGATIVE group	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	2,0000	2,0000	5,0000	1,0000
	N	90	90	90	90
Total	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	3,0000	2,0000	5,0000	1,0000
		H=9,54, df=2, p=0,008	H=6,14, df=2, p=0,046	H=3,89, df=2, p=0,142	H=6,43, df=2, p=0,040

With the H test, we examined whether there is a statistically significant difference between the three groups (the group that uses the original gel, the one that uses the second gel, and the group that uses placebo gel) on the measured indices of healing. **A statistically significant difference exists in the indices:** Landry index after the third day **and the index** Galli after the third and seventh day.

Groupe		DAY THREE Landry index	DAY THREE Index by Galli	DAY SEVEN Landry index	DAY SEVEN Index by Galli
I group – type 1 gel	N	30	30	30	30
	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	3,0000	1,0000	5,0000	,0000
II group - using the Type 2 gel	N	30	30	30	30
	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	2,0000	2,0000	5,0000	1,0000
		U=294,000, p=0,015	U=305,00, p=0,020	U=352,50, p=0,084	U=313,00, p=0,026

The alignment of the first and second group on the wound healing index

The **H-test** as a **nonparametric technique** doesn't have it's own Post Hoc test, to examine the difference between each group among themselves, we used a **U test**. Therefore, **first we used the H-test to determine if there is a difference between the three groups.**

But with this analysis **we did not receive information about the difference between the groups.** In order to determine this, we used a **U-test**. The following are the results:

With the **U test**, we examined whether there is a statistically significant difference between the **first and second group** measured with the **healing index**.

A statistically significant difference exists in the Landry index after the third day and the index Galli after the third and seventh day. Landry index after the third day is higher with first group (Me = 3) compared to the other group (2 = Me).

Thus, according to Landry index, the healing was better of those who used the nano-vitamin C 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 second group has higher score **after the third and seventh day (= Me 2, Me = 1) compared to the the first group (Me = 1, Me = 0).**

=> Nano-vitamin C gel showed better healing effect on the oral wounds according to Galli index.

Group		DAY THREE Landry index	DAY THREE Index by Galli	DAY SEVEN Landry index	DAY SEVEN Index by Galli
I group - using the Type 2	N	30	30	30	30
	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	2,0000	2,0000	5,0000	1,0000
III group – NEGATIVE Control Group	N	30	30	30	30
	Min	1,00	1,00	3,00	,00
	Max	4,00	3,00	5,00	2,00
	Me	2,0000	2,0000	5,0000	1,0000
		U=424,50, p=0,686	U=428,000, p=0,723	U=445,50, p=0,941	U=441,50, p=0,889

Alignment of the first and third group according to indices of wound healing

The following are the results using **U-test** **The statistical significance was below the threshold value of 0.05, and we can conclude that the wounds of the two groups heal similarly.**

Conclusions

The new formulated gel does appear that can improve oral wound healing particularly in the first three days after suturing. It gives good prevention against early complications from wound healing. Further trials are needed to better understand the potential role of nano-vitamin C in dental applications including the antibacterial, anti-inflammatory effects, the influence of the antioxidative potential on saliva and the reduction of pain after the surgery procedures in the oral and perioral area.

References

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