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ORIGINAL ARTICLE

Local application of hyaluronan gel in conjunction with periodontal surgery: a randomized controlled trial

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Abstract Hyaluronic acid application has been proven to be beneficial in a number of medical disciplines. The aim of the current study was to clinically evaluate the effect of local application of hyaluronan gel in conjunction with periodontal surgery. Fourteen patients with chronic periodontitis having four interproximal intrabony defects (≥3 mm) with probing depth values >5 mm were included in this split-mouth study. Following initial nonsurgical periodontal therapy and re-evaluation, defects were randomly assigned to be treated with modified Widman flap (MWF) surgery in conjunction with either 0.8% hyaluronan gel (test) or placebo gel (control) application. Clinical attachment level (CAL), probing depth (PD), gingival recession (GR), plaque index (PI), and bleeding on probing (BOP) values were taken at baseline and 3 and 6 months. Differences between test and control sites were evaluated using a Wilcoxon signed-rank and a McNemar test. A Friedman and a Cochran test were used to test equal ranks over time. Statistically significant differences were noted for CAL and GR ($P<0.05$) in favor of the test sites. No significant differences were found regarding PD, BOP, or PI values ($P>0.05$). Hyaluronan gel application in conjunction with periodontal surgery appears to result in significant improvement of CAL and in a reduction in GR. Hyaluronan gel application appears to improve the clinical outcome of MWF surgery.

Keywords Periodontal surgery · Hyaluronic acid · Clinical attachment level · Gingival recession

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Introduction

The ultimate goal of periodontal treatment is the regeneration of the supporting tissues lost due to periodontal disease [1]. Regeneration refers to the reproduction or reconstitution of a lost or injured part, in contrast to repair, which describes the healing by tissues that do not fully restore the architecture or the function of the lost part [2]. In periodontitis, as destruction reaches the deeper structures, regeneration becomes unpredictable.

However, in contrast to regeneration, periodontal repair remains a readily attainable goal. A variety of surgical procedures have been advocated to enhance periodontal tissue reparative response and to provide an environment, in which new attachment can readily occur. These include the modified Widman flap (MWF) [3], the excisional new

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attachment procedure [4], and the open flap debridement [5]. They all share minimal excision of gingival tissues, minimal extent of flap reflection with minimal exposure of the alveolar bone, close adaptation of the tissues, and replacement of the flap close to the pre-surgical position of the gingival margin [6]. Although most techniques resulted in significant gain in clinical attachment level in deep pockets, the modified Widman flap resulted in the greatest clinical attachment gain [7].

Hyaluronic acid (hyaluronan) is a linear, widely distributed glycosaminoglycan of the extracellular matrix of mammalian connective tissues [8, 9], primarily synthesized by mesenchymal cells [10] and forms the backbone for the organization of proteoglycans establishing links with collagen, fibrin, and different matrix molecules [11]. It was detected in periodontal tissues in varying quantities and is more prominent in the nonmineralized tissues, such as the gingiva and the periodontal ligament, than the mineralized ones such as the cementum and alveolar bone [12]. In the early inflammatory stages of wound healing, tissues are very rich in hyaluronan [13–15].

Hyaluronan has been the center of various studies, from early tissue development [16, 17], as an active bone matrix [18], in mesenchymal cells' differentiation [19], migration [20–22], and adhesion [23], to hydroxyapatite formation [24] and has been used as a vehicle for growth factors. The hydrophilic nature of hyaluronan creates an environment for cellular migration, while its free radical scavenging and protein exclusion properties offer protection to cells and extracellular matrix molecules [11]. Hyaluronan further promotes many blood cells' functions especially in the inflammatory response, e.g., phagocytosis and chemotaxis [14]. Its anti-inflammatory properties have made it of interest in various medical applications as in the treatment of radio-epithelitis [25], osteoarthritis of the knee [26–28], and rheumatoid arthritis [29].

Despite all previously mentioned properties, only few studies could be found on the potential of hyaluronan in periodontal wound healing. The aim of the present study was to clinically evaluate the adjunctive effects of hyaluronan gel application in conjunction with periodontal surgery.

