Anti-inflammatory and wound healing effects of an essential oils-based bioadhesive gel after oral mucosa biopsies: preliminary results

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Abstract:

Post-operative management of patients receiving oral biopsy includes the control of edema, pain, infection, and re-epithelization at the surgical site. This clinical study investigates the topical use of a bioadhesive gel, containing essential oils, to promote wound healing and prevent post-operative pain and infection, avoiding the need for surgical suture and chlorhexidine applications. Ten patients, who needed to receive oral biopsies (≤6 mm in diameter) for the diagnosis of mucosal oral lesions, were enrolled. The bioadhesive gel successfully controlled the post-surgical pain; at 1-week follow-up visit, no signs of infection nor side effects were reported and the surgical sites were completely healed. The bioadhesive gel resulted in a very promising agent for the post-operative management of oral biopsy site, without the need for surgical suture and chlorhexidine applications.

Keywords: bioadhesive gel, essential oils, pain, oral biopsy, wound healing

Introduction

A mucosal biopsy is a medical procedure where a fragment (incisional biopsy) or the entire (excisional biopsy) mucosal lesion is surgically collected to establish the histopathological diagnosis. This examination is the "gold standard" in terms of diagnostic sensibility and specificity and represents a routine procedure in oral medicine (1). Under local anesthesia, a scalpel blade or a punch are usually employed to collect the tissue, and the surgical suture is often placed. The post-operative management includes the application of topical antibacterial gel, and the control of post-operative pain using non steroidal anti-inflammatory drug (NSAID), as needed by the patient.

Despite a plethora of studies recognizes chlorhexidine as the gold-standard among antiseptic agents (2), this molecule is not exempted from adverse effects, most frequently represented by dental and mucosal stainings, long-lasting dysgeusia, burning sensation (3,4) and increased calculus deposition on tooth surfaces (5). Some *in vitro* studies also showed chlorhexidine possesses cytotoxic effects against human fibroblasts and lymphocytes, via oxidative stress pathways (6,7). Besides chlorhexidine, even the suture can be associated with complications, such as the bacterial colonization of the filaments which can occur with all type of suture, but increases while considering polyfilament, because of the micro-retaining pattern of the surface (8,9). Nonetheless, the suture often represents a source of stress and anxiety for patients.

To overcome chlorhexidine and suture limitations, the alternative use of natural products, in particular originating from plants, has been widely investigated, taking advantage from their bioactivities. Among the others, essential oils (EOs) showed many biological properties, such as the antioxidant, anti-inflammatory and analgesic ones. Among plants belonging to the Myrtaceae family, the extract from Manuka (*Leptospermum scoparium*) has shown the capacity to reduce free radicals and antibacterial effects, as well (10). Similarly, *Eucalyptus* spp. displayed antioxidant, cytoprotective and antibacterial properties, too (11,12). Tea tree (*Melaleuca alternifolia*), another plant belonging to this family, is characterized by anti-fungal (13), anti-inflammatory (14) and anti-bacterial effects (15,16). Furthermore, the extracts from the family of Lamiaceae, including *Thymus vulgaris* and *Mentha piperita*, exhibited *in vitro* antioxidant (17), antifungal and anti-cancer (18, 19) activities, as well as analgesic effects against inflammatory pain (20). Similar effects have been reported for *Commiphora myrrha* (21) and Licorice (*Glycyrrhiza glabra*) extracts (22). Among isolated compounds, (-)-α-bisabolol, the main component of the EO from *Matricaria chamomilla*, showed anti-bacterial, anti-mutagenic, anti-inflammatory, analgesic and cicatrizing properties (23,24), while anethole, from *Anethum graveolens* and *Illicium verum*, exhibited anesthetic and antioxidant effects (20,25), and decreased the synthesis and the release of several inflammatory mediators, thus explicating antinociceptive activity (26). Allantoin, physiologically present in a human body and in plants, especially in *Symphytum officinale* leaves, can promote wound healing and stimulate cell mitosis (27,28), displayed analgesic (29), anti-inflammatory and moisturizing effects (27), besides a certain keratolytic activity (30).

