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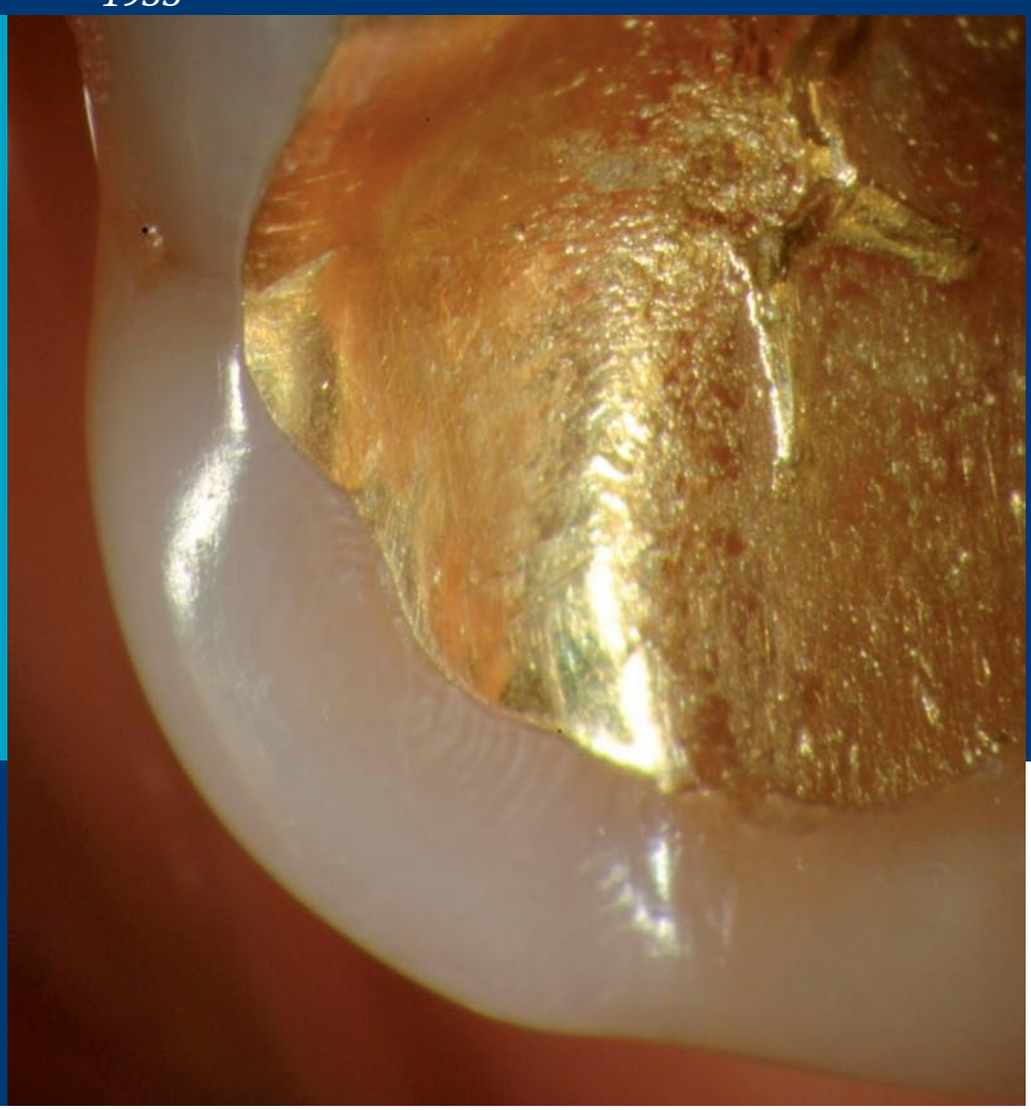

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Haemostatic effect of a bioadhesive gel based on essential oils in oral biopsies

Hemostatic effect of an essential oils-based bioadhesive gel in oral biopsies

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SUMMARY

OBJECTIVES

The general objective of this study was to evaluate the efficacy of a bioadhesive gel based on essential oils, used in patients undergoing biopsy sampling of oral lesions.

Primary outcome: hemostatic capacity. Secondary outcomes: antiseptic, pain-relieving and re-epithelializing capacities.

MATERIALS AND METHODS

This study was conducted at the Oral Medicine clinics of the Odontostomatology Unit II of the Santi Paolo e Carlo Territorial Health Authority, San Paolo Presidium (University of Milan). The ar-

The patient roll-over took place consecutively, during the course of the first visit.

After the operation, the surgical site was allowed to heal according to intention, by applying a thin layer of bioadhesive gel (Hoba- gel Plus®). Patients were instructed to repeat the application of the gel to the wound 3 times a day for 21 days.

At the end of the operation, patients were given questionnaires to be completed daily during the first week, to assess: presence of bleeding, degree of pain, intake of painkillers and any adverse effects.

Each patient was re-evaluated on the third, seventh, twenty-first and twenty-eighth days.

