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Non-Surgical Treatment of Moderate Periodontal Intrabony Defects With Adjunctive Cross-Linked Hyaluronic Acid: A Single-Blinded Randomized Controlled Clinical Trial

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ABSTRACT

Aim: To evaluate the clinical outcomes of moderate intrabony defects treated with minimally invasive non-surgical technique (MINST) with or without adjunctive delivery of cross-linked hyaluronic acid (xHyA) gel.

Materials and Methods: Forty-two patients with 42 interdental intrabony defects were randomly assigned to test (MINST + xHyA) or control procedures (MINST alone). Probing depth (PD), clinical attachment level (CAL), gingival recession (GR) and bleeding on probing (BOP) at the treated sites were assessed at baseline and at 3 and 6 months. Full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) were recorded at baseline and after 6 months. Radiographic evaluation was performed at baseline and after 6 months, assessing the defect fill (DF) and radiographic defect angle (RDA). The primary outcome variable was PD change.

Results: Thirty-eight patients completed the trial without any adverse events. At 6 months, a statistically significant improvement ($p < 0.05$) was measured in all clinical parameters except GR ($p > 0.05$). However, no statistically significant differences were found between the experimental and control procedures ($p > 0.05$). Statistically significant differences between the test and control sites were observed at 3 months for PD and CAL changes ($p < 0.05$). The DF change was statistically significant when comparing experimental and control procedures at 6 months ($p < 0.05$). Both procedures failed to show statistically significant differences in terms of RDA changes at 6 months ($p > 0.05$).

Conclusion: Within their limitations, the present results indicate that (a) treatment of intrabony defects with MINST, with or without application of xHyA gel, resulted in statistically significant improvements in the investigated clinical parameters at 3 and 6 months after therapy, and (b) although the adjunctive use of xHyA gel to MINST improved the clinical outcomes compared with MINST alone up to 3 months, statistically significant differences were not observed at 6 months. The study protocol was registered in [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT05188898).

1 | Introduction

At present, robust evidence is available demonstrating that professional mechanical removal of subgingival plaque using

machine-driven or hand instruments represents a crucial step in the periodontal treatment and is able to re-establish periodontal health without the need for additional periodontal surgery (Lang, Salvi, and Sculean 2019). Data from a

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systematic review (Suvan et al. 2020) reported a proportion of closed pockets (i.e., probing depth [PD] ≤ 4 mm) of 57% at 3 months, while it was 74% at 6 months. These results were independent of the non-surgical periodontal protocols applied (i.e., quadrant-wise or full-mouth approaches) or the instruments used (i.e., sonic/ultrasonic devices, hand instruments or a combination of both). Although the majority of periodontal pockets are 'closed' following non-surgical periodontal treatment, and there should be no difference in response to non-surgical therapy based on the presence of intrabony defects (Tomasi, Leyland, and Wennstrom 2007), in certain clinical situations (i.e., deep periodontal pockets) pocket closure may not be achieved.

After completion of active periodontal therapy, residual pockets (i.e., PD ≥ 5 mm) associated with intrabony defects present a risk factor for disease progression and may require additional surgical therapy (Matuliene et al. 2008; Papapanou and Wennstrom 1991). Usually, intrabony defects are considered candidates for periodontal surgical procedures using different regenerative biomaterials (Iorio-Siciliano et al. 2014; Matarasso et al. 2015; Nibali et al. 2020) and flap designs (Windisch et al. 2022). However, in the last years several authors have proposed treatment of intrabony defects by means of a minimally invasive non-surgical technique (MINST) based on the use of mini and micro instruments in combination with magnification loupes (Barbato et al. 2020). The MINST approach potentially reduces the post-operative trauma and gingival recessions, thus preserving the aesthetics (Iorio-Siciliano et al. 2021; Riberio et al. 2023) while yielding substantial clinical and radiographic improvements in intrabony defects (Nibali et al. 2015, 2018). In addition, similar PD reduction and clinical attachment level (CAL) gain were noted when comparing MINST with minimally invasive surgical approaches without biomaterials in the treatment of intrabony defects (Riberio et al. 2011). It has been suggested that these results depend on the accurate professional mechanical removal of subgingival plaque and formation of a stable blood clot. To enhance the clinical benefits of MINST in terms of blood clot stabilization and acceleration of healing processes, several authors have proposed the use of adjunctive therapies delivered alongside professional mechanical removal of subgingival plaque, such as the application of an enamel matrix derivative (EMD) (Graziani et al. 2019; Jentsch et al. 2021). However, the adjunctive use of EMD following non-surgical subgingival professional mechanical plaque removal does not seem to additionally improve the clinical outcomes when compared to non-surgical subgingival instrumentation alone (Rocuzzo et al. 2022). Likewise, Anoixiadou and co-workers did not find any statistically significant changes in clinical and radiographic parameters 12 months after treatment of intrabony defects using MINST with or without the local application of EMD (Anoixiadou, Parashis, and Vouros 2022).