A mixture of the above reported EOs and isolated phytochemicals could act in synergy to promote wound healing and show anti-inflammatory effects. Therefore, the aim of this study was to evaluate the efficacy of a topically applied bioadhesive gel, containing a mix of EOs as active ingredients, in terms of pain control and wound healing after oral biopsies.

Materials and methods

Study design - This was a pilot study, as the first stage of Phase II clinical trial, non-controlled and non-randomized, to preliminary investigate pain control and wound healing effects of an EOs-based bioadhesive gel (Hobagel Plus®, HOBAMA S.r.l. Milano) after oral mucosa biopsies. This study was conducted at the dental clinic of the UO Odontostomatologia II, ASST Santi Paolo e Carlo - San Paolo Hospital (University of Milan), where the interventions were performed and data collected and examined. The study included two phases: the former involved the surgical procedure and patient instructions for gel application; the latter involved the clinical follow-up visits and the recording of questionnaires.

Patients recruitment - From January 2018 to February 2018, ten patients referring to the dental clinic were recruited, in full accordance with ethical principles of the World Medical Declaration of Helsinki and under the approval of local Ethics Committee (ASST Santi Paolo e Carlo). Patients were consecutively enrolled during the first visit at the oral medicine unit. Inclusion criteria were being at least 18-years old and needing excisional or incisional oral biopsies (≤6 mm in diameter) for the histopathological diagnosis of oral mucosal lesions. Exclusion criteria included uncontrolled hypertension, uncontrolled diabetes, coagulation disorders, and absolute contraindications to surgery, pregnancy, and breastfeeding. Written informed consent of each patient was signed.

Personal and clinical data recording - For each patient, demographic and medical data were recorded during the first visit. An expert clinician, after having evaluated patient's eligibility to the study, performed the intraoral examination.

Intervention - Oral mucosal biopsies were carried out by trained practitioners. All surgical procedures were performed under local anesthesia, after obtaining written surgical consent. At the end of the surgical intervention, hemostasis was obtained with gauze soaked with the physiological saline solution, then, the surgical site was covered with a layer of gel (Hobagel Plus®, HOBAMA S.r.l. Milano). The gel contained Melaleuca Alternifolia Leaf Oil, Leptospermum Scoparium Branch/Leaf Oil, Ammonium Glycyrrhizate, Thymus Vulgaris Oil, Menthol, Mentha Piperita Oil, Eucalyptol, Anethole, Commiphora Myrrha Oil, Bisabolol, Tocopheryl Acetate, Allantoin, Cetylpyridinium Chloride, Hydrogen Peroxide, Sodium Hyaluronate Hydrolyzed, Sodium Hyaluronate and Triclosan as active ingredients, while Calcium/Sodium PVM/MA Copolymer, Paraffinum Liquidum, Petrolatum, Cellulose Gum, Polyvinylpyrrolidone as excipients.

Each patient was instructed to apply a thin layer of gel to the surgical wound, three times a day for one week. Clinical digital photographs of the surgical site were taken, using a reflex digital camera (Nikon D5300, 85 mm Micro Nikkor Af-s Lens, Metz 15 MS-1 digital Speedlight), after the biopsy and soon after topical gel application. Ordinary postoperative instructions were provided to the patient, as follows: do not disturb the area of surgery; do not spit or rinse the mouth for at least 24 hours; brush the teeth gently, taking care to not traumatize the surgical site; avoid physical activity for the first 48 hours; avoid hot/crunchy liquids or foods; if active bleeding would start, keep firm pressure by a gauze soaked with physiological saline solution for 15 minutes, and, in case of persisting bleeding, apply constant pressure at the surgical site for 15 minutes using

a tranexamic acid-soaked gauze. Acetaminophen 1000 mg tablets (max. three times a day) was prescribed with the recommendation to be used just as needed. After the biopsy, the patient also received the questionnaire to be filled during the following week, which included recordings about: daily pain (using Visual Analog Scale - VAS), eventual painkillers intake, presence of edema, secondary bleeding, and possible adverse reactions to the gel.

A follow-up visit was carried out one week later. Clinical photographs of the surgical site were taken again, as described above. An expert clinician (S.D.) performed the intraoral examination, particularly focused on the surgical site, recorded the presence/absence of infection or bleeding, and evaluated the wound healing in term of re-epithelization or presence of fibrin clot.