During the check-ups, an expert operator carried out an objective examination of the oral cavity with particular attention to the surgical site, verifying the possible presence of bleeding, signs of infection and the degree of re-epithelialization of the wound.

RESULTS

From January 2018 to July 2018, 25 patients were recruited, for a total of 32 biopsy samples. All patients were discharged with a competent doctor, without the need to resort to the use of sutures and were not

No perioperative bleeding.

There were 3 minor bleedings resolved by patients themselves. In all 3 cases, bleeding occurred during the day of surgery, following discharge. During the post-operative days, no cases of bleeding were found.

No surgical sites showed signs of infection during follow-up visits. Seven patients reported experiencing mild pain during the post-operative week, 5 of whom only on the first day. The remaining 18 patients reported total absence

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of pain. None of the 25 patients took painkillers. There were no delays in the healing of surgical wounds and no patients reported adverse effects related to the use of the bioadhesive gel.

CONCLUSIONS

The gel showed excellent hemostatic, antiseptic, antidote and antidotal re-epithelializing properties. The application of the gel has proven to be a valid alternative to the use of suture and chlorhexidine.

CLINICAL SIGNIFICANCE

The use of the gel can be particularly useful for the clinician in the case of biopsy samples carried out on mucous membranes that are difficult to suturate, such as in the case of tissues made brittle by chronic inflammatory pathologies or keratinized mucous membranes adhering to hard underlying tissues (hard palate, adherent gums). Further advantages deriving from the use of the gel are represented by the reduction of operating times, avoiding the suturing of the surgical wound, as well as by the reduction of the number of appointments, since a second meeting is not necessary for the removal of the stitches.

KEYWORDS

Oral Medicine
Oral Surgery
Oral Biopsy
Bioadhesive
Gel Essential
Oils

ABSTRACT

OBJECTIVES

The main objective of this study was to evaluate the efficacy of a bio-adhesive gel based on essen- tial oils, used in patients who re- ceived biopsies for oral mucosal lesions.

Primary outcome: to verify the he- mostatic activity of the gel. Sec- ondary outcomes: to verify anti- septic, pain- relieving and re-epi- thelizing properties of the gel.

MATERIALS AND METHODS

The present study was conducted at the Oral medicine clinics of the Azienda socio sanitaria territoriale Santi Paolo e Carlo, presidio San Paolo (Università degli studi di Mi- lano). Patients were consecutively recruited at first visit. After the surgical procedure, a thin layer of double-sided gel (Hobagel Plus®) was applied on the surgical site which healed by secondary inten- tion. Patients were instructed to repeat the application of the gel 3 times a day for 21 days. At the end of the intervention, the pa- tients received questionnaires, to be filled out daily during the first week, to evaluate presence of bleeding, degree of pain, intake of painkillers and possible adverse effects.

Each patient was assessed after 3, 7, 21 and 28 days.

During the follow-up visits, an ex- pert clinician made a careful ex- amination of the oral cavity with particular attention to the surgical wound, verifying the possible presence of bleeding, signs of in- fection and the degree of re-epi- thelialization of the wound.

RESULTS

From January 2018 to July 2018, 25 patients were recruited; 32 bi- opsies were performed. All pa- tients were discharged with a sta- ble clot, not needing sutures, and in absence of perioperative bleed- ing.

There were 3 minor wound bleedings, resolved autonomous- ly by the patients. In all 3 cases, the bleeding occurred during the same day of the intervention, soon after the patient's dis- charge. During the post- opera- tive days, no bleeding was re- ported.

No signs of in fection were re- corded during the follow-up vis- its. Seven patients experienced mild pain during the post-opera- tive week, 5 of them only on the first day. The remaining 18 pa- tients reported a total absence of pain. None of the 25 patients took painkillers. There were no delays in the healing of surgical wounds and no patient reported adverse effects related to the use of the bioadhesive gel. The gel showed excellent hemostatic, antiseptic, pain- relieving and re-epithelizing properties. There were no delays in the healing of surgical wounds, nor other ad- verse effects.

CONCLUSIONS

The gel showed excellent hemo- stat; antisept; pain-relieving and re-epithelizing properties and it could be proposed as promising alternative to the use of suture and chlorhexidine.

CLINICAL SIGNIFICANCE

The use of the gel can be particularly useful for the clinician in case of biopsy on oral mucosa difficult to be sutured, such as fibrotic tissues due to chronic in- flammatory diseases or keratinized mucosa, adherent to un- derlying hard tissues (hard pal- ate, adherent gingiva). Further advantages related to the use of the gel are the reduction of operating time, avoiding the suture of the surgical wound, and also the reduction of the number of ap- pointments, since the visit for the suture removal becomes, thus, unnecessary.

KEY WORDS

Oral medicine
Oral surgery
Oral biopsy
Bioadhesive gel
Essential oils

1. INTRODUCTION

Biopsy is a surgical procedure that consists of taking a portion of tissue in order to obtain an accurate histopathological diagnosis. The protocols used generally include, after the sample has been taken, the use of sutures and the application of topic antiseptics. Among these, chlorhexidine is the most widely used presi- dium.

