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Hyaluronic acid to improve healing of surgical incisions in the oral cavity: a pilot multicentre placebo-controlled randomised clinical trial

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Key words *hyaluronic acid, multicentre randomised placebo-controlled clinical trial, oral cavity, wound healing*

Purpose: To evaluate the efficacy of hyaluronic acid to improve the healing of surgical incisions in the oral cavity.

Materials and methods: Six Italian private practices participated in this trial, each centre provided 12 patients. After suturing, patients were randomised to receive either a single application of 0.8% hyaluronic acid or a placebo (the carrier). Outcome measures were: assessment of wound healing 10 days post-operatively on a Likert scale by the blind operators and by an independent and blinded outcome assessor on the photographs, adverse events and post-operative complications. Reproducibility was assessed by evaluating agreement between operators and the independent outcome assessor using the weighted Kappa statistic.

Results: Thirty-six patients were evaluated in each group, at ten days none had dropped-out. No post-operative complications or adverse events occurred. There were no statistically significant differences for wound healing, assessed clinically by the blinded operators or on photographs evaluated by a blinded and independent outcomes assessor. There was a substantial agreement between operators and the independent outcome assessor in the wound scoring.

Conclusions: Hyaluronic acid placed over surgical incisions in the oral cavity does not appear to improve wound healing. Further trials are needed to better understand the potential role of hyalulonic acid in dental applications.

■ Introduction

Hyaluronic acid (also known as hyaluronan or hyaluranate) is an extracellular constituent of the connective tissue belonging to the family of glycosaminoglycans. It is believed to play an important role in wound healing, facilitating cell migration and differentiation during embryonic development[1], and tissue repair[2]. In an *in vitro* study[3] it was found that hyaluronic acid possesses bacteriostatic (but not bactericidial) effects; the 1 mg/ml concentration of high molecular weight having the greatest overall bacteriostatic effect. Another interesting property of hyaluronic acid is that its molecular structure can be assembled into various molecular weights.

It has been suggested that hyaluronic acid could have a beneficial effect in the treatment of plaque-

induced gingivitis[4], in the maintenance of healthy peri-implant tissues[5] and in bone regeneration[6].

The aim of this randomised controlled clinical trial (RCT) was to compare the efficacy of a single application of hyaluronic acid with a placebo (the carrier) to improve wound healing of 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 hyaluronic acid, and those receiving a placebo, against the alternative hypothesis that a difference could be found. The present article is reported according to the CONSORT statement for improving the quality of reports of randomised trials[7].

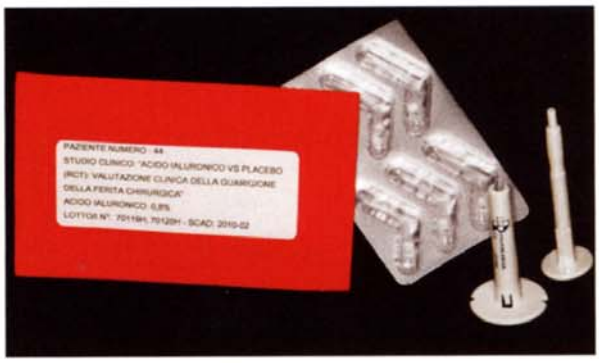


Fig 1 Each envelope contained a syringe, a blister with six hyaluronic acid or placebo capsules and a second envelope (illustrated) containing the exact content of the capsules to be opened only in case of an emergency such as an allergic reaction.

