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HUMAN RANDOMIZED CONTROL TRIAL

Clinical effects of the adjunctive use of polynucleotide and hyaluronic acid-based gel in the subgingival re-instrumentation of residual periodontal pockets: A randomized, split-mouth clinical trial

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Abstract

Background: Polynucleotides (PN) and hyaluronic acid (HA) have been effective in stimulating the growth of primary gingival fibroblasts and promoting wound healing. The aim of this study was to investigate the clinical efficacy of a gel containing PN and HA used in association with subgingival re-instrumentation in the treatment of residual periodontal pockets.

Methods: Fifty patients were enrolled in a randomized, split-mouth, single-blind, clinical trial. For each patient, two teeth with similar residual pockets with probing depth (PD) ≥5 mm were selected to receive re-instrumentation with (test group) or without (control group) the adjunctive use of a PN and HA-based gel. Differences in changes of PD, gingival recession, clinical attachment level (CAL), modified sulcular bleeding index (mSBI), plaque index (PI) from baseline to 6, 8, 24, 36, and 48 weeks were analyzed and the frequencies of sites with PD ≤4 mm at 48 weeks were compared.

Results: At 48 weeks, the test group showed better results in terms of PD reduction (2.08 ± 1.24 vs. 1.94 ± 1.19, $p = 0.533$) and sites with PD ≤4 mm (38/50 vs. 35/50, $p = 0.499$), although not statistically significant. Similarly, CAL gain was comparable between groups (test: 0.50 ± 1.85 vs. control: 0.36 ± 1.80, $p = 0.700$). Significantly higher reduction in mSBI was recorded in the test group only in sites with baseline PD ≥6 mm ($p = 0.004$).

Conclusions: The adjunctive use of a PN and HA-based gel could help to ensure a greater reduction of clinical parameters of inflammation in deep residual pockets.

KEYWORDS
hyaluronic acid, periodontitis, polynucleotides, retreatment, root planing

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1 | INTRODUCTION

After initial periodontal treatment a number of periodontal pockets, defined as "residual", often remain.[1] They could provide possible conditions for subgingival recolonization and recurrence of periodontitis with a greater risk of further attachment loss and tooth loss.[2–6] Therefore, they could require a further step of therapy consisting in either subgingival re-instrumentation with or without adjunctive therapies or periodontal surgery.[7,8]

Repeated subgingival instrumentation as exclusive therapy did not show to substantially improve the clinical outcomes in previously treated areas.[9,10] Tomasi et al. (2008) showed that retreatment alone is able to reach the end point of pocket closure (PC)—understood as probing depth (PD) ≤4 mm—at 9 months in the 53% of cases; however, considering only pockets with baseline PD >6 mm, the probability is only 17%.[11] Various adjunctive therapies to nonsurgical retreatment have been investigated to reduce the need for periodontal surgery, including locally delivered doxycycline, diode soft laser therapy, antimicrobial photodynamic therapy, 25% tetracycline fibers, 2% minocycline gel, 25% metronidazole gel, enamel matrix derivatives, and hyaluronic acid.[11–16] In the majority of studies, the additional PD reduction was reported to be between 0.7 and 1.8 mm.

Hyaluronic acid (HA) is synthesized by fibroblasts, keratinocytes, periodontal ligament cells, cementoblasts, and osteoblasts.[17,18] In addition to hygroscopic and viscoelastic characteristics, through which it plays the important role of maintaining the structural and homeostatic tissues integrity, bacteriostatic,[19,20] fungistatic,[21] anti-inflammatory,[22] anti-edema,[23] proangiogenic,[24] and osteoinductive[22,25] properties were also described. Furthermore, it seemed to be associated with scarless wound repair.[26,27] Data from clinical research provided evidence of the efficacy of HA in the surgical treatment of periodontal intrabony and mucogingival defects.[28,29] The periodontal wound healing/regeneration effects were further supported by in vitro studies showing that HA: (a) acts as lubricant and space filler[30]; (b) preserves human periodontal ligament cell viability and increased early osteogenic differentiation[31]; (c) controls the balance between self-renewal and differentiation during bone regeneration[32]; and (d) induces root cementum, periodontal ligament, and bone formation in experimentally created two-wall intrabony defects in dogs.[33] Based on the above-mentioned properties, HA effects on nonsurgical periodontal treatment were also investigated. In particular, it was applied in (a) initial periodontal treatment as a monotherapy or associated with subgingival instrumentation, showing moderate improvement of clinical parameters;[34,35] and (b) as an adjunctive therapy in conjunction to subgingival re-instrumentation of residual pockets, resulting in nonstatistically significant differences compared with nonsurgical retreatment alone.[16]