Despite its proven antiseptic properties, it is not free from the onset of adverse effects, typically represented by dysgeusia, pigmentation of the hard and soft tissues of the oral cavity, burning and increased accumulation of supragingival calculus[1]. More rarely, allergic or hypersensible episodes have been reported. Furthermore, several authors have shown, in *in vitro* studies, a certain degree of cytotoxicity associated with the use of chlorhexidine, which was found on various cell types (human fibroblasts, erythrocytes, lymphocytes)[2-4] and which seemed to be exerted through different mechanisms, including the inhibition of the activity of the sodium potassium ATPase pump[4] and some matrix metalloproteins[5], exocytosis of lyso-somal enzymes[6] and increased permeability of the plasmalemma[2].

The application of stitches, in addition to being a stressor for many patients, is associated with plaque retention at the stitches themselves and at the wound margins[7].

The search for new components that can be proposed as alternatives to the use of chlorhexidine remains a challenge for the scientific community, capable of arousing ever-increasing interest. In recent decades, attention has been focused mainly on various plant-derived agents and molecules with antiseptic, anti-inflammatory, antioxidant properties and adjuvants of the healing found in essential oils[8,9]. Several authors have in fact demonstrated the antibacterial, anti-inflammatory, antifungal and antioxidant effects of extracts from plants belonging to the *Myrtaceae family* (*Leptospermum scoparium*, *Eucalyptus* spp., *Melaleuca alternifolia*). Essential oils obtained from species belonging to the *Laminaceae family* (*Thymus vulgaris* and *Mentha piperita*) have antioxidant, antifungal and soothing effects against pain of inflammatory origin. Other studies have attributed similar properties to extracts of *Commiphora myrrha*, *Glycyrrhiza glabra*, *Matricaria chamomilla*, *Anethum graveolens* and *Symphytum officinale*[10].

The present work aims to evaluate the efficacy of topical application of a bioadhesive gel based on essential oils as an alternative to the use of suture and chlorhexidine in biooptic sampling sites or oral mucous membranes.

The primary outcome was the evaluation of the hemostatic action of the gel, while the secondary outcomes were the evaluation of the antiseptic, analgesic and re-epithelializing capacity of the gel.

2. MATERIALS AND METHODS

Study design

The present work was designed as a non-controlled and non-randomized PHASE II study, aimed at evaluating the hemostatic, re-epithelial, antiseptic and analgesic capacities of a bioa- desive gel based on essential oils (Hobagel Plus®, Hobama s.r.l., Milan, Italy), applied on surgical sites where oral biopsies are located. The study was conductedat the Oral Medicine Chambers of the Odontostomatology II Unit of the Santi Paolo and Santi Paolo Territorial Social Health Authority. Carlo, San Paolo Presidium (University of Milan), in accordance with the ethical principles for biomedical research established in the Declaration of Helsinki of the *World Medical Association* and following the approval of the study by the Ethics Committee of the ASST Santi Paolo e Carlo (Identification code BIOGEL-2017). The study was carried out in two phases: a first phase, during which the surgical procedures were performed and the correct instructions for applying the gel and filling in the questionnaires were given to the patients; a second follow-up phase in which, in subsequent 4 controls (at 3, 7, 21 and 28 days), the clinical variables investigated were monitored and documented.

Patient recruitment

The study design involved recruiting 25 patients, with consecutive enrollment during the first visit.

Inclusion criteria: all adult patients who needed to perform a biopticor incisional/excisional sampling at the level of the soft tissues of the oral cavity, ≤6 mm in diameter, carried out on an outpatient basis without the need for intravenous sedation, who agreed to participate in the study and who had no contraindications to oral surgery, were considered eligible.

Exclusion criteria: all patients suffering from coagulation deficiency, uncontrolled hypertension, uncontrolled diabetes, in a state of certain/presumed pregnancy, breastfeeding or with absolute contraindications to oral surgery were excluded from the study.

After proposing to the patient participation in the clinical trial, after careful information on the various aspects and methods of carrying it out, the informed consents of the clinical study were delivered to the patient.

and the intervention (biopticor incisional/excisional sampling) and the questionnaires for the evaluation of the predetermined outcomes. Informed consent was therefore obtained for each patient in written form.

Surgery for biopsy sampling

The surgery was performed according to the normal procedures of oral surgery below: local anesthesia with mepivacaine 2% with vasoconstrictor, excision of the le- sion or removal of a portion of it; hemostasis by compression with sterile gauze soaked in saline solution and kept under pressure on the surgical site for a minimum of 60 seconds. All surgical procedures were carried out by an experienced operator, a specialist in oral medicine. Once the hemostase by compression of the site concerned was reached, a thin layer of bioadhesive gel (Hobagel Plus®) was applied topically. At the end of the operation, the patient was instructed to apply a thin layer of gel to the surgical wound 3 times a day for 3 weeks. Clinical imaging was carried outusing a digital reflex camera (Nikon D5300, Af-s 85 mm Micro Nikkor lens, Metz 15 MS-1 digital flash, Moncalieri, Turin, Italy) at the end of the surgical procedure and undergone after the gel was applied.