■ Materials and methods

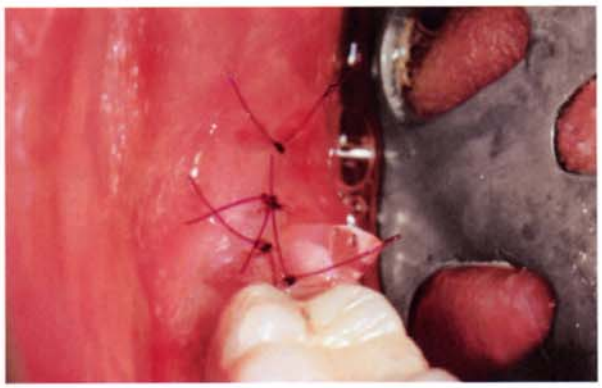
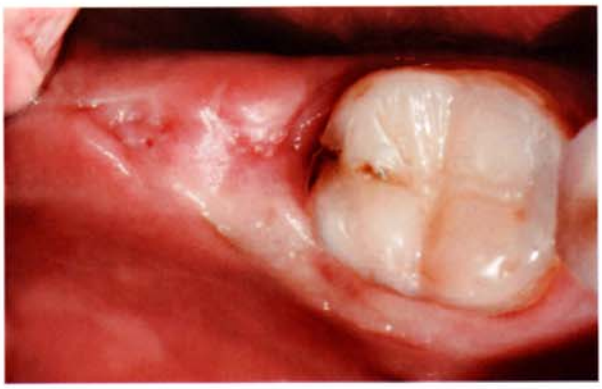
Any patient who required any dental surgical procedure was eligible for inclusion in this trial. They were not admitted in the study if any of the following exclusion criteria were present: patients affected by non-controlled diabetes, less than 18 years old or unable to sign an informed consent form.

Patients were grouped into 3 groups: non-smokers, light smokers (up to 10 cigarettes per day), and heavy smokers (more than 10 cigarettes per day).

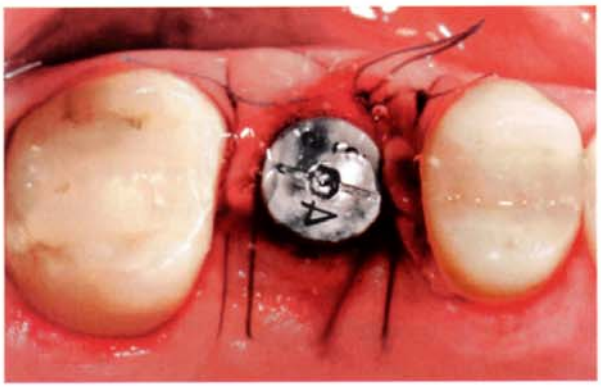
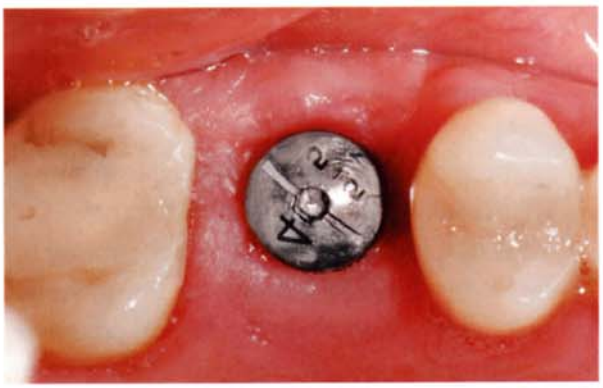
All patients received thorough explanations and signed a written informed consent form prior to being enrolled in the trial. Patients were recruited in Italian private dental clinics. Operators were allowed to include any surgical incisions (Table 1), as suggested by the manufacturer. After suturing of the surgical wounds with 5/0 sutures, patients were randomised to receive either a single application of 0.2 ml 0.8% high molecular weight hyaluronic acid (Gengigel

Table 1 Patients' and intervention characteristics of the two groups.