Material and methods

Patients

In this study, 800 patients from the outpatient clinic of the Oral Medicine and Periodontology Department at the Faculty of Oral and Dental Medicine, Cairo University were examined for conformity with the inclusion criteria. Seven hundred seventy-two patients were excluded (760 not meeting the inclusion criteria, 12 refused to participate). Twenty-eight patients were initially included in this split-mouth study. All patients had no relevant medical history that contraindicated periodontal surgery and were non-smokers. Inclusion criteria were chronic periodontitis cases [30], with at least four interproximal sites with moderate to deep intrabony defects (≥3 mm) on the radiographs, and clinical probing depths (PD) >5 mm using William's graduated periodontal probe on premolars or molars following initial nonsurgical periodontal therapy, with a minimum of 20 teeth in each patient. Patients should not have received antibiotic therapy or periodontal treatment within the 6-month period prior to the study. This study was approved by the Ethical Committee at Cairo University Hospital, Cairo, Egypt. The subjects gave their written informed consent to participate in the study. Fourteen patients were excluded due to a resultant probing depth <5 mm 8 weeks following the initial nonsurgical periodontal therapy and 14 patients continued the study.

Procedures

Pre-surgical phase

After initial examination, each patient was given detailed instructions on self-performed plaque control measures using a soft tooth brush and interdental cleaning devices when indicated. Initial nonsurgical professional periodontal therapy consisted of full mouth supra- and sub-gingival scaling and root planing (SRP). Eight weeks following the initial therapy, re-evaluation of the intrabony defects in the interproximal sites with a clinical probing depth >5 mm was performed by radiographs as well as by using William's graduated periodontal probe to confirm the indication for periodontal surgery. Pairs of premolar and molar teeth in the maxilla or the mandible were randomized (see below) to receive the test treatment (MWF with adjunctive hyaluronan gel application) or to serve as MWF controls. Prior to surgery, the values for clinical attachment level (CAL), PD, gingival recession (GR), bleeding on probing index (BOP), and plaque index (PI) were recorded. CAL, PDs, and GR were recorded to the nearest millimeter using a William's graduated periodontal probe, with a tip diameter of 0.5 mm. All teeth were evaluated at six sites: mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual, and mid-lingual.

Surgical phase and placement of hyaluronan gel in the defect

The defects in each patient were randomly assigned (see below) to be treated either with a mucoperiosteal flap, the MWF, with a placebo gel, or in conjunction with the application of hyaluronan gel. The MWF was selected as a surgical technique to ensure complete removal of the

pocket lining, thereby exposing the fresh underlying connective tissue to the hyaluronan gel's effect. Great care was exerted during the surgical procedure to minimize the width of the excised collar (around 0.5 mm in width) so as not to compromise the primary wound closure potential later. The root surface and bone defects were instrumented using Gracey curettes (Aesculap, Tuttlingen, Germany) to achieve complete debridement. Afterwards, 0.5 ml of 0.8% hyaluronan gel (Gengigel, Ricerfarma, Milan, Italy) was applied to the defect area, filling the defect from the bottom upwards. Closure of surgical sites was ensured with 4–0 black silk sutures (Ethicon, Johnson & Johnson, Livingston, Scotland, UK). A similar surgical procedure was conducted for the control site with the application of a placebo gel prepared at Cairo University (carboxymethylcellulose, xylitol, NaCl, and water).

Each patient was given both amoxicillin (GlaxoSmithKline, Cairo, Egypt) and metronidazole (Sanofi-Aventis Egypt, Cairo, Egypt) antibiotics each 500 mg t.d.s for 1 week postoperatively to optimize the surgical outcome [31]. Patients were advised to refrain from hot food and beverages for the first day, use soft diet, and avoid chewing on the surgical side for the first week following the surgery. Oral hygiene instructions using a soft tooth brush were stressed upon. For the following first week, salted water mouth bath four times per day, then for the second week 0.12% chlorhexidine mouthwash (Antiseptol, Kahira Pharmaceuticals, Cairo, Egypt) twice daily were implemented as an adjunct to oral hygiene measures. The rationale of the reliance on salted water mouth bath for the first week was based on the reported highly cytotoxic effects of chlorhexidine and the recommendations that it should be used with caution especially during surgery and the initial healing phase afterwards [32]. Sutures were removed 1 week following the surgery.