The patient was given the normal post-operative recommendations: maintain optimal oral hygiene at home without traumatizing the affected area, avoid spitting or rinsing for at least 24 hours, avoid hot food and drink, and engage in intense physical activity for the first 48 hours (to avoid blood pressure and secondary bleeding). Sterile gauze, a vial of sterile physiological solution and a vial of acid were given to the patient tranexamic 500 mg/5 ml, instructing him, in case of secondary bleeding, to maintain firm pressure on the area for 10 minutes with gauze soaked in physiological solution and, only in case of bleeding loss, after this procedure apply gauze soaked in tranexamic acid. Paracetamol 1000 mg tablets (maximum 3 per day) was also prescribed to be used as a painkiller in case of need.

Composition of the gel

The gel contains bisabolol, hydrogen peroxide, hydrolysed and non-hydrolysed sodium hyaluronate, hyaluronic acid oligomers, cetylpyridinium chloride, triclosan, *Thymus vulgaris essential oil*, *Mentha piperita*, *Melaleuca alternifolia*, *Commiphora myrrha*, *Leptospermum scoparium*, menthol, eucalyptol, anethole, tocopheryl acetate, allantoin and ammonium glycyrizate as active ingredients and Na/Ca mixed salt of the polyvinyl methyl ether copolymer / maleic acid, liquid paraffin, petrolate, cellulose gum and PVP (polyvinyl pyrrolidone) as excipients.

Outcome primario

Bleeding

Bleeding was assessed by the patient during the week by filling in the questionnaire and by an expert operator at the end of the surgical procedure and at the 3- and 7-day follow-up visits. The patients also reported daily on the appropriate sections of the questionnaire the possible presence of bleeding, through a dichotomous assessment (yes/no) and the possible way of controlling the bleeding. The same parameter was used by the operator to assess bleeding during follow-up visits at 3 and 7 days after surgery.

Outcome secondari

Pain

The presence and intensity of pain were assessed daily by the patient, during the first week, by filling in the appropriate section of the questionnaire, using the Visual Analogue Scale (VAS), and the possible intake of analgesics/anti-inflammatories as needed (indicating the day of intake and the amount of drug taken on that day). The VAS is a 100 mm long horizontal line contained within two vertical lines at the extremities, which correspond respectively to "absence of pain" (left extremity) and "worst conceivable pain" (right extremity). Patients were instructed to report the intensity of the pain perceived on the SEA daily and at the same time. According to Jensen et al.[11], the VAS scores were evaluated by dividing them into 4 categories: from 0 to 4 mm "no pain", from 5 to 44 mm "mild pain", from 45 to 74 mm "moderate pain", from 75 to 100 mm "severe pain".

Infection

The presence of infection was assessed and reported on a special questionnaire by the clinician during the check-ups – carried out by an expert operator 3, 7, 21 and 28 days after surgery – through the possible observation of swelling, erythema of the mucosa, presence of purulent or serum-purulent material.

Re-epithelialization

The degree of wound healing, in terms of the presence of fibrin or partial or total re-epithelialization of the surgical site, was measured by an expert operator at 7, 21 and 28 (T2, T3 and T4) days after surgery, through intraoral physical examination, with particular attention to the biopsy site.

3. RESULTS

From January 2018 to July 2018, 25 patients were recruited: 6 males and 19 females, aged between 31 and 87 years (± mean SD: 65.4 ± 14.2), of whom 21 were smokers and 4 were non-smokers. In total, 32 surgical sites were considered

In 7 patients it was necessary to take a double biotic or biotic sample toarrive at the diagnosis.

The anatomical site most affected by the samples was the gingiva (11 samples), followed by the hard palate (9), tongue (3), alveolar mucosa (3), genial mucosa, na (3), labial mucosa (2) and only 1 sample at the level of the soft palate. There were 8 excisional biopsies, while 24 incisional samples were taken **(Table I).**

In all cases, the gel showed excellent bioadhesive capabilities, remaining in place for a long time after application.

Tab. I Demographic characteristics of patients, characteristics of sampling and histopathological diagnosis