Primary outcome: pain - Pain intensity was assessed by visual analog scale (VAS). VAS is structured as a 100 mm horizontal line with two stop lines at the endings, which represent respectively "no pain" (left extreme) and "the worst pain conceivable" (right extreme). The patients were instructed to mark the VAS daily, always at the same hour. The VAS score was measured in millimeters, using a ruler, as the distance between and the starting point of the 100 mm line and the patient's mark (31). In accordance with Jensen et al. (32), VAS scores were further divided into four categories to quantify the postoperative pain: 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. Furthermore, the patients were instructed to record, daily, on the specific questionnaire section, the possible acetaminophen intake.

Secondary outcomes: infection, bleeding, and wound healing – Since the presence of infection, persistent inflammation and bleeding can contribute to the late repair of the surgical site, all these factors were assessed by the patient day-to-day, and by the clinician at 1-week follow-up. In particular, dichotomic visual analysis (presence/absence) of bleeding and edema was recorded every day by the patients throughout specific questionnaire sections. At 1-week follow up visit, a trained clinician (S.D.) visually verified the presence of local bleeding, edema, and signs of infection, and assessed the wound healing, in term of re-epithelization or presence of fibrin clot, comparing the surgical site with previously clinical photographs.

Results

Ten patients, requiring oral mucosa biopsies, participated in the study: nine females and one male (age range: 44-85 years, mean± SD: 65.7±11.3 years). In four patients, multiple biopsies (n = 2) were required for the accurate histopathological diagnosis, basing on the clinical features of the mucosal lesions, 14 surgical sites were, thus, considered in this trial. Clinical data are summarized in table 1.

Table 1. Demographic and clinical data of patients enrolled in the study. *Excisional biopsy: the lesion is completely removed; incisional biopsy: a tissue fragment of the lesion is collected

Patient	Gender	Age	Site	Biopsy Type*	Multiple biopsies	Adverse events	Pain		Edema	Infection	Bleeding	
							During the week	*At 1-week follow up*			*During the week*	*At 1-week follow up*
1	male	70	hard palate	incisional	-	none	no	no	no	none	yes	no
2	female	73	lateral surface of the tongue	incisional	-	none	mild	no	no	none	no	no
3	female	71	mandibular attached gingiva/alveolar mucosa	incisional	2	none	mild	no	yes	none	no	no

4	female	68	hard palate	incisional	2	none	no	no	no	none	no	no
5	female	85	hard palate	incisional	-	none	mild	no	no	none	no	no
6	female	71	maxillary attached gingiva/alveolar mucosa	incisional	2	none	no	no	no	none	no	no
7	female	61	soft palate	excisional	-	none	no	no	no	none	no	no
8	female	44	lingual aspect of the attached gingiva	excisional	-	none	no	no	no	none	no	no
9	female	58	maxillary attached gingiva/alveolar mucosa	incisional	2	none	mild	no	no	none	no	no
10	female	56	hard palate	incisional	-	none	mild	no	no	none	yes	No