Polynucleotides (PN) are natural-origin, highly purified DNA biopolymers from trout gonads. They are highly hydrophilic polymers that bind water molecules, providing a three-dimensional viscoelastic gel that provides persistent hydration and viscosupplementation.[36] Data from in vitro and in vivo studies showed that PN in a fixed coformulation with HA: (a) has a high trophic effect and accelerates venous lower limb ulcers healing rate[37]; and (b) improves properties of synovial fluid and reduces pain in patients with knee osteoarthritis.[38,39] Recently, an in vitro study investigating the effect of PN with or without HA on gingival fibroblasts concluded that the addition of HA further increased the effectiveness of PN and that this combination has been effective in stimulating primary gingival fibroblast growth, thus promoting wound healing.[40]

However, to the best of our knowledge, no study has been performed to evaluate whether subgingival re-instrumentation of residual pockets after active initial periodontal therapy could benefit from the adjunctive nonsurgical application of a gel containing PN and HA.

Therefore, the aim of this randomized controlled clinical trial was to investigate the clinical efficacy of a gel containing PN and HA used in association with subgingival re-instrumentation in the treatment of residual periodontal pockets.

2 | MATERIALS AND METHODS

2.1 | Study design and ethical aspects

The study was designed as a randomized, split-mouth clinical trial of 12-months duration. The research protocol (ClinicalTrials.gov- NCT05210686) was approved by the Ethical Committee of Sapienza, University of Rome (#4766; 947/17; approval date: 12/10/2017) and was in accordance with the Declaration of Helsinki, as revised in 2008. Informed consent was obtained for all the participants. The trial was conducted at the Section of Periodontics of the Department of Oral and Maxillofacial Sciences of Sapienza, University of Rome between February 2018 and December 2019.

2.2 | Patient selection

Inclusion criteria were: (a) males and females aged ≥18 years; (b) stage 3 generalized periodontitis; (c)

3–6 months after step 1 and 2 of periodontal treatment, at least two nonadjacent teeth showing one residual pocket with PD ≥5, without mobility and furcation involvement.

Exclusion criteria were: (a) full-mouth plaque score and full-mouth bleeding score > 20%; (b) inadequate restorative therapy or malocclusion; (c) uncontrolled systemic disease; (d) immunosuppressive therapy or therapy with corticosteroid/bisphosphonates; (e) oral cavity inflammatory and autoimmune diseases; (f) history of malignancy, radiation therapy or chemotherapy in the last 5 years; (g) insulin-dependent diabetes; (h) smoking (> 10 cigarettes per day); (i) drug and alcohol abuse; and (j) pregnant or lactating.

2.3 | Sample size

The sample size calculation was based on a previous study on periodontal re-instrumentation (Aimetti et al. 2004).[13] PD reduction (change) at 48 weeks was selected to determine the sample size. Considering an alpha error of 0.05, the power calculation based on the detection of a 0.85-mm difference in mean PD reduction between treatment sites (assuming a standard deviation (SD) of 1.2 mm) revealed that 42 sites were required for each treatment modality to have a power of 90%. Considering a possible dropout of 20%, 50 sites in each treatment group were estimated to be necessary for the study.

2.4 | Initial periodontal treatment

Three to six months before the study, initial periodontal therapy was performed in the same center and consisted in oral hygiene instructions (OHI), local and systemic risk factor for periodontitis onset and progression control, supragingival and subgingival biofilm, and calculus removal with manual and/or ultrasonic instrumentation under local anesthesia. No adjunctive local or systemic antibiotics, host-modulating agents, and physical or chemical agents were used at this stage. This phase was not part of the study design, and it was accomplished by different operators within the center using the same treatment modalities and instruments.

2.5 | Periodontal re-instrumentation

For each patient, periodontal retreatment was performed in the two selected residual pockets at the same appointment. For both test and control sites, re-instrumentation involved the use of mini curettes[†] and/or ultrasonic instrumentation with periodontal tips,[‡] under local anesthesia. In the test sites, after rinsing with sterile saline solution and gently air-drying (with extreme caution not to direct the tip of the air syringe towards the epithelial attachment on the gingival crevice), a viscoelastic gel containing a fixed combination of natural origin PN (10 mg/ml, 1%) and HA with molecular weight > 1500 kDa (10 mg/ml, 1%)[§] was gently applied using a sterile prefilled (1 ml) glass syringe with a blunt tip until overflowing from the periodontal pocket.