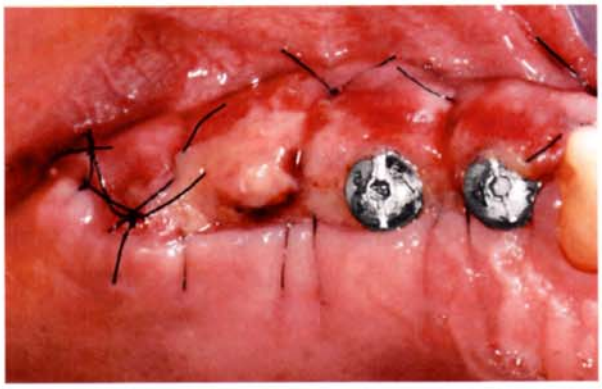
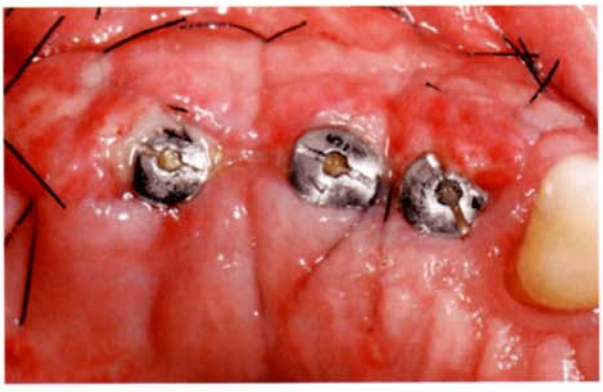
	Hyaluronic acid (n = 36)	Placebo (n = 36)
Females	19 (53%)	22 (57%)
Mean age (range)	50 (27 to 73)	49 (26 to 77)
Non-smokers	27 (75%)	30 (83%)
Smoking < 10 cigarettes/day	6 (17%)	5 (14%)
Smoking > 10 cigarettes/day	3 (8%)	1 (3%)
Controlled diabetes	2 (6%)	1 (3%)
Took prophylactic antibiotics	31 (86%)	31 (86%)
Type of surgical intervention		
Dental implant placement	14 (39%)	13 (36%)
Dental implant placement in fresh extraction socket	5 (14%)	6 (17%)
Use of resorbable barrier	3 (8%)	1 (3%)
Surgical tooth extraction	4 (11%)	3 (8%)
Periodontal surgery	8 (22%)	9 (25%)
Sinus lift	2 (6%)	4 (11%)
Intraoperative complications	1 (3%)	3 (8%)

Figs 2a and b Clinical photos illustrating the wound closure after removal of a mandibular third molar: (a) immediately after suturing; (b) after 10 days of healing. The patient received hyaluronic acid and the wound was scored as 0 (complete closure – no fibrin) by both the masked treating clinician and the independent outcome assessor.

Figs 3a and b Clinical photos illustrating the wound closure after placement of a postextractive immediate implant: (a) immediately after suturing; (b) after 10 days of healing. The patient received hyaluronic acid and the wound was scored as 0 (complete closure – no fibrin) by both the masked treating clinician and the independent outcome assessor.