On the contrary, a systematic review reported a moderate benefit of local application of hyaluronic acid (HA) on the clinical outcomes following non-surgical periodontal therapy (Eliezer et al. 2019). HA stimulates blood clot formation and shows a bacteriostatic effect on periodontal bacterial pathogens (Scully et al. 1995). Moreover, HA plays a crucial role in each phase of wound healing by stimulating cell proliferation

(Olczyk et al. 2008) and inducing angiogenesis and osteogenesis (Bezerra et al. 2012). In the last years, a new formulation of cross-linked hyaluronic acid gel of non-animal origin with high molecular weight (xHyA) was proposed to improve wound healing and regenerate the periodontal tissues (Mendes et al. 2008). A series of histological studies reported that intrabony defects, gingival recessions and furcation defects treated using xHyA gel showed a greater area of new cementum and new periodontal ligament (Shirakata, Imafuji, et al. 2021; Shirakata, Nakamura, et al. 2021; Shirakata et al. 2022). These preclinical observations have been corroborated by clinical studies indicating a substantial benefit of xHyA in the treatment of gingival recessions and intrabony defects (Pilloni, Rojas, et al. 2021; Pilloni, Zeza, et al. 2021; Pilloni et al. 2019).

However, it is currently unknown to what extent the use of xHyA in conjunction with MINST may further improve clinical outcomes in intrabony defects compared to the use of MINST alone. Therefore, the aim of present study was to clinically and radiographically evaluate the outcomes obtained at 6 months following the treatment of moderate intrabony defects using MINST with or without adjunctive delivery of xHyA.

2 | Materials and Methods

2.1 | Study Design

The study was designed as a superiority, parallel-arm, single-blinded randomized controlled trial (RCT) with a 6-month follow-up. The idea was to test the null hypothesis of no statistically significant differences with respect to PD change.

In each patient, one intrabony defect was selected for the investigation. The intrabony defects were randomly assigned at test or control procedure. Intrabony defects of the test group were treated by means of MINST and xHyA gel as adjunct, while in the control group MINST alone was performed. The study was conducted at the Department of Periodontology, University of Naples Federico II, from January 2022 to March 2023. The Research Protocol was submitted to and approved by the Institutional Review Board (IRB) of the University of Naples Federico II (Approval Number: 141/21) and the study protocol was registered at [ClinicalTrial.gov](https://clinicaltrials.gov) (No. NCT05188898). Furthermore, written consent was obtained from all patients before the investigation. The study is reported according to CONSORT statement and it was conducted in observance to the Principles of the Declaration of Helsinki on experimentation involving human subjects.

2.2 | Patient Sample

From the patient pool of the Department of Periodontology, University of Naples Federico II, patients diagnosed with periodontitis according to Tonetti, Greenwell, and Kornmann (2018) were invited to participate in the study.

After initial screening, an accurate periodontal exam confirming the diagnosis of periodontitis was made. Patients who met the eligibility criteria were enrolled in the study.

The inclusion criteria were as follows:

- Males and females aged ≥ 18 years.
- Patients with diagnosis of periodontitis (stage III or IV) (Tonetti, Greenwell, and Kornmann 2018).
- Single-rooted and multi-rooted teeth in both arches.
- Presence of interdental periodontal defects with PD ≥ 5 mm associated with an intrabony component ≥ 2 mm at single-rooted teeth or at molars with \leq class I furcation involvement.
- One intrabony defect was treated per patient. If multiple teeth presented pockets associated with an intrabony defect, only the site with the deepest PD was selected for the study. In case of same PD in two or more intrabony defects per patient, the site with the deepest radiographic intrabony component was selected.

The exclusion criteria were as follows:

- Patients with systemic diseases.
- Pregnant or lactating females.
- Tobacco smokers (≥ 10 cigarettes per day).
- Multi-rooted teeth with class II and class III furcation defects.
- Third molars.
- Teeth with grade III mobility.
- Peri-apical pathology and acute abscess.
- Non-surgical or surgical periodontal treatment in the past 12 months.
- Prolonged treatment with antibiotics or anti-inflammatory agents within 6 months prior to periodontal therapy.
- Patients without adequate level of oral hygiene following step 1 of periodontal therapy (full-mouth plaque score [FMPS] $\geq 20\%$).
- Patients without adequate level of oral hygiene at the 1-, 3- and 6-month follow-up visits (FMPS $\geq 20\%$).

The initial periodontal screening took place from October 2021 to December 2021, while the trial was conducted from January 2022 to March 2023.

2.3 | Clinical and Radiographic Outcome Measures

2.3.1 | Primary Outcome

The primary outcome was the change in PD measured from the gingival margin to the bottom of the pocket.