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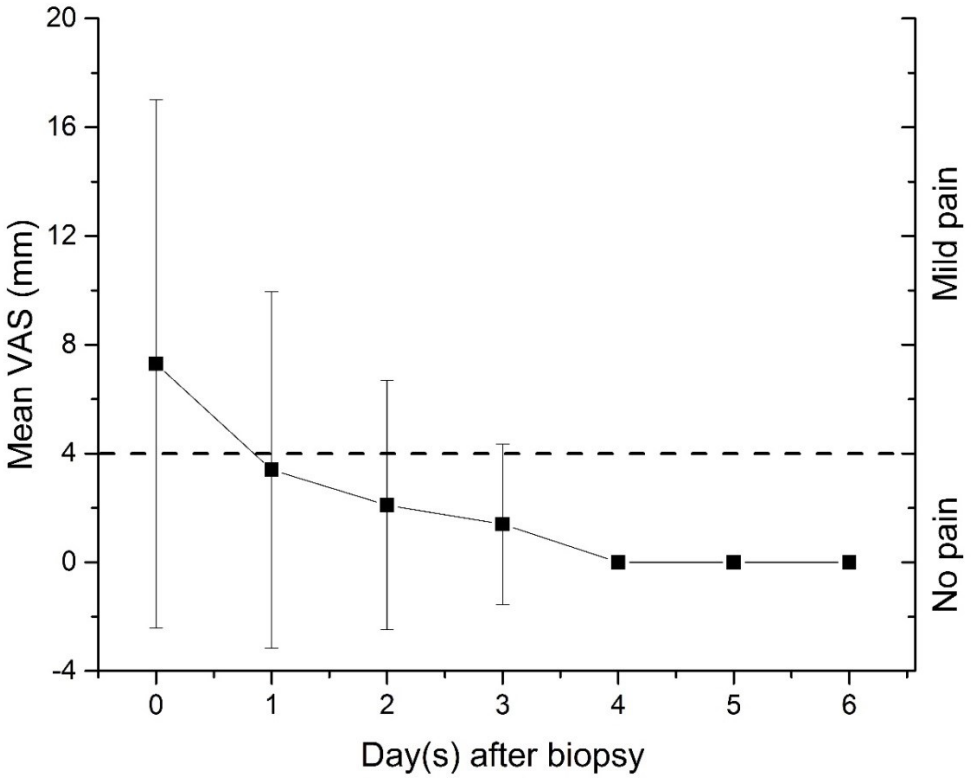
Five biopsies were taken from the palatal mucosa and seven from the keratinized gingiva/alveolar mucosa, while in one case from the lingual margin and in a further one from the soft palate. In two patients were performed excisional biopsies as diagnostic and therapeutic procedure (excisional biopsy), while in the other ones a fragment of the lesion was removed for just diagnostic purposes (incisional biopsy).

In all case, the EOs-based gel showed high and long-lasting bioadhesion, remaining in place for several minutes after a single application.

Primary outcome: pain - During the week following the procedure, each patient was asked to record daily the level of pain using VAS, the presence/absence of secondary bleeding, the presence/absence of edema, painkillers use and experience of adverse reactions to the gel. In five cases, a mild level of pain was reported: 3 of them recorded mild pain just during the day of the intervention (patients number 3, 9, and 10 – Table 1), another one exclusively during eating and speaking, and the pain lasted for the following four days (patient number 2 – Table 1), a further patient reported mild pain when wearing the denture. In the latter case, a double biopsy was performed at the hard palate, localized exactly on the mucosa under denture bases (patient number 5 - Table 1). The remaining five patients reported no pain during the week after surgery. After the fifth day, all patients did not record any pain.

Mean VAS values, recorded each day after the biopsy for one week, are shown in Figure 2. Overall, a mild pain resulted during the same day of the intervention (day 0), while from postoperative day 1 to day 6 no pain was recorded. None of the patients reported the use of acetaminophen or other painkillers.



158

159 **Figure 2.** Distribution of daily means VAS score.

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161 *Secondary outcomes: infection, bleeding, and wound healing* – The gel, placed after gauze compression on
162 the surgical site, successfully controlled post-operative bleeding in all patients (Fig. 1a and b). Only one
163 patient reported a light edema at the surgical site soon after the biopsy, which resolved within the first 2 days
164 (patient number 3 – Table 1).

165 In terms of secondary bleeding, two patients reported one episode during the first day: one of them occurred
166 two hours after the surgery, while the patient was eating, and was easily controlled by the compression of the
167 site with saline impregnated gauze (patient number 1 – Table 1). The other case of secondary bleeding
168 occurred after the patient had performed physical activity and taken his dinner, thus largely increased the risk
169 of this complication. The same patient controlled the bleeding using tranexamic acid-soaked gauze, instead
170 of a saline one as we recommended (patient number 10 – Table 1).

171 None of the patients enrolled reported adverse events to the gel.

172

At 1-week follow-up visit, no signs of infection at the surgical sites could be observed (Figure 1c). In 10 surgical sites, complete or partial re-epithelization occurred, in other 4 the presence of fibrin clot could be detected (Figure 1c, as an example).