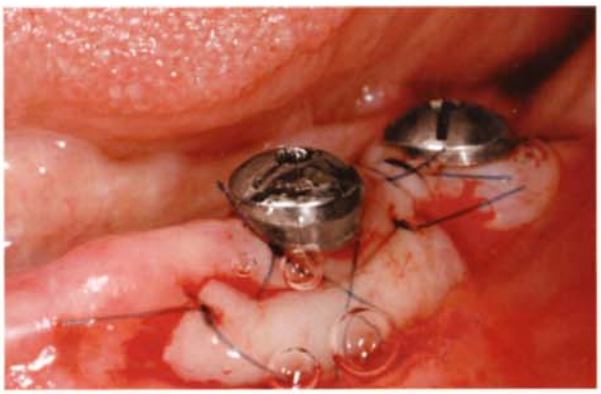



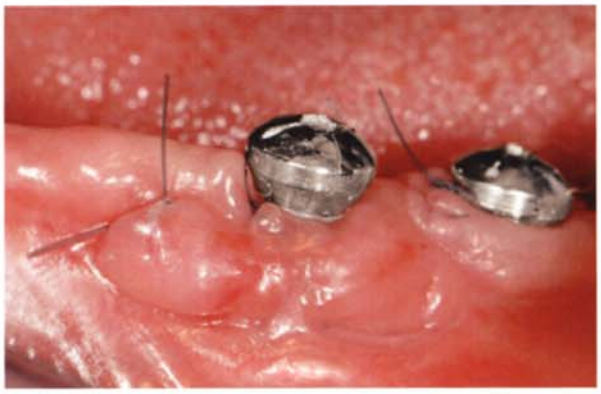
Figs 4a and b Clinical photos illustrating the wound closure after implant placement: (a) immediately after suturing; (b) after 10 days of healing. The patient received the placebo and the wound was scored as 2 (complete closure with presence of fibrin) by both the masked treating clinican and the independent outcome assessor.

Prof®/Gengi Pro® Bulbs, Ricerfarma s.r.l., Milan, Italy) or an identical placebo (the carrier). A film of gel was applied using a syringe (Fig 1) to the closed incision wound with a gentle massage for 2 minutes, avoiding saliva contamination. No post-operative chlorhexidine mouthwash was prescribed so as to better evaluate the effects of the tested intervention.

The outcome measures were as follows.

- Wound healing evaluation – the following scoring system was adopted:
  0. Complete wound closure without presence of fibrin (Figs 2b, 3b and 5b)
  1. Complete wound closure with a thin line of fibrin present





Figs 5a and b Clinical photos illustrating the wound closure after a 'periodontal' type intervention: (a) immediately after suturing; (b) after 10 days of healing. The patient received the placebo and the wound was scored as 0 (complete closure – no fibrin) by both the masked treating clinician and the independent outcome assessor.

2. Complete wound closure with presence of fibrin (Fig 4b)
3. Incomplete wound closure (dehiscence)
4. Incomplete wound closure (necrosis).

Assessments were made at suture removal 10 days after the interventions by the individual clinicians who performed the surgical procedure. Clinicians also took standardised digital pictures after suturing (Figs 2a, 3a, 4a and 5a) and of the healed wounds (Figs 2b, 3b, 4b and 5b) that were subsequently assessed by a single blinded and independent outcome assessor.

- Post-operative complications (such as suppuration, fistula, abscess etc).
- Post-operative adverse events.

These outcomes were recorded 10 days after surgical incision. Clinical assessments were made by the treating clinicians who remained unaware of group allocation for the entire duration of the study.

A sample size was not calculated. Thirty-six patients were included in each group. Six centres agreed to participate in this trial. Each centre recruited 12 patients: six randomised to the hyaluronic acid and six to the placebo.

Six computer-generated restricted randomisation lists with equal groups of participants were prepared by the manufacturer. None of the investigators was aware of the randomisation sequence. The randomised product (active agent and placebo) were enclosed in sequentially numbered, identical, opaque, sealed envelopes together with a second opaque, sealed envelope (Fig 1) containing information on the exact contents, to be opened only in an emergency situation (e.g. allergic reaction). Envelopes were opened sequentially, after suture placement, and the product contained in the envelope was delivered to the wound. Therefore, treatment allocation was concealed to the investigators in charge of enrolling and treating the patients, and both patients and operators/outcome assessors were blinded to the tested intervention. In addition the statistician was kept blind and performed all analyses unaware as to which group the patients were allocated. All data analysis was carried out according to a pre-established analysis plan. The patient was the statistical unit of the analyses. Afte the statistical analyses were completed the authors became aware which group used the placebo and which group used the active gel. The wounds were assessed on a Likert scale from 0 to 4. Comparison between the median wound scores for

Table 2 Interpretation of Kappa statistics according to Landis and Koch[8].