2.3.2 | Secondary Outcomes

The following secondary clinical and radiographic outcomes were assessed:

- FMPS, representing the percentage of sites covered with plaque (O'Leary, Drake, and Naylor 1972).
- Full-mouth bleeding score (FMBS), representing the percentage of sites with bleeding on probing (Claffey et al. 1990).
- CAL, measured from the cemento-enamel junction (CEJ) to the bottom of the pocket.
- Gingival recession (GR), measured from the CEJ to the gingival margin.
- CEJ–bottom of the defect (CEJ-BD), measured from the CEJ to the most apical extension of the bone defect.
- Defect fill (DF), calculated as the difference between CEJ-BD at baseline and after 6 months.
- Radiographic defect angle (RDA), defined as angle between the line connecting the CEJ of the tooth presenting the intrabony defect to the most apical point of the defect and the line connecting the most apical point of the defect and the point where the bone crest touched the neighbouring tooth (Steffensen and Weibert 1989).

All clinical variables were recorded using a manual periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA), applying a probing force of 0.2 N. The radiographic examination was performed at baseline and at the 6-month follow-up. Radiographs were acquired using a parallel cone technique with a Rinn holder, and radiographic measurements were performed using a computer software (VistaSoft 2.4.3. Durr Dental Italia S.R.L).

The distortion in the radiographic measurements was adjusted using the correction factor method (Tu et al. 2010). In brief, the correction factor was calculated using the vertical distance between the CEJ and the root apex (RA) at baseline and after 6 months:

$$\text{Correction factor} = (\text{baseline CEJ-RA}) / (6\text{-month CEJ-RA})$$

The corrected radiograph change in defect fill (DF) was derived as

$$\text{DF} = \text{baseline CEJ-BD} - (6\text{-month CEJ-BD} \times \text{correction factor})$$

In addition, information on gender, age and smoking habits was also collected. All data were collected in the Department of Periodontology, University of Naples Federico II.

2.4 | Sample Size Calculation

Sample size calculation was performed using a computer software (IBM-SPSS, IBM Inc.). Based on data presented in a previous study (Rajan et al. 2014), in order to detect a statistically significant difference of 1.12 ± 0.9 mm with a power of 0.80 for the primary outcome (PD change at 6 months) between test and control procedures, a sample size of 11 patients with one intrabony defect was required in each group. To avoid an underpowered sample size due to an overestimation of the expected difference between both procedures, a total of 21 patients with 21 intrabony defects were enrolled in each group. Potential dropouts were not included in the sample size calculation.

2.5 | Investigator Calibration

All parameters were recorded by two expert periodontists (B.A. and M.L.). Examiners attended a training and calibration session on a total of 40 patients (20 patients per examiner) not involved in the trial. Repeated measurements of PD were assessed once by each examiner. Furthermore, the inter-examiner variability for the radiographic measurement (RDD) was also checked. Twenty radiographs of patients not enrolled in the study were used for the calibration. A contingency coefficient (Cohen's kappa coefficient) was used to test the agreement between examiners. A value of 0.954 was obtained for the clinical variable (PD), while a value of 0.814 was found for the radiographic parameter (RDD).

2.6 | Randomization and Blinding

The patients were randomly assigned to test or control procedures by means of a simple randomization without restrictions and using a 1:1 allocation ratio. Minimization and stratification were not done. Randomization was made using a computerized random number generator (Random.org; www.random.org). Allocation concealment was effected by associating even numbers to the test group and odd numbers to the control group. The cards with numbers were enclosed in opaque envelopes, and treatment allocation was performed after professional mechanical subgingival plaque removal of the intrabony defect selected for the study by opening the envelope containing the number. The random allocation sequence was generated by a clinician not involved in the investigation. The examiners of outcome measures were masked with respect to test and control procedures, while the periodontist performing the treatments and patients were not masked. At baseline and after 6 months follow-up, radiographs were taken by two masked examiners (A.B. and L.M.).

Clinical parameters were recorded at baseline by A.B. and after 3- and 6-months follow-up by M.L., while the radiographic

variables were measured by the same masked examiners at baseline and after 6 months.

2.7 | Intervention

2.7.1 | Pre-Treatment

Prior to the pre-treatment phase, FMPS and FMBS were assessed. In the pre-treatment session (first step of periodontal therapy) (Sanz et al. 2020), all participants received supragingival professional mechanical plaque removal to eliminate the supragingival biofilm and calculus in combination with oral hygiene instructions and motivation. After 4 weeks, the clinical parameters of the intrabony defects selected for the trial were assessed (i.e., PD, CAL, GR and BOP) (Figure 1A,B).