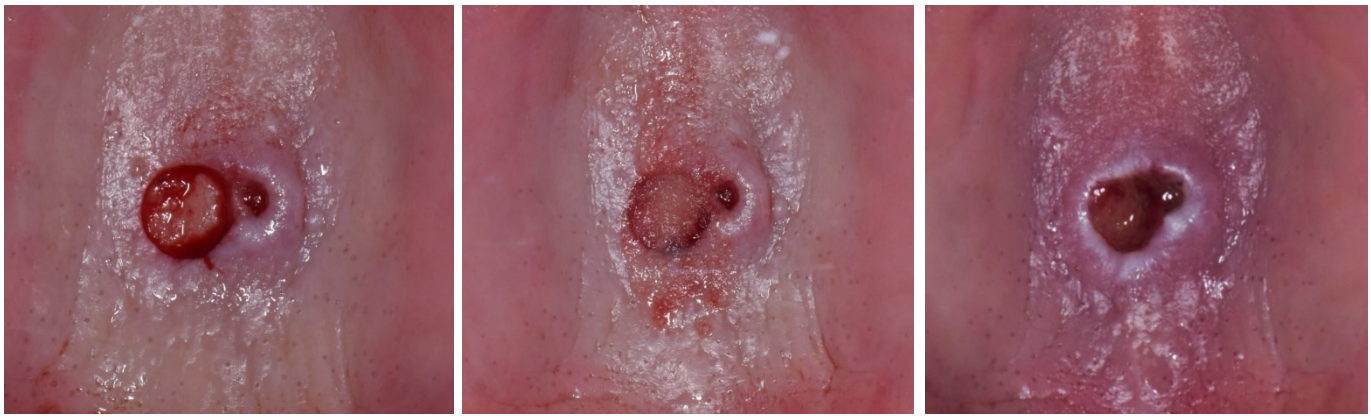

Figure 1. Intraoral photographs: a) surgical site, b) wound covered by a thin layer of gel, c) clinical appearance of the surgical site at 1-week follow up.

Discussion

The need of new agents as alternatives to surgical suture and chlorhexidine is still demanding, and, in recent decades, high attention has been directed towards several plant-derived compounds with antiseptic, anti-inflammatory and wound healing properties. The role of chlorhexidine application over the suture is to reduce bacterial colonization of filaments (9,33), usually associated with both aerobic (*Streptococcus* spp., *Staphylococcus warneri*, *Neisseria* spp., *Actinomyces* spp., *Pasteurella* spp.) and anaerobic (*Veillonella parvula*, *Peptostreptococcus* spp., *Actinobacillus* spp., *Prevotella* spp., *Fusobacterium* spp.) microorganisms. Chlorhexidine, however, has the major drawbacks to stain oral hard and soft tissue and to modify the perception of taste (34,35). Recently, Vouzara and colleagues (36) demonstrated its cytotoxicity, which resulted significantly higher than sodium hypochlorite and ethylenediaminetetraacetic acid. Hidalgo et al. (6) found that chlorhexidine produced mitochondrial injury and had anti-proliferative effects against human fibroblasts, consistently with Salimi and colleagues (7) who demonstrated its cytotoxicity in human lymphocytes.

This pilot study demonstrated the promising effects of a high bioadhesive gel containing a mixture of EOs in controlling post-operative inflammation and wound healing after minor surgery, i.e. oral mucosal biopsies. Noteworthy, just two patients out ten reported a mild pain up to three days from intervention, three patients reported mild pain just for one day, while, in the remaining 5, no pain was ever perceived. This finding was consistent with the findings reported by Kearns and Lodi (37,38). Interestingly, none of the patients, in this study, declared the use of painkillers after the intervention. This finding indicates a better outcome than data available from the literature, which reports about 18-26% of patients taking painkillers after oral mucosa biopsies, usually during the same day of surgery (38,39). One week later, 10 surgical sites experienced partial or complete re-epithelization, while the remaining ones had a stable fibrin clot; in all cases, no signs of infection were detectable. Interestingly, partial or complete re-epithelization of surgical wounds occurred in five patients out six who were affected by immune-mediated inflammatory oral mucosal diseases (oral lichenoid lesions, mucous membrane pemphigoid, and oral lichen planus).