Kappa value	Strength of agreement
< 0.00	Poor
0.00 to 0.20	Slight
0.21 to 0.40	Fair
0.41 to 0.60	Moderate
0.61 to 0.80	Substantial
0.81 to 1.00	Almost perfect

Scoring by each clinician	Hyaluronic acid (n = 36)	Placebo (n = 36)
0 Complete closure – no fibrin	25 (69%)	22 (61%)
1 Complete closure – thin line of fibrin	7 (19%)	8 (22%)
2 Complete closure – fibrin	3 (8%)	6 (17%)
3 Incomplete closure – dehiscence	1 (3%)	0 (0%)
4 Incomplete closure – necrosis	0 (0%)	0 (0%)
Mann-Whitney U-test $P = 0.45$		

Table 3 Wound assessment by each clinician 10 days after incision.

Scoring by independent outcome assessor	Hyaluronic acid (n = 36)	Placebo (n = 36)
0 Complete closure – no fibrin	22 (61%)	23 (64%)
1 Complete closure – thin line of fibrin	9 (25%)	8 (22%)
2 Complete closure – fibrin	3 (8%)	5 (14%)
3 Incomplete closure – dehiscence	2 (6%)	0 (0%)
4 Incomplete closure – necrosis	0 (0%)	0 (0%)
Mann-Whitney U-test $P = 0.79$		

Table 4 Wound assessment by independent outcome assessor using photographs.

the two groups was made by using the Mann-Whitney U-Test. The wound assessment of the clinicians was compared with those of the independent outcome assessor using photographs. Weighted Kappa and the intra-class correlation coefficients were used to assess inter-assessor agreement. The weighting modifies the Kappa values to better reflect the relative importance of the degree of disagreement in an ordered scale so that disagreement by one category is less important than disagreement by two categories. The interpretation of Kappa statistics follows the recommendations of Landis and Koch[8] and is illustrated in Table 2. Differences in the proportion of post-operative complications and adverse events were to be compared among the groups, using Fisher's exact probability test. All statistical comparisons were conducted at the 0.05 level of significance.

■ Results

Seventy-two patients were enrolled, randomised and treated at the six centres and no patients were excluded from the analysis. In total, data from 72 patients were evaluated: 36 patients in the hyaluronic acid group and 36 in the placebo group. Numbers and reasons of patients not willing or not eligible to participate into this trial were not recorded. No deviations from the research protocol occurred. All patients were treated according to the allocated interventions and no patients dropped out. Patients were recruited and treated from September 2007 to December 2007. The follow-up focused on the time between the surgical intervention and the 10 days following .

The main baseline patient and intervention characteristics are presented in Table 1. There were no apparent relevant baseline imbalances between the two groups. Intraoperative complications (perforation of the sinus membrane during a sinus lift procedure) occurred in four patients (Table 1).

The outcome of the wound assessment (Figs 2a and b, 3a and b; 4a and b; 5a and b) performed by individual clinicians 10 days after the interventions is presented in Table 3, whereas the scoring of the

independent assessor on digital pictures is presented in Table 4. There was no evidence of a statistically significant difference between the medians of the groups for either of the wound assessments (Mann-Whitney U-test: treating operator $P = 0.45$, independent assessor $P = 0.79$).

There was a substantial agreement between the blinded operators and the blinded and independent outcome assessors, with weighted kappa 0.76 (SE 0.092), and an intra-class correlation coefficient of 0.85.

No post-operative complications or adverse events were recorded. The independent assessor judged that two wounds, belonging to the hyaluronic acid group, presented a dehiscence. The incisions were made to extract a tooth and to perform periodontal surgery.

■ Discussion

This trial was not able to disclose any statistical significant differences or trends when comparing the efficacy of hyaluronic acid versus a placebo to improve wound healing after a surgical procedure in the oral cavity. No adverse events were reported, and this supports the safety of hyaluronic acid.