2.7.2 | Treatment

All patients received step 2 of periodontal therapy based on a quadrant-wise approach, that is, in four appointments. Teeth with degree 2 mobility were splinted before the subgingival instrumentation. All periodontal pockets in each quadrant were treated with subgingival professional mechanical plaque removal using an ultrasonic scaler under local anaesthesia. Only the periodontal pocket associated with intrabony defect selected for the investigation was treated with the experimental procedure. MINST was performed by means of subgingival application of thin ultrasonic tips (Figure 1C). Additional subgingival instrumentation using Gracey mini-curettes (Hu-Friedy) was also performed to achieve biofilm removal in areas with difficult access (Figure 1D). Subgingival rinses were not performed in order to achieve blood clot stabilization following subgingival instrumentation. All therapies were performed using $\times 4.0$ magnification loupes (Univet, Italy) (Nibali et al. 2015). After completion of MINST, the defects

EXPERIMENTAL PROCEDURE



CONTROL PROCEDURE



FIGURE 1 | (A) Probing depth (PD) at baseline. (B) Radiographic view at baseline. (C) Subgingival instrumentation using ultrasonic scaler. (D) Additional subgingival instrumentation using mini-curette. (E) Application of xHyA after completion of MINST approach. (F) Clinical view at 6 months follow-up. (G) Radiographic view at 6 months follow-up. (H) Clinical view at baseline. (I) X-ray exam at baseline. (J) Subgingival instrumentation with ultrasonic scaler. (K) Subgingival instrumentation with mini-curettes. (L) Probing depth (PD) at 6 months follow-up. (M) X-ray exam at 6 months follow-up.

were randomly assigned to the test group or the control group. In the patients of the test group, at the end of subgingival professional mechanical plaque removal, the pockets associated with intrabony defects were filled once using xHyA gel (Hyadent BG, Regedent AG, Zürich, Switzerland) (Figure 1E). The defects of the control group were treated only with the MINST approach (Figure 1H–K). After completion of both procedures, oral hygiene instructions were reinforced. No antiseptic mouthwashes or antibiotics were prescribed for either group. All clinical procedures were performed by the same expert operator (V.I.S.).

2.7.3 | Post-Operative Follow-Up

All patients were recalled at 1, 3 and 6-months following treatment for supragingival professional mechanical plaque removal and motivation. After 3 months, only clinical parameters were recorded, while at 6 months the final clinical and radiographic evaluations were performed (Figure 1F,G,L,M). During the follow-ups at 1 and 3 months, no additional subgingival professional mechanical plaque removal or application of xHyA was performed.

2.8 | Statistical Analysis

All data were collected and analysed at the Department of Periodontology, University of Naples Federico II. Data analysis was conducted using a statistical software package (IBM-SPSS, IBM Inc.); the statistician was not blinded with respect to the research protocol. Since each patient contributed only one intrabony defect to the study, the patient was considered as the statistical unit. The variables PD, CAL, GR, CEJ-BD and DF were expressed in millimetres and FMPS and FMBS were expressed in percentages, while the radiographic angles were reported in degrees. Means and standard deviation (SD) were calculated for each parameter. The assumption of normal distribution was checked for all parameters by means of the Shapiro–Wilk test, and normal or non-normal distribution was checked using parametric or not parametric tests. A Chi-square test was used to compare gender and smoking habits between test and control procedures, while the Mann–Whitney *U* test was used for evaluating the age and teeth location (mandible/maxillae). The inter-group and intra-group analyses of FMPS and FMBS were carried out using an unpaired and a paired *t*-test, respectively. An intra-group analysis for the variables full-mouth probing depth (FMPD), PD, CAL, GR, BOP, CEJ-BD and defect angle was performed using the Wilcoxon test, while the inter-group evaluation was made by means of the Mann–Whitney *U* test. The inter-group analysis for DF was performed by means of the unpaired *t*-test.

Intra-group analysis of differences in number and percentages of sites with $PD \leq 4$ mm was carried out using the lambda test, while the inter-group evaluation was done by means of the McNemar test. To compare the frequency distribution of sites with residual PD and CAL gain between the test and control procedures, the lambda test was used. A *p*-value < 0.05 was set to accept a statistically significant difference.

3 | Results

3.1 | Patient Accountability

Seventy patients diagnosed with periodontitis (Tonetti, Greenwell, and Kornmann 2018) were invited to participate in the study. After initial screening, 20 patients not meeting the inclusion criteria were excluded, while 8 patients declined to participate in the study. Finally, 42 patients with 42 intrabony defects were included in the study. At 6 months, 38 patients with one intrabony defect each (a total of 38 intrabony defects) were available for the analysis.

Four patients were lost to follow-up. In the control group, two patients were excluded during the follow-up based on insufficient level of oral hygiene, while in the test group two patients declined to continue at 1 and 2 months, respectively (Appendix S1).

During the follow-up, no adverse events were recorded and no teeth were lost.

3.2 | Patient Characteristics

The characteristics of the sample enrolled in the trial are given in Table 1. Fourteen females and 5 males (mean age 49.3 ± 11.6 years) and 10 females and 9 males (mean age 50.8 ± 10.8 years) with a diagnosis of generalized stage III, grade C periodontitis were allocated to the test group and the control group, respectively. Nine patients were tobacco smokers (four in the test group and five in the control group). Five intrabony defects in the mandible and 14 in the maxilla received the experimental procedure, while 8 intrabony defects in the mandible and 11 in the maxilla were treated by means of the control procedure alone. No statistically significant differences were found between the test and control groups ($p > 0.05$) (Table 1).