All these findings might be ascribed to the strong bioadhesion of the gel and to the synergic effects of its active ingredients. The Manuka EO, indeed, possesses high antioxidant and antibacterial properties, even against Methicillin-Resistant Staphylococcus Aureus (MRSA) (39), besides anti-inflammatory activity, reducing migration of inflammatory cells at the wound site and stimulating the proliferation of fibroblasts and epithelial cells (10). Similarly, to Manuka EO, *Thymus vulgaris* and *Melaleuca alternifolia* EOs showed high antibacterial and antioxidant properties (16, 18). Terpinen-4-ol, a component of many EOs, is one of the most investigated compounds and evidence suggests that it can suppress inflammatory mediators by monocytes, and reduce histamine-induced skin inflammation (40,41). Nogueira (14) investigated the capacity of terpinen-4-ol and α-terpineol (another EO constituent) to modulate macrophage response towards bacterial stimulation and found that these compounds significantly reduce the production of several interleukins (IL-1β, IL-6, IL-8, and IL-10). Furthermore, EO from *Mentha piperita*, mainly composed by menthol, exhibited anti-inflammatory properties, too, as shown in animal models, by inhibiting the production of nitric oxide and prostaglandin E2 in lipopolysaccharide-activated macrophages (42). A review by de Cassia (20) reported, in particular, a significant analgesic activity of menthol in controlling acute and inflammatory pain. This could act in combination with both (-)-α-bisabolol (24), *Commiphora myrrha* (21), allantoin (20), anethole and licorice (27,43), which display analgesic activity together with anti-inflammatory and wound healing effects.

No adverse events were found. One case of post-operative swelling occurred in a patient affected by a chronic inflammatory oral disease (oral lichen planus), which could have exacerbated the response to surgical trauma. Just two cases of minor the secondary bleeding, during the same day of the intervention, were reported, but they could be explained since patients disobeyed the post-surgical instructions provided by the clinician. Indeed, one patient reported that the bleeding occurred while he was eating crunchy food in

the same day of surgery, while the second one carried out intense physical activity, and, again, the bleeding occurred while eating.

Within the limitations of this pilot study (having little sample size and following a not controlled and not randomized design), the here proposed EOs-based bioadhesive gel may represent a promising alternative to the use of suture and chlorhexidine, promoting wound healing, showing anti-inflammatory effects, and reducing operative time, patient distress, and chlorhexidine side effects as well. It may be particularly useful in those cases difficult to suture (for example gingiva and hard palate mucosa), or in presence of highly inflamed and friable tissue. The further second stage of this Phase II clinical trial is needed to better explore these preliminary findings, especially throughout the controlled randomized clinical trial on large patient population.

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Conflicts of interest

The authors declare no conflicts of interest.

Author's contributions

GL conceived the study design, FS and SD performed the biopsies and collected the data, SD executed the follow-up visits. FS, SD, and EV analyzed the data and drafted the article, GL, AS and MI critically reviewed the article. All authors approved the final version to be published.

References:

1. Mota-Ramírez A, Silvestre FJ, Simó JM. Oral biopsy in dental practice. Med Oral Patol Oral Cir Bucal. 2007 Nov 1;12(7):E504-510.

2. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. Minerva Stomatol. 2012 Sep;61(9):399-419.

3. Marinone MG, Savoldi E. Chlorhexidine and taste. Influence of mouthwashes concentration and of rinsing time. Minerva Stomatol. 2000 May;49(5):221-226.

4. Flötra L, Gjermo P, Rölla G, Waerhaug J. Side effects of chlorhexidine mouth washes. Scand J Dent Res. 1971;79(2):119-125.

5. Richards D. Chlorhexidine mouthwash plaque levels and gingival health. Evid Based Dent. 2017 Jun 23;18(2):37-38.

6. Hidalgo E, Dominguez C. Mechanisms underlying chlorhexidine-induced cytotoxicity. Toxicol In Vitro. 2001 Aug-Oct;15(4-5):271-276.

7. Salimi A, Alami B, Pourahmad J. Analysis of cytotoxic effects of chlorhexidine gluconate as antiseptic agent on human blood lymphocytes. J Biochem Mol Toxicol. 2017 Aug;31(8).

8. Banche G, Roana J, Mandras N, Amasio M, Gallesio C, Allizond V et al. Microbial adherence on various intraoral suture materials in patients undergoing dental surgery. J Oral Maxillofac Surg. 2007 Aug;65(8):1503-1507.

9. Grigg TR, Liewehr FR, Patton WR, Buxton TB, McPherson JC. Effect of the wicking behavior of multifilament sutures. J Endod. 2004 Sep;30(9):649-652.

10. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Giampieri F. The Composition and Biological Activity of Honey: A Focus on Manuka Honey. Foods. 2014 Jul 21;3(3):420-432.

11. Rocha Caldas GF, Oliveira AR, Araújo AV, Lafayette SS, Albuquerque GS, Silva-Neto Jda C, Costa-Silva JH, Ferreira F et al. Gastroprotective Mechanisms of the Monoterpene 1,8-Cineole (Eucalyptol). PLoS One. 2015 Aug 5;10(8):e0134558.

12. Abd El-Moneim MR, Fatma SA, Turky AF. Control of Tetranychus urticae Koch by extracts of three essential oils of chamomile, marjoram and Eucalyptus. Asian Pac J Trop Biomed. 2012 Jan;2(1):24-30.

13. Tobouti PL, Mussi MC, Rossi DC, Pigatti FM, Taborda CP, de Assis Taveira LA et al. Influence of melaleuca and copaiba oils on Candida albicans adhesion. Gerodontology. 2016 Sep;33(3):380-385.

283 14. Nogueira MN, Aquino SG, Rossa Junior C, Spolidorio DM. Terpinen-4-ol and alpha-terpineol (tea tree
284 oil components) inhibit the production of IL-1β, IL-6 and IL-10 on human macrophages. Inflamm Res. 2014
285 Sep;63(9):769-778.

286 15. Santamaria M Jr, Petermann KD, Vedovello SA, Degan V, Lucato A, Franzini CM. Antimicrobial effect
287 of Melaleuca alternifolia dental gel in orthodontic patients. Am J Orthod Dentofacial Orthop. 2014
288 Feb;145(2):198-202.

289 16. Sharifi-Rad J, Salehi B, Varoni EM, Sharopov F, Yousaf Z, Ayatollahi SA et al. Plants of the Melaleuca
290 Genus as Antimicrobial Agents: From Farm to Pharmacy. Phytother Res. 2017 Oct;31(10):1475-1494.

291 17. Mamadalieva NZ, Youssef FS, Ashour ML, Sasmakov SA, Tiezzi A, Azimova SS. Chemical
292 composition, antimicrobial and antioxidant activities of the essential oils of three Uzbek Lamiaceae species.
293 Nat Prod Res. 2018 Feb 22:1-4.

294 18. Alexa E, Sumalan RM, Danciu C, Obistioiu D, Negrea M, Poiana MA et al. Synergistic Antifungal,
295 Allelopatic and Anti-Proliferative Potential of Salvia officinalis L., and Thymus vulgaris L. Essential Oils.
296 Molecules. 2018 Jan 16;23(1). pii: E185.

297 19. Rozza AL, Meira de Faria F, Souza Brito AR, Pellizzon CH. The gastroprotective effect of menthol:
298 involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. PLoS One. 2014 Jan
299 21;9(1):e86686.

300 20. de Cássia da Silveira E, Sá R, Lima TC, da Nóbrega FR, de Brito AEM, de Sousa DP. Analgesic-Like
301 Activity of Essential Oil Constituents: An Update. Int J Mol Sci. 2017 Dec 9;18(12). pii: E2392.

302 21. Mahmoud AM, Zaki AR, Hassan ME, Mostafa-Hedeab G. Commiphora molmol resin attenuates
303 diethylnitrosamine/phenobarbital-induced hepatocarcinogenesis by modulating oxidative stress,
304 inflammation, angiogenesis and Nrf2/ARE/HO-1 signaling. Chem Biol Interact. 2017 May 25;270:41-50.

305 22. Dastagir G, Rizvi MA. Review - Glycyrrhiza glabra L. (Liquorice). Pak J Pharm Sci. 2016
306 Sep;29(5):1727-1733.

307 23. Woollard AC, Tatham KC, Barker S. The influence of essential oils on the process of wound healing: a
308 review of the current evidence. J Wound Care. 2007 Jun;16(6):255-257.

309 24. Hartman D, Coetzee JC. Two US practitioners' experience of using essential oils for wound care. J
310 Wound Care. 2002 Sep;11(8):317-320.

311 25. Ghelardini C, Galeotti N, Mazzanti G. Local anaesthetic activity of monoterpenes and phenylpropanes of
312 essential oils. Planta Med. 2001 Aug;67(6):564-566.