The gel is a commercially available as a Class III medical device, as no-prescription medical device. All procedures were performed by the same investigator.

Subjects received the following postoperative instructions. First, rinse with 0.12% chlorhexidine[‖] (1 min, twice a day for 4 weeks). They were instructed to avoid oral hygiene procedures for the first 2 weeks in the treated areas. Subsequently, a soft toothbrush[¶] was indicated. Normal hygiene practices with interdental cleaning devices[#] were resumed after the fourth week.

Follow-up visits were scheduled at 6, 8, 24, 36, and 48 weeks. At each recall appointment, OHI instructions were reinforced and the supragingival plaque was eliminated if present.

2.6 | Randomization and allocation concealment

A randomization list was generated using dedicated software.[**] For each patient, the study treatment was assigned to each of the two eligible sites according to the randomization list. Allocation concealment was performed using sealed envelopes to be opened after subgingival re-instrumentation. The generation of the random allocation sequence, the enrollment of participants, and the assignment of participants to interventions were performed by an investigator, other than the clinical examiner and the operator who provided the treatment. As indicated inside the envelope, the investigator applied a gel containing PN and HA (test site) or no additional treatment (control site).

[†] Hu-Friedy, Chicago, IL
[‡] Perio Slim, E.M.S. Electro Medical System S.A., Nyon, Switzerland
[§] REGENFAST, Mastelli S.r.l., Sanremo, Italy
[‖] Paroex, Sunstar Americas, Schaumburg, IL
[¶] TePe Select Compact Soft Toothbrush, TePe Munhygien produkter AB, Malmö, Sweden
[#] TePe Interdental Brushes Original, TePe Munhygien produkter AB, Malmö, Sweden
[**] PROC PLAN of SAS 9.4 for Windows, SAS Institute, Cary, NC

2.7 | Clinical measurements

Clinical evaluations were performed by a masked calibrated examiner. At baseline and at 6, 8, 24, 36, and 48 weeks (visits 1–6), the following parameters were evaluated using a periodontal probe[††]:

- Plaque index (PI),[41] as described by Silness and Löe (1964);
- Modified sulcus bleeding index (mSBI)[42], as proposed by Mombelli et al. (1987), modified as follows: score 0: no bleeding when inserting a periodontal probe into the periodontal pocket; score 1: isolated bleeding spots visible; score 2: blood forms a confluent red line on margin; score 3: heavy or profuse bleeding;
- Probing depth (PD);
- Gingival recession (REC);
- Clinical attachment level (CAL).

The examiner underwent a training and calibration session on five patients not included in the investigation, in which he was asked to evaluate PI, mSBI, PD, REC, and CAL at six sites per each tooth in two occasions 120 min apart. Calibration was not considered adequate if the intraclass correlation coefficient was < 0.81.

At each visit, the investigator had to record possible complications or adverse events related to the study device or study interventions, as well as to collect those reported by patients.

2.8 | Statistical analysis

Data analysis was accomplished by a statistician using statistical software.[‡‡] Continuous variables were expressed as mean and SD. Categorical variables were summarized as frequency and percentage. Adherence to normal distribution was evaluated with Shapiro-Wilk test. Considering the nature of the split-mouth design in which both test and control groups are related, the changes in PD (primary outcome) and REC, CAL, mSBI, and PI (secondary outcomes) between test and control sites and within each group from baseline to each follow-up visit were analyzed using paired T-test (in presence of normal distribution) or Wilcoxon signed-rank test (in presence of non-normal distribution). Chi-square test was used to determine whether there was a statistically significant difference between test and control groups in the proportions of sites showing PD ≤4 mm at 48 weeks. A significance level of 0.05 was used for all statistical tests.

[††] PCP-UNC 15, Hu-Friedy, Chicago, IL

[‡‡] SAS 9.4 for Windows, SAS Institute, Cary, NC

3 | RESULTS

Fifty patients (age range, 31–71 years; mean age, 56.15 ± 8.87; 24 females and 26 males; nine smokers [≤10 cigarettes/day]) were enrolled in this study. All patients completed the investigation with no dropouts. Figure 1 shows the CONSORT flowchart of patient enrollment, allocation, follow-up, and analysis.