3.3 | FMPS and FMBS

All patients showed a statistically significant improvement in FMPS and FMBS after 6 months ($p < 0.05$). At 6 months, FMPS and FMBS decreased from $58.6 \pm 6.0\%$ and $53.4 \pm 6.6\%$ to $18.7 \pm 2.2\%$ and $14.3 \pm 3.6\%$ in patients of the test group, while FMPS and FMBS changed from $59.5 \pm 6.5\%$ to $18.9 \pm 1.8\%$ and from $55.8 \pm 6.6\%$ to $14.7 \pm 2.5\%$ in the control group. Inter-group comparison did not show statistically significant differences ($p > 0.05$) (Table 2).

3.4 | FMPD and Number of Sites With $PD > 4$ mm and $PD > 6$ mm of the Entire Dentition

At baseline, the mean FMPD was 3.9 ± 0.5 mm and 4 ± 0.4 mm in patients of the test and control group, respectively. After 6 months, FMPD was 3.3 ± 0.2 mm in the test group and 3.4 ± 0.2 mm in the control group. Intra-group comparison (i.e., baseline to 6 months) showed statistically significant differences ($p < 0.05$), while no statistically significant differences between the test and control groups were found

($p > 0.05$). At 6 months, the number of sites with PD > 4 mm and ≤ 6 mm changed from 384 to 330 and from 480 to 322 in the test and control groups, respectively. At baseline, patients of the test and control groups had 546 and 490 sites with PD > 6 mm, respectively. No sites with PD > 6 mm were recorded after 6 months. Hence, overall, sites with PD > 4 mm varied from 930 to 330 in the test group and from 970 to 322 in the control group (Table 2).

3.5 | Changes in PD

The primary outcome, namely PD, decreased statistically significantly at 3 and 6 months in both groups ($p < 0.05$). At baseline, intrabony defects treated with the experimental procedures showed a PD of 6.7 ± 1.4 mm, while in the control group the PD was 6.8 ± 0.8 mm. At 3 months, PD was 3.3 ± 1.0 and 5.2 ± 0.7 mm in the test and the control group, respectively, while after 6 months, PD of 4 ± 0.8 and 4.2 ± 0.8 mm was recorded for the test and the control procedure, respectively. No statistically significant differences between the test and control groups were recorded at baseline and after 6 months ($p > 0.05$). However, a statistically significant difference was noted at 3 months ($p < 0.05$) (Table 2).

3.6 | Changes in CAL

Statistically significant changes were found between baseline, 3 months and 6 months in both groups ($p < 0.05$). In the test group, CAL changed from 8.4 ± 2.8 to 4.9 ± 2.0 and to 5.5 ± 1.9 mm between baseline and 3 and 6 months, while the defects of the control group showed a CAL change from 8.2 ± 1.7 to 6.8 ± 1.9 and to 6 ± 2.4 mm. Inter-group comparison showed no statistically significant difference at baseline and after 6 months ($p > 0.05$). However, a statistically significant difference was noted at 3 months ($p < 0.05$). No statistically significant differences ($p > 0.05$) were noted between test and control procedures at baseline and at the 6 months follow-up (Table 2).

3.7 | Changes in GR

At baseline, patients treated with the test procedure reported a GR of 1.6 ± 1.7 mm, while patients of control group showed a GR 1.4 ± 1.5 mm. At the 3-month follow-up, the GR was 1.6 ± 1.6 and 1.6 ± 1.9 mm in the test and the control group,

respectively, while after 6 months these parameters were 1.5 ± 1.7 mm in the test group and 1.8 ± 2.2 mm in the control group (Table 2).

3.8 | Number and Percentage of BOP-Positive Sites

The number and percentage of sites with positive BOP at baseline and at 3- and 6-months follow-up are summarized in Table 3. At baseline, BOP-positive sites were assessed at 12 defects (63.1%) of the test group and at 11 defects (57.9%) of the control group. After 3 months, the number (percentage) of BOP-positive sites was 2 (10.5%) and 5 (26.3%) in the test and control groups, respectively. At 6 months, two (10%) sites in the test group and five (21%) in the control group were BOP-positive. A statistically significant improvement was observed when the number and percentage of BOP-positive sites were compared at baseline and after 3 months ($p < 0.05$). Comparable results were observed when evaluating the presence of BOP-positive sites between baseline and 6 months ($p < 0.05$). No statistically significant changes were recorded between 3 and 6 months ($p > 0.05$) (Table 3).

3.9 | Number and Percentage of Defect Sites With 'Pocket Closure'

The number and percentage of sites that displayed a PD ≤ 4 mm without BOP (i.e., pocket closure) are shown in Table 4. After 3 months, the number of sites with pocket closure was 16 (84.2%) and 2 (10.5%) in the test and control group, respectively, while after 6 months, in 15 (78.9%) test sites and 12 (63.1%) control sites PD ≤ 4 mm was recorded. Statistically significant differences were observed between the test and control groups at 3 months ($p < 0.05$), while at 6 months no statistically significant differences were recorded ($p > 0.05$). Intra-group analysis showed no statistically significant changes between 3 and 6 months in the test group ($p > 0.05$), while a statistically significant difference was observed in the control group ($p < 0.05$) (Table 4).