Among the possible limitations of the present trial are that the wound healing assessment was made too late (10 days after surgical incision) to be able to detect any significant difference; that the scoring system used to clinically evaluate wound healing was too 'crude' and 'subjective'; that other outcome measures, such as post-operative pain and swelling could have also been assessed to investigate on the possible anti-inflammatory effect of hyaluronic acid; and that the sample size was too small to detect a statistically significant difference. On the other hand, the study was conducted using a placebo (the carrier without the active ingredient), stored in an identical dispenser as the active intervention and produced by the same manufacturer, which kept patients and investigators blinded for the entire duration of the trial. The randomised codes were broken only after all the statistical calculations were completed. The scoring assessment of wound healing was done in duplicate by the masked operators and, on digital pictures, by a single independent and masked outcomes assessor. Interestingly, a substantial agreement between the scoring was achieved, which suggests that the wound healing scoring system used in the present investigation was reproducible. The sample size was the largest so far published with respect to clinical trials testing the efficacy of hyaluronic acid in the oral environment. The tested products were kindly donated by the manufacturer, who also prepared the randomisation sequence. However, the data of the study were analysed and the reports written independently by the investigators.

When comparing the present findings with other similar studies, it appears that the authors' results are in agreement with the majority of published studies (with a couple of exceptions[4,9]).

There are two early studies published in Italian[10,11], but the authors were unable to retrieve one of these studies, so conclusions could not be drawn from that paper[11]. The retrieved study is a poorly described uncontrolled case series of 10 patients; six affected by a slight form of marginal gingivitis and four affected by gingival inflammation because of periodontal surgery performed 7 days before using a periodontal pack. Patients were subjected to professional oral hygiene procedures and instructions and only patients able to maintain a good oral hygiene were included. A 0.2% hyaluronic acid gel was used on both arches three times a day for an unspecified period of time. The authors concluded, using subjective outcome measures, that in nine out of 10 patients, the gel had a positive effect after approximately 7 days, and in one patient a dubious effect was seen. The lack of a control group and of objective outcome measures challenge the authors' conclusions, as patients may have healed because of the conventional oral hygiene procedures.

The conclusions of a narrative review[12] on the role of hyaluronic acid in the management of periodontal diseases concluded that clinical studies carried out up until the late 1990s have shown a high effectiveness and tolerance of hyaluronic acid. However, the authors could not find any clinical evidence of efficacy in the review. On the other hand, the authors were unaware of any problems with respect to safety and tolerance.

A split-mouth RCT[6] evaluated hyaluronic acid in the treatment of infra-bony pockets. Six patients were treated with a resorbable barrier with or

without hyaluronic acid, whereas nine patients received only scaling with or without hyaluronic acid. The only statistically significant difference that the authors found was in the bone height at 12 months, which favoured hyaluronic acid in surgically treated patients. All other assessment times and outcome measures were not significant. Due to the limited sample size (only six patients), and to the huge variability of the reported bone height measurements at 1, 3, 6 and 12 months, it is likely that observed significance is casual.

In a placebo-controlled RCT of split-mouth design, the effect of hyaluronic acid gel on cell proliferation was evaluated using immunohistochemistry in 21 patients[13]. Gingival bleeding index and probing pocket depths were also assessed. Patients were instructed to apply, after tooth brushing, the active and placebo gels with fingers twice-a day. After 1 month the gingival biopsies of the test sites showed a statistically significant reduction in inflammatory infiltrate and cell proliferation index in the gingival epithelium. There were no differences in the bleeding index (actual figures not given). Curiously, although probing depths remained substantially stable in the test sites (baseline: 2.71 ± 0.85 mm; after 1 month: 2.71 ± 0.82 mm), they deteriorated slightly in a statistically significant way in the placebo sites (baseline: 2.84 ± 0.94 mm; after 1 month: 3.05 ± 0.82 mm). These clinical findings are difficult to explain. However, patients with a previous history of periodontitis were included in the study. They may have been completely healthy or severely affected by periodontal disease, and 1 month of follow-up might have been too short a time period to show any improvement in probing depths.