Table 1 shows mean values of clinical parameters at baseline and changes at 6, 8, 24, 36, and 48 weeks at test and control sites. At baseline, 31/50 test and 30/50 control sites showed PD ≥6 mm ($p = 0.591$). Re-instrumentation with and without adjunctive therapy resulted in statistically significant PD reduction at each follow-up. At 48 weeks, the mean PD in test and control sites was reduced by 2.08 ± 1.24 mm ($p < 0.0001$) and 1.94 ± 1.19 mm ($p < 0.0001$), respectively (Table 1, Figure 2A). However, there was no statistically significant difference between treatments in any time interval. When sites were subdivided according to their baseline PD (< 6 mm or ≥6 mm), the test treatment obtained a greater reduction in PD than the control treatment at 48 weeks (sites with baseline PD < 6 mm: 1.74 for test group vs 1.60 for control group; sites with baseline PD ≥ 6 mm: 2.32 for test group vs 2.13 for control group); but statistically significant differences were not yet observed between the two groups throughout the study (Figure 2C). In addition, test sites showed a greater tendency for PC at 48 weeks with a higher percentage of residual pockets reaching PD ≤4 mm (76%) versus re-instrumentation alone (70%), although this difference was not significant ($p = 0.499$) (Table 2).

Similarly, mSBI decreased statistically significantly during the study in both groups. At 48 weeks, the reduction from baseline was 0.38 ± 0.92 and 0.30 ± 0.99 at test and control sites, respectively (Table 1, Figure 2B).

In the sites that showed baseline PD values ≥6 mm, statistically significant differences between groups were observed at 48 weeks (test sites, 0.50; control sites, 0.18; $p = 0.004$) (Figure 2D).

In both groups, the PD reduction was accompanied by a CAL gain at 48 weeks (0.50 ± 1.85 vs. 0.36 ± 1.80, for test and control sites, respectively) and an REC increase (test sites: 1.60 ± 1.16 vs. control sites:1.60 ± 1.34), with no differences between groups at any follow-up visit (Table 1).

No difference was observed between the two groups in plaque deposits. In both cases, the presence of supragingival biofilm was minimal, with a slight increase over the course of the study (Table 1).

No complications or adverse events related to the study device were observed or referred by patients.

The investigational device confirmed to be a very well tolerated and safe product when used in periodontal therapy.

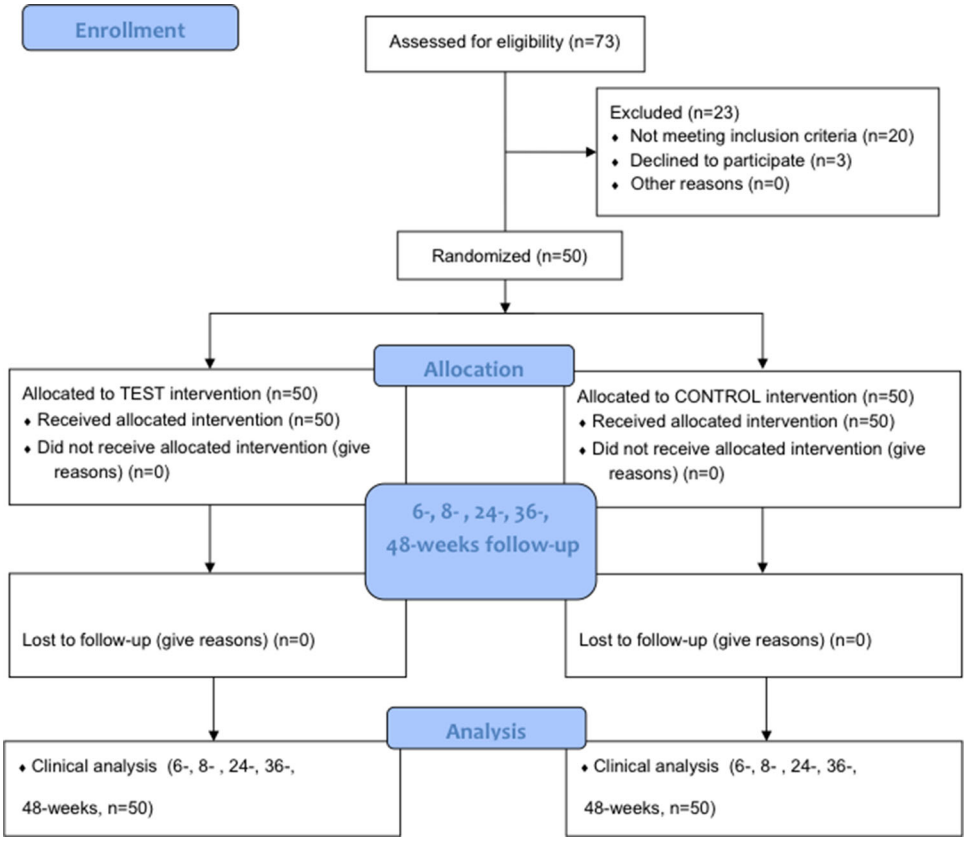

FIGURE 1 CONSORT flowchart of patient enrollment, allocation, follow-up, and analysis.