3.10 | Frequency Distribution of Sites With Residual PDs and CAL Changes

Table 5 summarizes the frequency distribution of residual PDs with or without BOP and CAL changes after 3 and 6 months.

TABLE 1 | Patient characteristics.

	Test group (N=19)	Control group (N=19)	p
Gender (F/M)	14/5	10/9	0.179*
Mean age (years)	49.3 ± 11.6	50.8 ± 10.8	0.452*
Smoking habit (y/n)	4/15	5/14	0.703*
Periodontitis stage and grade	19 (stage III, grade C)	19 (stage III, grade C)	
Intrabony defects location (mand./max.)	5/14	8/11	0.418*

Abbreviations: F, female; M, male; mand., mandible; max., maxilla; n, no; y, yes.

*No statistically significant difference.

TABLE 2 | Changes in FMPS, FMBS, FMPD, number of sites >4/>6 mm, PD, CAL and GR between baseline and follow-up.

Parameters		Parameters		Parameters						
FMPS (%)	Baseline	6 months	p	FMBS (%)	Baseline	6 months	p			
Test group (19)	58.6 ± 6.0	18.7 ± 2.2	0.001**	Test group (19)	53.4 ± 6.6	14.3 ± 3.6	<0.001**			
Control group (19)	59.5 ± 6.5	18.9 ± 1.8	0.001**	Control group (19)	55.8 ± 6.6	14.7 ± 2.5	<0.001**			
p	0.625*	0.686*		p	0.237*	0.642*				
Parameters		Parameters		Parameters						
FMPD (mm)	Baseline	6 months	p	No. of sites with PD > 4 mm	Baseline	6 months	No. of sites with PD > 6 mm			
Test group (19)	3.9 ± 0.5	3.3 ± 0.2	<0.001**	Test group (19)	384	330	Test group (19)			
Control group (19)	4 ± 0.4	3.4 ± 0.2	<0.001**	Control group (19)	480	322	Control group (19)			
p	0.343*	0.280*								
Parameters (intra-bony defects)		Baseline	3 months	6 months	Δ BL-3 m	Δ 3-6 m	Δ BL-6 m	p (BL-3 m)	p (3-6 m)	p (BL-6 m)
PD (mm)										
Test group (N=19)	6.7 ± 1.4	3.3 ± 1.0	4 ± 0.8	3.4 ± 1.4	0.7 ± 1.0	2.7 ± 1.5	<0.001**	0.011**	<0.001**	
Control group (N=19)	6.8 ± 0.8	5.2 ± 0.7	4.2 ± 0.8	1.6 ± 0.6	0.9 ± 0.8	2.6 ± 1.1	<0.001**	0.002**	<0.001**	
p	0.258*	0.001**	0.435*							
CAL (mm)										
Test group (N=19)	8.4 ± 2.8	4.9 ± 2.0	5.5 ± 1.9	3.4 ± 1.8	0.6 ± 0.9	2.8 ± 2.1	<0.001**	0.017**	<0.001**	
Control group (N=19)	8.2 ± 1.7	6.8 ± 1.9	6 ± 2.4	1.3 ± 0.9	0.5 ± 1.1	1.9 ± 1.6	<0.001**	0.034**	<0.001**	
p	0.729*	0.015**	0.563*							
GR (mm)										
Test group (N=19)	1.6 ± 1.7	1.6 ± 1.6	1.5 ± 1.7	0.05 ± 1.0	0.05 ± 0.6	0.1 ± 1.0	0.665*	0.705*	0.516*	
Control group (N=19)	1.4 ± 1.5	1.6 ± 1.9	1.8 ± 2.2	0.3 ± 0.6	0.4 ± 0.7	0.7 ± 1.1	0.238*	0.339*	0.119*	
p	0.795*	0.954*	0.795*							

Note: Δ BL-3 m = difference baseline-3 months; Δ 3-6 m = difference 3-6 months; Δ BL-6 m, difference baseline-6 months.

Abbreviations: CAL, clinical attachment level; FMBS, full-mouth bleeding score; FMPD, mean full-mouth probing depth; FMPS, full mouth plaque score; GR, gingival recession; PD, probing depth.

*No statistically significant difference.

**Statistically significant difference.

TABLE 3 | Number and percentage of sites with BOP-positive at baseline, 3- and 6-months follow-up.

Parameters						
BOP-positive (N/%)	Baseline	3 months	6 months	<i>p</i> (BL-3 m)	<i>p</i> (3-6 m)	<i>p</i> (BL-6 m)
Test group (N=19)	12/63.1	2/10.5	2/10	0.002**	0.999*	0.012**
Control group (N=19)	11/57.9	5/26.3	4/21	0.031**	0.999*	0.016**
<i>p</i>	0.795*	0.418*	0.795*			

Abbreviation: BOP, bleeding on probing.