Follow up and re-evaluation

Patients were seen weekly for the first month to check their oral hygiene performance and then at 3- and 6-month intervals to record all clinical indices.

Outcome measures

The primary outcome measure defined in the study was the change in CAL between the test and control sites. Secondary outcome measures outlined were changes in GR, PD, BOP, and PI.

Sample size

This split-mouth study was conducted as a randomized controlled clinical trial (RCT). In the absence of any data from previous studies on the beneficial effect of hyaluronan gel on the CAL, sample size was calculated on the basis of data from a study on the effect of Emdogain (Straumann, Freiburg, Germany) on the CAL [33]. Assuming a difference of 0.5 mm between the means and a standard deviation of the difference in means of 0.35 mm (the CAL gain in their test group was 1.7 mm with a SD of 1.3. The SD for the difference in CAL gain between the groups was not given. However, since the SD in the CAL gain was about 70% of the mean in the test group, we assumed a SD for the difference of 0.35 mm, 70% of 0.5 mm), with $\alpha=0.05$ and $1-\beta=0.9$, we calculated that a total of seven pairs would be needed to find significant differences. With a dropout rate of 75%, 28 patients were enrolled for this RCT.

Randomization

Case and control were taken from the same patient using a split-mouth design so that each patient served as his own control. Randomization of the sites for each patient was performed using a coin toss method by a single clinician in charge of randomization. The random sequence was then allocated and concealed till the end of the 6-month follow-up phase. Since the number of patients' withdrawals after randomization of the sites was zero, no loss of information biased the data. For each patient, sites were allocated to receive either material A (placebo) or B (hyaluronan gel).

Surgeries with application of hyaluronan as well as placebo gel were performed after filling both materials in plastic syringes and designating them A and B by the clinician in charge of randomization before handing them to the surgeon. During surgery, the patients as well as the surgeon did not know which site received the hyaluronan gel. To evaluate the surgeon's as well as patients' blinding, they were asked individually after the surgeries if they noted any differences, by which they could tell which site received the hyaluronan or the placebo gel. All patients and the surgeon answered negatively. On subsequent re-evaluation visits, a single clinician who performed the pre-surgical examinations and did not perform the surgeries performed the follow-up examinations for all patients at 3- and 6-month intervals without knowledge about the test versus control sites. Only the study statistician and the clinician in charge of randomization of sites knew about the unblinded data.

Statistical analysis

A Shapiro–Wilk test was performed to test for the normal distribution of data. Descriptive statistics were computed for the variables of CAL, GR, PD, BOP, and PI. The nonparametric Wilcoxon signed-rank test was used for CAL, GR, PD, and PI and a McNemar test was used for BOP to test hypotheses about equal ranks between test and control groups. The nonparametric Friedman test was used

for CAL, GR, PD, and PI and the Cochran test for BOP to test hypotheses about equal ranks over time in each group. All data were statistically analyzed using the SPSS software (SPSS 15, Chicago, IL, USA). The level of significance was set at 0.05 for all tests.

Results

Statistically significant differences ($P<0.05$) were noted between the test and the control sites at 3 and 6 months, where the test sites showed greater gain in CAL and reduction in GR values (Table 1). No statistically significant differences were noted regarding PD (Table 1), BOP, and PI values (Table 2) ($P>0.05$). Over time, a significant improvement of the CAL, PD, and PI values was noted for both test and control sites as well as of BOP values of the control sites ($P<0.05$). Frequency distribution for CAL and PD values was further calculated (Tables 3 and 4). Twelve test (86%) and seven control (50%) sites showed 3 mm or greater gain in CAL, while one test (7%) and three control (22%) sites demonstrated a residual PD of 4 mm or greater by the end of the study time.

Discussion

The current study evaluated the adjunctive effect of hyaluronan gel application in conjunction with periodontal surgery. Hyaluronan application proved to be promising in wound healing in a number of medical disciplines [34]. Its application in conjunction with periodontal surgery was thought to achieve similar beneficial effects.