4 | DISCUSSION

Based on scientific evidence, the recent EFP guidelines for the treatment of patients with Stage I–III periodontitis have suggested additional nonsurgical treatment for residual pockets up to 5 mm and surgical therapy for sites >5 mm.[7,8] In the present randomized, split-mouth study, it was evaluated whether, using a gel containing PN and HA, it was possible to enhance the results of subgingival re-instrumentation in residual pockets ≥5 mm, to reduce the cases for which the surgery is needed. For these reasons, the results of this trial could be of high clinical interest, although no statistically significant differences were observed for most of the clinical parameters evaluated, since the results have shown that: (a) the additional use of this gel tends to promote superior results in terms of PD reduction; (b) at 48 weeks, there were more sites with PD < 5 mm in the test group; (c) the PD and mSBI reduction occurred to a greater extent in the first weeks and it was greater in the deeper pockets; (d) at 48 weeks, test sites showed a higher tendency for PC; (e) similarly in both groups, the CAL gain was higher at the first follow-up visits and then decreased during the follow-up visits due to REC increase.

The concentration of the fixed combination of PN and HA used in this trial was supported by previous studies in other medical fields where its use has shown to be promising for the treatment of wounds and ulcers and in intradermal and intra-articular infiltrations.[37–39] Furthermore, the concentration used for this clinical in vivo study did not cause adverse events or complications and was well tolerated and safe.

At 48 weeks, the PD reduction was 2.08 ± 1.24 for test and 1.94 ± 1.19 for control group, respectively. These values are slightly higher than those of previous studies that evaluated adjunctive local therapies in association with subgingival re-instrumentation including locally administered doxycycline,[11] 25% tetracycline fibers, 2% minocycline gel, 25% metronidazole gel,[12] and tetracycline-loaded fibers.[13] The discrepancy between the present trial and the above-mentioned studies could probably be due to the differences in site selection and in the study design. In the Tomasi and Wennström study, furcation lesions were treated while in the present study teeth with furcation involvement were not included.[11] Moreover, in the present investigation, moderate sites were selected (baseline PD: 5.84 ± 0.82 and 5.88 ± 1.04 for test and control group, respectively), while in the other studies baseline PD values

TABLE 1 Mean ± SD of clinical parameters at baseline and changes at 6, 8, 24, 36, and 48 weeks at test and control sites

	Baseline		△ Baseline – 6 weeks		△ Baseline –8 weeks		△ Baseline – 24 weeks		△ Baseline –36 weeks		△ Baseline –48 weeks	
Parameter	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**
PD (mm)												
Test	5.84 ± 0.82	–	- 1.60 ± 0.97	* < 0.0001	- 1.90 ± 0.97	* < 0.0001	- 2.02 ± 1.20	* < 0.0001	- 1.92 ± 1.08	* < 0.0001	- 2.08 ± 1.24	* < 0.0001
Control	5.88 ± 1.04	–	- 1.68 ± 1.06	* < 0.0001	- 2.08 ± 1.14	* < 0.0001	2.08 ± 1.16	* < 0.0001	- 2.08 ± 1.26	* < 0.0001	– 1.94 ± 1.19	* < 0.0001
Test versus Control		0.8314		0.6874		0.4226		0.8074		0.4553		0.5334
CAL (mm)												
Test	5.94 ± 0.89	–	- 1.28 ± 1.21	* < 0.0001	-1.44 ± 1.20	* < 0.0001	- 1.02 ± 1.56	* < 0.0001	- 0.54 ± 1.61	*[0].0214	- 0.50 ± 1.85	0.0624
Control	5.98 ± 1.12	–	- 1.40 ± 2.16	* < 0.0001	- 1.62 ± 1.44	* < 0.0001	- 1.12 ± 1.75	* < 0.0001	- 0.88 ± 1.85	*[0].0015	- 0.36 ± 1.80	0.1646
Test versus Control		0.8433		0.5951		0.4695		0.7727		0.3205		0.7002
REC (mm)												
Test	0.10 ± 0.36	–	0.32 ± 0.68	*0.0017	0.58 ± 0.86	* < 0.0001	1.00 ± 0.99	* < 0.0001	1.40 ± 1.16	* < 0.0001	1.60 ± 1.16	* < 0.0001
Control	0.10 ± 0.51	–	0.28 ± 0.67	*0.0049	0.48 ± 0.91	*[0].0005	0.96 ± 1.14	* < 0.0001	1.24 ± 1.30	* < 0.0001	1.60 ± 1.34	* < 0.0001
Test versus Control		1.0000		0.7189		1.0000		0.8523		0.4918		1.0000
mSBI												
Test	0.60 ± 0.95	–	- 0.30 ± 1.04	*[0].0458	- 0.34 ± 1.06	*[0].0280	- 0.28 ± 1.11	0.0800	- 0.26 ± 1.03	0.0794	- 0.38 ± 0.92	*0.0054
Control	0.66 ± 0.96	–	- 0.44 ± 0.93	*[0].0016	- 0.38 ± 0.95	*0.0065	-0.24 ± 1.06	0.1160	- 0.38 ± 0.99	*0.0090	- 0.30 ± 0.99	*0.0380
Test versus Control		0.7539		0.2538		0.7275		0.7664		0.3710		0.5518
PI												
Test	0.06 ± 0.24	–	0.12 ± 0.48	0.0832	0.20 ± 0.44	*0.0062	0.28 ± 0.57	*0.0011	0.22 ± 0.51	*0.0035	0.18 ± 0.52	*0.0062
Control	0.06 ± 0.24	–	0.14 ± 0.45	*0.0334	0.06 ± 0.37	0.2610	0.20 ± 0.49	*0.0062	0.20 ± 0.45	*0.0029	0.20 ± 0.49	*0.0185
Test versus Control		1.0000		0.8111		0.0897		0.3509		0.7846		0.8212