Patient	Sex	Age	Pickup site	Type of biopsy	Multiple biopsy	Histopathological diagnosis
1	Male	70	Hard palate	Engraving	-	Epithelial hyperplasia and orthokeratotic hyperkeratosis
2	Female	73	Lingual margin	Engraving	-	Lichenoid injury
3	Female	71	Mandibular adherent gingiva/ alveolar mucosa	Engraving	2	*Lichen planus*
4	Female	68	Hard palate	Engraving	2	*Lichen planus*
5	Female	85	Hard palate	Engraving	-	Leucoplachia
6	Female	71	Maxillary adherent gingiva/ alveolar mucosa	Engraving	2	Pemphigoid
7	Female	61	Soft palate	Excisional	-	Fibroma
8	Female	44	Mandibular adherent gingiva (lingual side)	Excisional	-	Papilloma squamoso
9	Female	58	Mandibular adherent gingiva/ alveolar mucosa	Engraving	2	*Lichen planus*
10	Female	56	Hard palate	Engraving	-	*Lichen planus*
11	Female	31	Mandibular free gingiva/ lower labial mucosa	Excisional	2	Traumatic pseudofibroma/traumatic pseudofibroma
12	Female	44	Lower labial mucosa	Excisional	-	Traumatic pseudofibroma
13	Female	69	Genienal mucosa	Engraving	-	*Lichen planus*
14	Male	34	Mandibular adherent gingiva (lingual side)	Excisional	-	Papilloma squamoso
15	Female	81	Hard palate/hard palate	Engraving	2	*Lichen planus*
16	Female	62	Mandibular adherent gingiva	Engraving	-	*Lichen planus*
17	Female	63	Lingual apex	Excisional	-	Giant cell fibroid
18	Male	87	Mandibular adherent gingiva/ mandibular adherent gingiva	Engraving	2	Pemphigoid
19	Female	67	Hard palate	Excisional	-	Papilloma squamoso
20	Male	75	Genienal mucosa	Engraving	-	*Lichen planus*
21	Female	71	Mandibular adherent gingiva	Engraving	-	Leucoplachia
22	Male	78	Lingual margin	Engraving	-	*Lichen planus*
23	Female	69	Genienal mucosa	Engraving	-	*Lichen planus*
24	Male	72	Hard palate	Engraving	-	Leucoplachia
25	Female	66	Mandibular adherent gingiva	Engraving	-	Macula melanotica

Outcome primario

Bleeding

The gel applied to the surgical site at the end of the procedure showed excellent hemostatic capabilities, controlling bleeding in all cases analyzed. In fact, perioperative hemostasis was always maintained through the application of the gel and no patient showed bleeding of the surgical site in the time between the application of the gel on the wound and discharge. During the first week, only 3 patients reported secondary bleeding **(Table II),** corresponding respectively to 12% of the sample, taking into account the number of patients, and to 9.4% of the sample, taking as reference the total number of surgical sites (n = 32). All episodes occurred during the same day of surgery (T0), were minimal and easily manageable by the patients themselves, following the instructions received. One case occurred about 2 hours after the surgical procedure, during the course of the surgery (patient 1 - **table II).** The patient has hurt to have eaten hot and hard food, despite the recommendations. The san- guination was resolved by tamping with gauze soaked in physiological solution left in place for 5 minutes. In patient 10, bleeding occurred during dinner, after a few hours of intense physical activity, a behavior that significantly increases the risk of this complication and is not recommended in the instructions given after surgery. Despite the slight bleeding, the patient reported having compressed the wound with gauze soaked in tranexamic acid for the management of the complication, without using physiological solution in the first instance, as suggested in the post-surgical indications. The last case of sanguinamento occurred immediately after hospital discharge and was resolved with a few seconds of rear-end collision using dry gauze (patient 24 - **table II).** In all 3 cases, the site involved in the sampling was the hard palate and the biopsy was incisional with a single sampling.

Outcome secondari

Pain

During the week following the intervention, each patient marked daily, in the specific sections of the questionnaire, the level of pain by VAS, as well as the possible intake of painkillers. The trend of pain perceived by each patient, based on the VAS of the day, is schematized in **graph 1**.

Seven patients reported mild pain: 5 only during the day of surgery (patients 3, 9, 10, 15, 25 - **graph 1**), one in the first 4 days, but only at the same time as meals (patient 2 - **graph 1**) and the last during the first 4 days (patient 5 - **graph 1**). In the latter case, the patient reported the presence of pain only with the use of the prosthesis. In this patient, in fact, 2 incisions were taken on the hard palate, in mucous areas corresponding to the prosthetic base. The remaining 18 patients reported no pain during the post-operative week.

Tab. II Characteristics and methods of control of post-operative bleeding

Patient	Bleeding		Days when bleeding occurred	Type of bleeding	Bleeding management mode
	During the week	*At the 1-week follow-up visit*			
1	Yes	No	T0 (operating day)	During lunch (the patient reports having eaten hot and hard foods)	Haemostasis obtained by tamponade for 5 min with gauze soaked in saline solution
10	Yes	No	T0 (operating day)	During dinner (the patient reports having eaten hot food and having carried out intense physical activity during the afternoon)	Haemostasis obtained by tamponade for 5 min with gauze soaked in tranexamic acid (without any previous attempt at tamponade with gauze soaked in saline)
24	Yes	No	T0 (operating day)	Immediately after discharge	Haemostasis obtained after a few seconds of tamponade with dry gauze

The values of the average VAS per day, shown in **graph 2**, show the absence of pain during all the days of the first week. In addition, none of the 25 patients used acetaminophen or other painkillers in the first week after the biopsy.

Infection

No signs of infection were observed during follow-up visits at 3, 7, 21 and 28 days after surgery.