Previous studies investigated the adjunctive effect of hyaluronan application in the treatment of chronic periodontitis [35, 36]. In these studies, SRP without a flap reflection was instituted prior to the application of the material. The current study applied hyaluronan in conjunction with a MWF. The rationale behind this surgical technique was to surgically remove the entire pocket lining as well as to clean the intrabony defects more thoroughly, via an open approach, prior to the hyaluronan gel application. The removal of the pocket lining by this flap design was to place the hyaluronan gel in direct contact with the connective tissue to perform all its alleged beneficial effects.

This study is the first to report on a significant improvement of CAL following hyaluronan gel application. The present study demonstrated a significant improvement of the CAL, PD, and PI values for the test and control sites over time, as well as a statistically significant difference between the test and control sites for CAL and GR values in favor of the test sites. The improvement in CAL, PD, and

Table 1 Median CAL, GR, and PD values (Q25/Q75)

Treatment	CAL				GR				PD			
	Baseline (mm)	3 months (mm)	6 months (mm)	*P* value	Baseline (mm)	3 months (mm)	6 months (mm)	*P* value	Baseline (mm)	3 months (mm)	6 months (mm)	*P* value
Control sites	5.50 (5.00/8.00)	3.50 (3.00/5.00)	3.00 (2.00/5.00)	<0.001	0.00 (0.00/2.00)	1.00 (0.00/2.25)	0.00 (0.00/1.50)	0.193	5.00 (5.00/6.00)	3.00 (1.75/3.00)	3.00 (2.00/4.00)	<0.001
Test sites	5.50 (2.00/7.00)	3.00 (1.75/3.50)	2.00 (1.00/3.50)	<0.001	0.00 (0.00/0.25)	0.00 (0.00/1.00)	0.00 (0.00/0.00)	0.58	5.00 (5.00/6.00)	2.50 (1.75/3.00)	2.00 (1.00/3.00)	<0.001
P value	0.666	0.032	0.027		0.129	0.026	0.041		0.083	0.603	0.059	

Table 2 Median BOP and PI values (Q25/Q75)

Treatment	BOP				PI			
	Baseline	3 months	6 months	*P* value	Baseline	3 months	6 months	*P* value
Control sites	1.00 (0.75/1.00)	0.00 (0.00/0.00)	0.00 (0.00/1.00)	0.002	0.00 (0.00/1.00)	0.00 (0.00/0.00)	0.00 (0.00/0.50)	0.041
Test sites	0.50 (0.00/1.00)	0.00 (0.00/1.00)	0.00 (0.00/1.00)	0.236	0.00 (0.00/1.00)	0.00 (0.00/0.00)	0.00 (0.00/0.50)	0.022
P value	0.219	0.250	1.000		1.000	0.157	1.000	

PI noted in both groups over time is consistent with an earlier systematic review on the positive effect of surgical debridement on the clinical parameters of chronic periodontitis [37]. The current study results are further consistent with the similarly designed study on the effect of enamel matrix derivative's application in conjunction with the MWF on the clinical outcome of the surgical treatment of intrabony periodontal defects [33].

In contrast to earlier studies by Xu et al. [35] and Johannsen et al. [36], who found no statistically significant difference in CAL between sites receiving hyaluronan as an adjunct to SRP and sites receiving SRP alone, the present study reported an improvement in CAL in the test sites. This is also in contrast to the study of Engström et al. [38], who found no difference in clinical measures between sites treated by guided tissue regeneration (GTR) with hyaluronan and control sites treated with GTR alone. Variations in hyaluronan gel formulations employed in the different studies, treatment modalities and surgical techniques, disease severity, and follow-up intervals may explain the differences in the results between these studies and the present one.