Abbreviations: CAL, clinical attachment level; mSBI, modified sulcular bleeding index; PD, probing depth; PI, plaque index; REC, gingival recession; SD, standard deviation.

*Indicates statistically significant differences ($p < 0.05$) between treatment groups ($p > 0.05$).

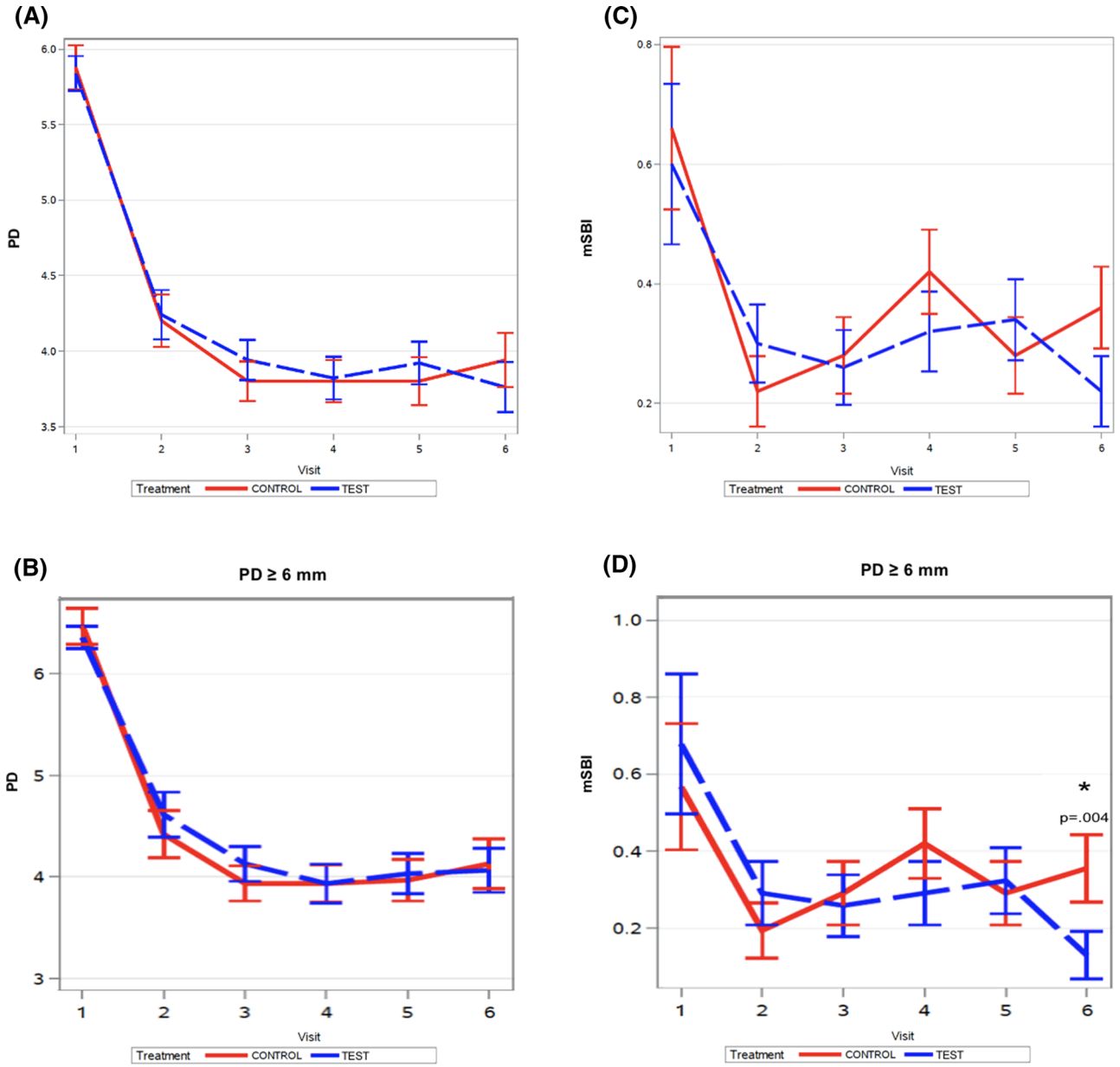

FIGURE 2 (**A** and **C**) Mean values and SDs of probing depth (PD) and modified sulcus bleeding index (mSBI) in test (re-instrumentation + polynucleotide and hyaluronic acid-based gel) and control (re-instrumentation alone) sites at visit 1 (baseline), 2 (week 6), 3 (week 8), 4 (week 24), 5 (week 36), and 6 (week 48). (**B** and **D**) Mean values and SDs of PD and mSBI in test and control sites with the baseline PD ≥6 mm. * Statistically significant, $p < 0.05$.

TABLE 2 Proportion of sites with PD ≤4 mm

	Test	Control	*p* value
Baseline	0/50	0/50	
48 Weeks	38/50 (76%)	35/50 (70%)	0.499

There were no statistically significant differences between treatment groups ($p > 0.05$).

were lower.[12,13] Nevertheless, the PD reduction after 6 months in the group treated with PN and HA was similar to those reported after the use of antimicrobial photodynamic therapy (2 mm), and enamel matrix derivatives (2.1 mm) as adjunctive therapies.[14,15] Furthermore, the values observed in both groups of the present study were also similar to those reported in a previous recent study evaluating the effect of HA-based gel in the residual pockets treatment.[16]

At the end of the trial, a higher tendency for achievement of PC at test sites was observed, although the difference with control sites was not statistically significant. Comparably, in the above-mentioned study evaluating the effect of an HA-based gel in the treatment of periodontal residual pockets, at 3 months from the first application the percentage of patients that reached PC was greater in the

HA group, despite not statistically significant, and it was maintained almost stable over time.[16] The results of the present investigation on the effectiveness of PN and HA showed a similar pattern compared with the previously quoted study and resulted in a similar percentage of test sites (76% vs. 77%) and a lower percentage of control sites (70% vs. 78%) which achieved PC at 12 months.