Re-epithelialization

The intraoral examination showedexcellent levels of healing of surgical wounds at the **level of the bioptic sites (graph 3):**

- T2 (7 days after surgery), on 7 sites there was evidence of fibrin, 15 showed partial re-epithelialization and 10 complete re-epithelialization;
- T3 (at 21 days after surgery), on 1 partial re-epithelialization site and on 29 complete re-epithelialization;
- T4 (at 28 days after surgery), all observed sites showed complete summarization.

All patients enrolled in the study presented at scheduled 3- and 7-day visits (T1 and T2). One patient did not show up for the follow-up visit after 21 days (T3), 2 patients at the 28-day check-up (T4) and one patient did not show up for the check-up after 21 days or the check-up after 28 days.

For each clinical case, in addition to intra-operative photographs, photographs of the surgical site were taken at 3, 7, 21 and 28 days after sampling **(figs. 1-5).**

4. DISCUSSION

This study evaluated the hemostatic, re-epithelial, analgesic and antiseptic effectsof a highly bioadhesive gel containing several essential oils in the healing of minor surgical wounds, in particular biopsies of the oral mucosa.

In terms of maintaining haemostasis, the results were encouraging, with

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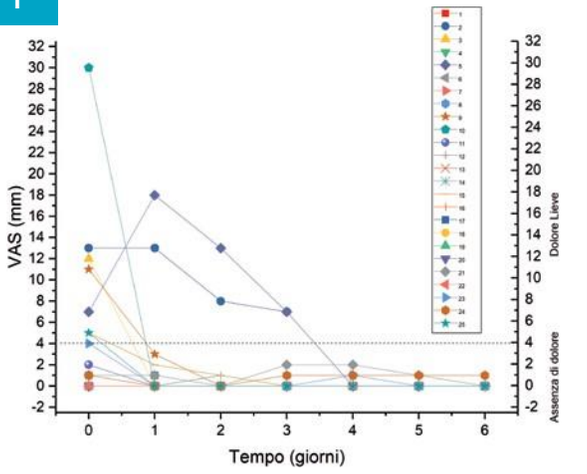

Graph 1 Punctual trend of pain for each individual patient during the First post-operative week

2

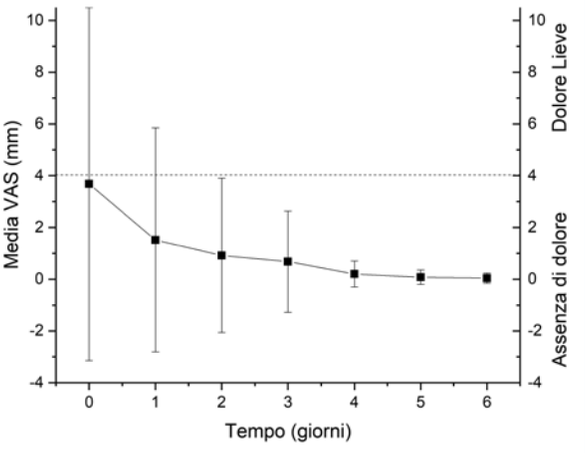

Figure 2 Distribution of average SEA per day

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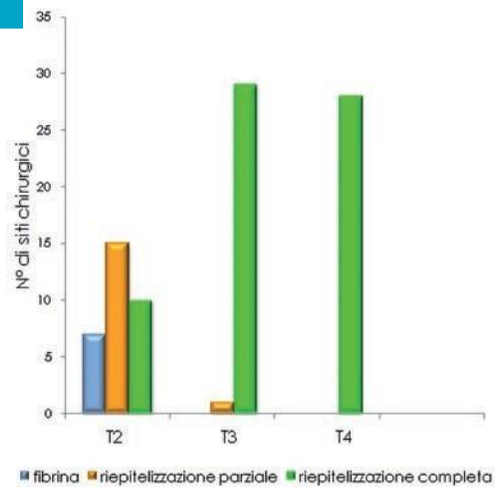

Graph 3 Trend of healing of surgical sites during controls at 7 (T2), 21 (T3) and 28 (T4) days

Figs. 1a-c Patient no. 9 - Adherent gingiva/maxillary alveolar mucosa Quadrant I: surgical wound (a), gel placement (b) and 7-day follow-up (c)

Figs. 2a-c Patient no. 2 - Left lingual margin: surgical wound (a), gel placement (b) and 7-day follow-up (c)

Figs. 3a-c Patient no. 5 - Hard palate: surgical wound (a), gel placement (b) and 7-day follow-up (c)

Figs. 4a-c Patient no. 13 - Right genienal mucosa: surgical wound (a), gel placement (b) and 7-day follow-up (c)

Figs. 5a-c Patient no. 16 - Mandibular adherent gingiva IV quadrant: surgical wound (a), gel placement (b) and 7-day follow-up (c)

3 cases of bleeding out of a total of 25 patients, of which 2 probably occurred due to failure of the patients to respect the post-operative proto-neck. In a study by Lodi et al.[12] The onset of prolonged intra-operative bleeding is reported in 9 out of 286 cases of oral biopsies, of which 3 concerned areas that could not be sutured due to the inextensibility of the tissues involved (hard palate and keratinized gingiva). Of the total sample, only 203 patients presented for the one-week check-up, of which 12 (6%) reported the onset of secondary haemorrhage, mainly found on the first day, without specifying whether the sites had been sutured or not. According to the data collected in this study, it has always been possible to achieve primary haemostasis intraoperatively and, although the rates of secondary bleeding are higher (12%) compared to the study by Lodi et al.[12], 2 of the 3 secondary bleedings are consistent with reduced *patient compliance* with post-surgical instructions. In addition, the difference in the percentage figure could be at least partly related to the smallness of the sample selected here and to the fact that the surgical sites considered have never been managed through the use of stitches.