The findings of the present study are in accordance with the study of Prato et al. [39], who concluded that the use of an autologous cell hyaluronic acid graft in tissue engineering results in an increase of gingival height in a very short time without discomfort to the patients. In the present study, sites receiving hyaluronan showed a statistically significant improvement in CAL and GR values at 3 and 6 months compared to the control sites. This may be attributed to the fact that hyaluronan facilitated cell division [34], promoted cell–cell and cell–substrate adhesion, cell migration, proliferation, and activation [40], while acting as a storehouse and a vehicle for growth factors [41, 42]. By interacting with the fibrin clot during the early stages of healing, hyaluronan could have provided a structural framework to modulate the infiltration of various cells into the wound site including fibroblasts, keratinocytes, cementoblasts, and osteoblasts and to stimulate them to produce a series of pro-inflammatory cytokines [42]. It is alleged to be involved in the activation of micro and macrophages, stimulating their migration, adherence at the wound site, phagocytosis, and killing of pathogens making the local condition more favorable for the healing process. Additionally, it is believed to stabilize the granulation tissue at the wound site by scavenging cell-derived oxygen radicals and inhibiting cell-derived enzymes as serine proteases that degrade the extracellular matrix proteins [43]. During this process, hyaluronan contributes to extracellular matrix formation, cell migration into the wound matrix, cell proliferation, and granulation tissue organization, thus allowing the re-attachment of the basal layer of the gingival epithelium to the basal lamina [44]. All these effects may

Table 3 Frequency distribution of CAL values

CAL (mm)	% Control sites			% Test sites		
	Baseline	3 months	6 months	Baseline	3 months	6 months
1			7.1		21.4	28.6
2		14.3	28.6		14.3	42.9
3		35.7	14.3		42.9	21.4
4		21.4	14.3	7.1		
5	50.0	21.4	28.6	42.9	21.4	
6	21.4			21.4		
7			7.1	14.3		7.1
8	28.6	7.1		14.3		

be responsible together or in turn for the improvement of the CAL and GR in sites treated with the hyaluronan.

The current results of an absence of any statistically significant PD reductions between the test and control sites are further in concert with the results of earlier studies [35, 38]. This absence of significant PD reduction may be explained by the fact that the improvement in CAL on the test sites, in which the absence of an improvement in GR could have meant an eventual reduction in PD, was in the current study compensated by the improvement in GR values.

An anti-inflammatory effect of hyaluronan was not substantiated by a statistically significant reduction in BOP index in favor of the test sites by the end of the study. These results are in accordance with the studies of Engström et al. [38] and Xu et al. [35], who failed to demonstrate any significant improvement in BOP. The current study's results are, however, in contrast to the results of Johannsen et al. [36], who found a statistically significant improvement in BOP, in sites treated with hyaluronan gel in conjunction with SRP by the end of their 12-week study. The divergence in follow-up intervals of this study to the current one may account for this discrepancy. The hyaluronan gel may have exerted its reported anti-inflammatory effect during the early phases of wound healing of cellular proliferation and granulation tissue maturation. Yet, this effect was not substantiated at 3 or 6 months postoperatively.

The reduced plaque formation, noted by an improvement in the PI values for the test and control sites over the study time, likely resulted from improved gingival health following the MWF, the reinforcement of oral hygiene instructions given to the study subjects postsurgically, in addition to the improved oral hygiene routines commonly observed in study subjects [45, 46]. However, comparing the test and control sites continued to show a nonsignificant difference regarding their PI values. Similar to earlier investigations [35, 36, 38], the current study did not demonstrate an additional bacteriostatic effect of the hyaluronan gel in terms of a reduced plaque accumulation. This remains in contrast to an earlier in vitro study [44]. The bacterial complexity of the oral environment as opposed to their in vitro investigation could account for this difference to the current results.

Conclusions

Hyaluronan gel application in conjunction with periodontal surgery appears to significantly favor the periodontal surgical outcome in terms of a gain in clinical attachment level as well as a reduction in gingival recession values.

Table 4 Frequency distribution of PD values

PD (mm)	% Control sites			% Test sites		
	Baseline	3 months	6 months	Baseline	3 months	6 months
1		21.4	7.1		21.4	28.6
2		21.4	42.9		28.6	50.0
3		42.9	28.6		35.7	14.3
4	14.3		7.1	7.1	7.1	
5	57.1	14.3	14.3	50.0	7.1	
6	28.6			35.7		
7				7.1		7.1

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