The importance of bleeding on probing as an indicator of inflammation and its association with a state of activity of periodontitis and a greater risk of progression is well known.[43] With the scope of having the opportunity to detect more subtle differences in the variations of periodontal inflammation, an index with values from 0 to 3 (mSBI),[42] based on the magnitude of bleeding, was used. A greater reduction in mSBI in the test group after 48 weeks in deep residual pockets (PD ≥6 mm) was observed, and this difference was statistically significant ($p = 0.004$). However, the significance in terms of bleeding reduction at 48 weeks may not be clinically relevant, as there is usually a shorter time interval between retreatment and the decision for the need of further treatment of the residual pocket. Therefore, this result could be interpreted with caution. The beneficial effects on periodontal inflammatory parameters by the combination of PN and HA compared with HA alone could be further hypothesized if it is considered that in a previous study on the additional use of HA in step 3 of periodontal therapy there were no significant changes observed for the bleeding on probing compared with re-instrumentation alone after 12 months.[16] However, in another study the authors reported that bleeding index improved significantly after HA application, but the final evaluation was performed at 3 months.[44]

Baseline PD, tooth anatomy, bony defect morphology, furcation involvement, mobility, and smoking affect the likelihood of subgingival re-instrumentation to determine PC.[11,45–47] According to the literature, the smoking history was recorded but smoking was not considered an exclusion criterion.[11,14] Similar to the most recently published studies on periodontal retreatment, this research only selected subjects who consumed no more than 10 cigarettes per day.[15,16] Nine out of 50 patients included in this study were smokers, similarly to a previous study using enamel matrix derivatives in which 11 of 44 were smokers.[15] The split-mouth design did not allow smoking to influence the healing response of one treatment over the other.