*No statistically significant difference.

**Statistically significant difference.

TABLE 4 | Number and percentage of site with PD ≤4 mm (pocket closed) at baseline and after 3 and 6 months.

	Test group (N=19)	Control group (N=19)	<i>p</i>
<i>N</i> /% of sites with PD ≤4 mm			
3 months	16/84.2	2/10.5	<0.001**
6 months	15/78.9	12/63.1	0.360*
<i>p</i>	0.999*	0.004**	
<i>N</i> /% of sites with PD ≥5 mm			
3 months	3/15.8	17/89.5	<0.001**
6 months	4/21.05	7/36.8	0.360*
<i>p</i>	0.999*	0.004**	

Abbreviation: PD, probing depth.

*No statistically significant difference.

**Statistically significant difference.

A statistically significant improvement in residual PDs and CAL changes was seen after 3 months when xHyA gel was used ($p < 0.05$), while no statistically significant changes were noted at 6 months ($p > 0.05$) (Table 5).

3.11 | Radiographic Outcomes

A statistically significant difference was noted between baseline and 6 months for both procedures when CEJ-BD values were compared ($p < 0.05$). CEJ-BD was 6.3 ± 2.3 and 4.3 ± 2.3 mm in the test group, while a mean of 6.9 ± 1.7 and 5.6 ± 1.9 mm was assessed in the control group. After 6 months, a statistically significant difference was found while comparing CEJ-BD values of intrabony defects treated with MINST + xHyA compared with MINST alone ($p < 0.05$). At 6 months, DF was 2.1 ± 0.9 and 1.4 ± 1.1 mm for the test and control procedures, respectively. A statistically significant difference was found ($p < 0.05$).

At baseline, an RDA of $36 \pm 14.8^\circ$ and $44.7 \pm 12.9^\circ$ was assessed in the test and control patients, respectively. At 6 months, in the test group RDA was $53.9 \pm 23.8^\circ$ and in the control group $48.4 \pm 19.8^\circ$. Intra-group comparison (i.e., baseline to 6 months) showed a statistically significant difference ($p < 0.05$) in the test group but no statistically significant difference in the control

group ($p > 0.05$). At 6 months, inter-group analysis did not show statistically significant differences ($p > 0.05$).

No statistically significant differences ($p > 0.05$) were found when inter-group analysis was made. However, the intra-group comparison (i.e., baseline to 6 months) showed a statistically significant change for patients of the control group ($p < 0.05$) (Table 6).

4 | Discussion

The present study aimed at comparing the healing of intrabony defects treated by means of MINST + xHyA gel application with MINST alone during non-surgical periodontal therapy. After 6 months, a significant improvement of all clinical and radiographic parameters was observed in both groups. However, no statistically significant difference between test and control procedures was found. Therefore, the null hypothesis, namely no statistically significant difference between procedures, was accepted. These data seem to suggest that the adjunct of xHyA gel to MINST did not yield superior results when compared with MINST alone after 6 months. The results of the present study must be interpreted with caution, however, because the intrabony defects displayed moderate severity, both in terms of clinical and radiographic measurements.

The clinical improvements of sites treated with the experimental procedure at 6 months (i.e., PD reduction of 2.7 mm and CAL gain of 2.8 mm) agree with those reported in previous studies. Diehl and co-workers achieved a PD reduction of > 2 mm associated with a reduction of the sites with BOP in suprabony defects (Diehl et al. 2022). Ramanauskaite and co-workers reported a PD reduction of 2.9 mm and a CAL gain of 2.6 mm in suprabony defects (Ramanauskaite, Machiulskiene, Dvyliene, et al. 2023). However, in our trial the control procedure (i.e., MINST alone) showed similar results when compared with local application of xHyA following MINST. These findings agree with those reported by Pilloni and coworkers on the efficacy of xHyA application as adjunct to subgingival professional mechanical plaque removal of suprabony defects (Pilloni, Rojas, et al. 2021; Pilloni, Zeza, et al. 2021). The authors of that study noted that the use of xHyA showed a tendency for better results but there was no statistically significant difference between test and control procedures. On the contrary, statistically significant differences were found by Ramanauskaite and co-workers for all parameters investigated (i.e., PD reduction and CAL gain) when

TABLE 5 | Frequency distribution (N/%) of residual PD and CAL gain at 3 and 6 months.