313 26. Ritter AM, Domiciano TP, Verri WA Jr, Zarpelon AC, da Silva LG, Barbosa CP et al.
314 Antihypernociceptive activity of anethole in experimental inflammatory pain. Inflammopharmacology. 2013
315 Apr;21(2):187-197.

316 27. Araújo LU, Grabe-Guimarães A, Mosqueira VC, Carneiro CM, Silva-Barcellos NM. Profile of wound
317 healing process induced by allantoin. Acta Cir Bras. 2010 Oct;25(5):460-466.

318 28. Loots JM, Loots GP, Joubert WS. The effect of allantoin on cellular multiplication in degenerating and
319 regenerating nerves. S Afr Med J. 1979 Jan 13;55(2):53-56.

320 29. Shestopalov AV, Shkurat TP, Mikashinovich ZI, Kryzhanovskaia IO, Bogacheva MA, Lomteva SV et
321 al[Biological functions of allantoin]. Izv Akad Nauk Ser Biol. 2006 Sep-Oct;(5):541-545.

322 30. Veraldi S, Menter A, Innocenti M. Treatment of mild to moderate seborrhoeic dermatitis with MAS064D
323 (Sebclair), a novel topical medical device: results of a pilot, randomized, double-blind, controlled trial. J Eur
324 Acad Dermatol Venereol. 2008 Mar;22(3):290-296.

325 31. Kjeldsen HB, Klausen TW, Rosenberg J. Preferred Presentation of the Visual Analog Scale for
326 Measurement of Postoperative Pain. Pain Pract. 2015 Aug 27.

327 32. Jensen MP, Chen C, Brugger AM. Interpretation of visual analog scale ratings and change scores: a
328 reanalysis of two clinical trials of postoperative pain. J Pain. 2003 Sep;4(7):407-414.

329 33. Otten JE, Wiedmann-Al-Ahmad M, Jahnke H, Pelz K. Bacterial colonization on different suture
330 materials--a potential risk for intraoral dentoalveolar surgery. J Biomed Mater Res B Appl Biomater. 2005
331 Jul;74(1):627-635.

332 34. al-Tannir MA, Goodman HS. A review of chlorhexidine and its use in special populations. Spec Care
333 Dentist. 1994 May-Jun;14(3):116-122.

334 35. Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent. 2008 Nov-Dec;33(6):710-716.

335 36. Vouzara T, Koulaouzidou E, Ziouti F, Economides N. Combined and independent cytotoxicity of sodium
336 hypochlorite, ethylenediaminetetraacetic acid and chlorhexidine. Int Endod J. 2016 Aug;49(8):764-773.

337 37. Kearns HP, McCartan BE, Lamey PJ. Patients' pain experience following oral mucosal biopsy under
338 local anaesthesia. Br Dent J. 2001 Jan 13;190(1):33-35.

339 38. Lodi G, Sardella A, Demarosi F, Canegallo L, Moneghini L, D'Orefice S et al. Oral biopsy. A
340 prospective study on 286 consecutive procedures. Minerva Stomatol. 2007 May;56(5):241-251.

341 39. Blaser G, Santos K, Bode U, Vetter H, Simon A. Effect of medical honey on wounds colonised or
342 infected with MRSA. J Wound Care. 2007 Sep;16(8):325-328.

343 40. Halcón L, Milkus K. Staphylococcus aureus and wounds: a review of tea tree oil as a promising
344 antimicrobial. Am J Infect Control. 2004 Nov;32(7):402-408.

345 41. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and
346 other medicinal properties. Clin Microbiol Rev. 2006 Jan;19(1):50-62.

347 42. Sun Z, Wang H, Wang J, Zhou L, Yang P. Chemical Composition and Anti-Inflammatory, Cytotoxic and
348 Antioxidant Activities of Essential Oil from Leaves of Mentha piperita Grown in China. PLoS One. 2014
349 Dec 10;9(12):e114767.

350 43. Yang R, Yuan BC, Ma YS, Zhou S, Liu Y. The anti-inflammatory activity of licorice, a widely used
351 Chinese herb. Pharm Biol. 2017 Dec;55(1):5-18.

352