The efficacy of subgingival application of hyaluronic acid gel as an adjunctive to scaling to treat periodontitis was evaluated in a controlled clinical trial of split-mouth design[14]. Manual scaling was performed at baseline, 2, 4 and 6 weeks, whereas 1 ml of 0.2% hyaluronic acid was delivered once per week for 7 weeks. After 12 weeks no statistically significant difference between the two interventions was observed. The antimicrobial effect was not observed, thus the authors questioned the clinical significance of a previous in vitro study that showed a bacteriostatic effect of hyaluronic acid[3].

Another RCT evaluated the effect of hyaluronic acid sprayed 5 times a day for 1 week in 60 patients affected by gingivitis[15]. Various clinical outcome measures were used including approximal plaque index, sulcus bleeding index and papilla bleeding index. No placebo was used, as it was claimed that the manufacturer was unable to produce one. Forty patients were randomised to the test group and 20 to the control group. However, such an unequal randomisation may not be a good choice, as the power of the sample size is actually reduced to 20 per group. While plaque levels remained substantially stable, both the sulcus and papilla bleeding indexes showed a statistically significant reduction in the test group, whereas the *P* values for the control group were not given and the differences between tests and control groups (i.e. the most useful clinical information) were not calculated. Though the study was poorly conducted and reported, it might be possible that hyaluronic acid has an anti-inflammatory effect, and, therefore, better designed clinical trials are needed to evaluate the potential efficacy of hyaluronic acid.

Hyaluronic acid gel (0.2%) was also compared in a RCT with 0.2% chlorhexidine gel in the maintenance of 30 patients with implant-supported bridges[5]. Patients used only a toothbrush and these two gels as hygiene procedures for 6 months after implant placement. The trial showed a statistically significant reduction in marginal bleeding using a hyaluronic acid gel during the first 2 months of healing. These findings were based only on 15 subjects per group so the result could be due to chance. However, at 6 months no differences in plaque, changes in probing depth and modified bleeding index were observed.

The efficacy of a 3-week application of hyaluronic acid gel on gingivitis was evaluated in double-blind placebo-controlled RCT, which included 50 subjects[4]. Hyaluronic acid gel had a statistically significant beneficial effect on the treatment of gingivitis for plaque and papilla bleeding index over the placebo. A split-mouth placebo-controlled RCT evaluated a single application of 0.8% hyaluronic acid gel after scaling in 52 patients affected by periodontal disease[9]. After 3 months, the sites treated with hyaluronic acid gel showed a statistically significant reduction (almost double) in bleeding on probing

and pocket depths compared with the placebo treated sites. These two studies are the only ones that clearly showed a statistically and clinically significant additional effect of hyaluronic acid in the treatment of gingivitis. Additional RCTs should be conducted to confirm these interesting results.

Another interesting, but still experimental, application of hyaluronic acid is in the healing of extraction sockets. In a split-mouth pilot study including 8 rabbits, 2 of which were killed at 7, 13, 20 and 30 days, an earlier and increased percentage of alveolar bone filling after teeth extractions was observed in alveoli filled with 0.8% hyaluronic acid gel when compared with unfilled controls[16]. If these preliminary findings can be substantiated by more robust animal data, it would be interesting to investigate in humans whether or not hyaluronic acid could be used to enhance bone healing at immediate implants in post-extractive sites.

This trial was designed as a pilot study in order to see if there could be any beneficial effect on surgical wound healing by applying hyaluronic gel. No statistically significant differences, not even trends, supporting the hypothesis of the efficacy of hyaluronic acid in this specific application, could be found. The present study was conducted at six Italian private dental practices, included a variety of surgical interventions and the inclusion criteria were very relaxed, therefore the present results can be generalised to similar settings.

Conclusions

A single-dose of 0.8% hyaluronic acid placed over surgical incisions in the oral cavity does not appear to improve wound healing. Further trials are needed to better understand the potential role of hyaluronic acid in dental applications.

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