In this study, a considerable amount of follow-up visits were scheduled. The early control appointments were performed at both 6 and 8 weeks, differently from previous researches on this topic which selected only one of these time intervals.[12,14] However, since PN and HA showed favorable properties in early phases of wound healing [22-24,37,40] it was considered of interest to investigate the earliest time point at which the present adjunctive treatment could have reached its greatest efficacy. Interestingly, in both groups PD and mSBI reduction occurred to a greater extent after 8 weeks. Conversely, 6 weeks seems not enough to achieve the highest treatment response. For analogous reasons, the long-term efficacy was assessed at 6, 9, and 12 months to better elucidate how long the treatment effects were sustained. This did not cause higher costs for the present study, since usually patients showing residual pockets with PD ≥ 5 mm are susceptible to further periodontal breakdown and should be targeted with additional periodontal treatment or shorter recall intervals during supportive periodontal therapy, namely, every 3 to 4 months.[48] In view of this, motivational reinforcement and repetition of OHIs at each follow-up visit of the study, as well as the removal of dental plaque present, do not seem far from clinical practice. In general, the effects on PD reduction were well maintained up to 12 months in both groups, while those on mSBI reduction starts declining from the sixth month in the control group while they improved up to 12 months in the test group.

Limitations of this clinical study should be mentioned. First, initial periodontal therapy of patients treated in this trial was not part of the study design. It consisted in OHI, local and systemic risk factor for periodontitis onset and progression control, supragingival and subgingival biofilm and calculus removal with manual and/or ultrasonic instrumentation under local anesthesia, but it was accomplished by different operators within the same center. Therefore, a possible different quality of performance of the pre-study phase could have affected the results of the subsequent retreatment. However, this aspect is common to all previously published comparable studies.[11–16] Second, using a split-mouth design, it was possible to eliminate patient variability effect, but the carryover effect, that is, the possibility that the performed treatment at one site affects the outcomes of the treatment performed at another site, must be considered. In addition, multiple sites could have been evaluated in the same patients to better compare treatment efficacy.

Further studies to confirm the findings of this investigation should evaluate these shortcomings, as well as considering performing a microbiological and biochemical analysis. Finally, the effects of the repeated application of the gel based on PN and HA on the periodontal clinical parameters could be studied. However, in this regard a recent study showed that a second application of HA-based gel did not add substantial additional benefits.[16] Furthermore, it has been reported that, because of the synergy between PN and HA (based in viscoelastic and mechanical properties) and the high molecular weight HA used in the formulation, it may not be necessary to perform repeated applications of this gel to obtain better and/or stable results over time.[39]

5 | CONCLUSIONS

The present clinical investigation showed that the additional use of a gel containing PN and HA in the re-instrumentation of residual periodontal pockets could be a safe, nonpharmacological, topical treatment that might help to improve periodontal wound healing and reduce the clinical parameters of inflammation in deep periodontal pockets. Nevertheless, it does not seem to provide further benefits when compared with subgingival re-instrumentation alone.

This medical device could be useful to avoid periodontal surgery in sites where it could be possible to achieve PC without bleeding, or to improve the condition of the surgical site—by promoting the wound healing process and decreasing parameter related to local inflammation—before intervention.

AUTHOR CONTRIBUTIONS

Andrea Pilloni: Conceptualization; Formal Analysis; Methodology; Project Administration; Supervision; Visualization; Writing – Original Draft Preparation; Writing – Review and Editing. Mariana A. Rojas: Formal Analysis; Visualization; Writing – Original Draft Preparation; Writing – Review and Editing. Cinzia Trezza: Data Curation; Investigation. Mauro Carere: Data Curation; Investigation. Alessandro De Filippis: Data Curation; Investigation. Rosalia L. Marsala: Data Curation; Investigation. Lorenzo Marini: Formal Analysis; Supervision; Visualization; Writing – Original Draft Preparation; Writing – Review and Editing. All authors critically revised the manuscript, gave their final approval and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors report that they have no conflicts of interest related to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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JOURNAL OF Periodontology

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