In a 1992 paper by Eisen[13], 140 biooptic punch samplesof the oral mucous membranes without suture were analyzed. The authors report 6 cases in which the use of intra-operative diathermocoagulation was necessary for the control of hemostasis and the absence of post-operative bleeding that required prompt intervention by health personnel, although without specifying the possible presence of secondary bleeding that resolved independently or through patient intervention. The authors specify that all those patients who required biopsy samples localized from the hard palate, the only site where secondary bleeding was found in this study, were not included in the study.

Among several published scientific papers on the experience of pain following oral biopsies, Kearns et al.[14] report, out of a total of 76 VASs obtained on the first day, 40% of patients with no pain, followed by 37% with mild pain, 18% with moderate pain and, finally, 4% with intense pain. In this same work, the intake of analgesic drugs by patients in the days following the collection is also investigated, reporting an incidence of 26% (20 cases) on the day of the biopsy, with percentages progressively reducing up to 3% (2 cases) of intake on the seventh day.

In another study from 2008[15], 84 cases of oral biopsy were analyzed with a prescription of paracetamol 650 mg to be taken as needed in the days following surgery. The peakof perceived painis identified two hours after surgery, when the effect of the anesthetic topicor used in the surgical phase was lost. This study shows an average VAS score of 1.3 two hours after biopsy, which, in accordance with the interpretation mode used in this work, falls into the "mild pain" category. The authors report a significant reduction in the "pain" experience starting from the second day, although the VAS values of the days following that of the sample are not reported. The survey on the use of analgesics shows the intake of paracetamol 650 mg every 8 hours for an average duration of 2 days in 50% of cases (42 patients). The use of analgesic molecules has also been studied by Lodi and colleagues, who report the intake of painkillers in 18% of cases (out of 203 patients), mainly on the same day of sampling[12]. Comparing these data with those obtained in this study, it is interesting to note that, in this work, the average VAS per day indicates the absence of pain on all days of the week following the time of the biopsy. In addition, none of the 25 patients in the sample of this study had to resort to the use of paracetamol or other painkillers for post-operative pain control.

In terms of healing of the surgical site, the results have also proved to be encouraging. At 7 days after sampling, only 7 out of 25 sites showed only the presence of fibrin at the wound level, while in 15 sites the re-epithelialization process was underway but not completed; 10 sites were completely covered by epithelial tissue. The 3-week follow-up showed total re-epithelialization for all but 1 sites, while at 4 weeks all surgical sites were correctly healed, with the formation of mature epithelium.

In the literature, the dental discipline in which the re-summarization of surgical sites is most investigated is periodontology. In a paper by Keceli et al. [16], the re-epithelialization of the donor site (hard palate) in the surgical procedure of epithelial-con- nectival harvesting is investigated. After the operation, the patient was given a protective device for the palatine vault, personalized on the basis of previously obtained models. The authors showed the absence of re-epithelialization, even partial, at the control at 1 week, while at 21 days after the sampling, the

approximately 75% of partial re-epithelialization and about 18% of total re-epithelialization. At 4 weeks, however, the summarizations were complete for the entire sample. In this study, 7 patients underwent bioptic or hard palate sampling, for a total of 9 sites (in two cases, a double sampling was performed for diagnostic purposes related to the clinical appearance of the lesions). Four out of 9 sites (44%) were partially re-epithelized at 1 week, while at 3 weeks all sites were correctly healed, with the presence of mature epithelium overlying the entire area of the surgical wound. Although the methods of intervention are different from the survey by Keceli et al.[16] (the bioptic or bioptic samplinghas a smaller size and greater thickness than the collection for the purpose of free gingival grafting), the results obtained in this work are remarkably encouraging, suggesting a possible faster wound healing process thanks to the use of the gel. It should also be considered that most of the sample analyzed here is represented by patients suffering from oral or chronic diseases, such as *lichen planus* and pemphigoid **(tab. I),** and that therefore many surgical operations have been carried out at the level of fragile and inflamed mucous membranes that require longer healing times than physiological tissue.

During the course of the study, no adverse effects or allergic reactions were found to the gel used. Three out of 25 patients reported the onset of mild edema in the days following the sampling. In 2 of these, it was possible to subsequently make a histopathological diagnosis of oral *lichen planus*, a condition related to the onset of swelling related to the re-inflammatory displacement resulting from surgical trauma. In one of the two cases, the sensation of mild swelling was reported only on the first day, while the second case of *lichen planus* was characterized by a progressive reduction of the edema in the first two days. The third case showed the presence of oede- but only on the second and third days, located much posteriorly with respect to the bioticsite and therefore not correlated to the use of the gel or the surgical procedure. In this same patient, in the area indicated by him, during the check-up on the third day it was possible to observe a small lesion of probable traumatic origin, which completely regressed at the time of the 7-day visit. In addition, no signs of infection were detected at any site.

It is likely that the results described are attributable to the important bio-adhesive capacity of the gel associated with the synergistic action of its active components. The bioadhesive vehicle of the gel, in addition to having demonstrated an excellent haemostatic capacity, may have acted as a barrier on the surgical wound, protecting it from external stimuli, reducing post-operative pain and assisting the analgesic action of the active components present in the formulation. The anti-inflammatory and antimicrobial properties of the essential oils contained in the formulation of the gel[10] used in the study may, on the other hand, have limited the main local post-surgical complications, avoiding any delays in wound healing.

A different formulation of the product, characterized by lower mucoadhesive capacity, but similar composition, has been tested by other authors by evaluating the control of bacterial plaque and the non-surgical treatment of mucoadhesive peri-implant sites, demonstrating a improvement of the clinical parameters investigated[17,18].

Manuka essential oil, in fact, has important antioxidant and antimicrobial properties, even against *Staphylococcus aureus* methicillin-resistant[19]. Its anti-inflammatory activity is also recognized in the literature, which is expressed by reducing the chemo-rates of proinflammatory cells in the wound bed, and the stimulation of fibroblasts and keratinocytes[20]. Thyme and tea tree essential oils have shown similar properties, among which antibacterial and antioxidant are the most represented[21,22]. Terpinen-4-ol, the main component of tea tree essential oil (but also present in many other oils), is one of the most investigated components in the literature: evidence suggests that this molecule is able to inhibit the production of pro-inflammatory mediators by monocytes, and to reduce skin inflammatory states induced by histamine[23,24]. Nogueira et al. investigated the properties of terpinen-4-ol and α-terpineol (another monoterpene present in several essential oils) to modulate the antimicrobial response of macrophages, highlighting their ability to significantly reduce the production of pro-inflammatory interleukins such as interleukins 1β, 6, 8 and 10[25]. Menthol, moreover, the main constituent of the essential oil extracted from *Mentha piperita*, has been found to be able to carry out anti-inflammatory activities through the inhibition of the production of nitric oxide and prostaglandin E2 in macrophage colonies activated by lipopolysaccharides[26]. In a recent review of the reading, the analgesic efficacy of menthol in the control of acute and inflammatory pain is emphasized[27]. In addition, it is

It has been widely demonstrated in the literature that hyaluronic acid, present in the bioadhesive gel tested, is able to assist the healing processes of wounds[28], promoting the proliferation of epithelial cells[29] and the organization of granulation tissue at the wound level[30]. Thanks toits reduced molecular weight, its oligomers are able to penetrate deeply into the tissues[31], stimulate the proliferation of endothelial cells[32] and express important antioxidant qualities, superior to those of hyaluronic acid high molecular weight[33]. These components seem to work synergistically together with bisabolol[34], *Commiphora myrrha* extract[35], allantoin[36], anethole and licorice extract[27,37] which are endowed with analgesic, anti-inflammatory and assistive healing processes.

Among the limitations of the study, it is important to remember that, although the composition of the gel includes the presence of many components of natural origin, there are also molecules of synthetic origin, which may have played a role in favoring the healing process. The small size of the sample examined and the lack of a control group also represent further elements to be taken into account for the correct interpretation of the results and their external validity.

Based on the promising results described here, further randomised controlled trials are needed in larger populations to confirm the efficacy of the bioadhesive gel.

5. CONCLUSIONS

Taking into account the methodological limitations of the study (small sample size and uncontrolled and non-randomized design), the gel used demonstrated excellent bioadhesive, hemostatic, re-epithelializing and antiseptic capabilities. Among the advantages associated with its use are the reduction of operating times, as there is no need to affix stitches, as well as the possibility of avoiding a second appointment for their removal. Most of the study participants expressed considerable satisfaction with the lack of sutures, which most likely reflects a state of anxiety and stress that often accompanies patients who have to undergo such a procedure.

The use of the gel can also be of help to the clinician in cases of localized samples in portions of the oral cavity that are difficult to sutulate, both because of access problems and because of the characteristics of the tissues, such as in the case of mucous membranes made fragile by inflammatory and/or pathological processes or mucous membranes lining hard tissues that are inextensible (adherent gingiva and hard palate). In addition, the gel based on essential oils could be proposed as an alternative to chlorhexidine in cases of allergic or hypersensitive patients to the molecule.

Although further studies are needed, the gel appears to be a promising, convenient, safe, well-tolerated and patient-appreciated alternative to the use of sutures and chlorhexidine in oral biopsy samples.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

STUDY FUNDING
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INFORMED CONSENT
The authors state that the patient's informed consent was obtained for the publication of the case, including photos.

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