	Test group (N=19)			Control group (N=19)			p
	0-4 mm	5 mm	≥ 6 mm	0-4 mm	5 mm	≥ 6 mm	
3 months							
Residual PD with BOP negative	15/78.9	2/10.5	0	2/10.5	9/47.4	3/15.8	<0.001**
Residual PD with BOP positive	1/5.3	1/5.3	0	0	4/ 21.1	1/5.3	
6 months							
Residual PD with BOP negative	13/68.4	2/10.5	1/5.3	10/52.6	4/21.1	1/5.3	0.311*
Residual PD with BOP positive	2/10.5	1/5.3	0	2/10.5	2/10.5	0	

	0-1 mm	2 mm	3 mm	4 mm	≥ 5 mm	0-1 mm	2 mm	3 mm	4 mm	≥ 5 mm	p
3 month											
CAL gain	2/10.5%	1/5.3%	9/47.4%	4/21.1%	3/15.8%	8/42,1%	10/52.6%	0	1/5.3%	0	p < 0.001**
6-month											
CAL gain	3/15.8%	2/26.3%	6/31.6%	3/15.8%	2/10.5%	6/31.6%	5/26.3%	4/21.1	2/10.5%	2/10.5%	p = 0.442*

*No statistically significant difference.

**Statistically significant difference.

TABLE 6 | Radiographic defect changes at baseline and after 6 months follow-up.

	Test group (N=19)	Control group (N=19)	Δ	p
CEJ-BD (mm)				
Baseline	6.3 ± 2.3	6.9 ± 1.7	0.6 ± 2.7	0.271*
6 months	4.3 ± 2.3	5.6 ± 1.9	1.3 ± 2.6	0.023**
p	0.001**	0.001**		
DF (mm)				
Baseline	2.1 ± 0.9	1.4 ± 1.1	0.7 ± 1.3	0.026**
RDA (°)				
Baseline	35.8 ± 14.3	44.7 ± 12.9	8.8 ± 18.9	0.053*
6 months	53.9 ± 23.8	48.4 ± 19.8	5.4 ± 29.4	0.450*
ΔBL-6 m	18 ± 18.8	3.74 ± 12.6		
p	0.001**	0.492*		

Note: CEJ-BD, vertical distance measured from the CEJ to the most apical extension of the bone defect; DF, defect fill, calculated as CEJ-BD - (6-month CEJ-BD × correction factor); RDA, radiographic defect angle, defined as angle between the line connecting the CEJ of the tooth presenting the intrabony defect to the most apical point of the defect and the line connecting the most apical point of the defect and the point where the bone crest touched the neighbouring tooth.

*No statistically significant difference.

**Statistically significant difference.

xHyA was added to MINST (Ramanauskaite, Machiulskiene, Shirakata, et al. 2023). Potential explanations may be related to the type of periodontal defects and adjunctive delivery of sodium hypochlorite/amino acids. In the study by Ramanauskaite, Machiulskiene, Shirakata, et al. (2023), the authors tested the combination xHyA and sodium hypochlorite/amino acids as adjunct to MINST in patients with primarily suprabony defects,

while in the present study, only isolated intrabony defects were selected.

It may thus be anticipated that the reasons for the lack of difference between test and control groups at 6 months depend on the efficacy of MINST in the treatment of intrabony defects (Nibali et al. 2015) with or without adjunctive use of biological agents. In the present study, the benefit of using xHyA gel as adjunct on the PD reduction and CAL gain was only noted during the first 3 months of healing. At 3 months, the differences in PD reduction and in CAL gain were statistically significant between procedures, and the sites treated with MINST + xHyA gel showed faster healing compared to those treated by means of MINST alone. The statistically significant improvement of the defects treated by MINST + xHyA seems to depend on the delayed response of the defects of the control group rather than on the experimental procedure. Certainly, intrabony defects treated by means of MINST alone require a healing period of > 3 months. However, one of the objectives of this study was also to evaluate the potential of xHyA in accelerating the healing process. For these reasons, the clinical parameters were also recorded at 3 months in both groups. It is important to point out that patients treated with the experimental procedure showed a higher percentage of pocket closure at 3 months (84.2% vs. 10.5%), and no sites with PD ≥ 6 were recorded. These results seem to indicate a possible stimulating effect of xHyA in the early healing phase of intrabony defects. Indirect biological evidence showed that xHyA accelerated the phase of wound healing by stimulating cell migration and proliferation (Olczyk et al. 2008) and by providing a sealing effect of the periodontal pocket. After local application, xHyA increased the dentin surface texture, and then an increase in the number and improvement in spreading of periodontal ligament cells on this surface were noted (Mueller et al. 2017). However, these observations should be interpreted with caution because histological evidence is lacking

in humans. The statistically significant improvement in clinical parameters of test sites may also be related to the bacteriostatic effect of xHyA on the strains associated with periodontitis. Microbiological evidence has shown that the adjunctive application of xHyA prevents recolonization by periodontopathogens of the treated sites (Eick et al. 2013). In addition, the counts of *Treponema denticola* and *Compylobacter rectus* are significantly reduced in sites treated with xHyA, while *Prevotella intermedia* and *Porphyromonas gingivalis* increased in sites that did not receive xHyA gel (Eick et al. 2013). In all patients, both clinical procedures were delivered attempting to minimize potential trauma to the gingival tissue. For these reasons, gingival curettage was avoided and changes in GR within 1 mm were observed in both groups.

Both treatment modalities led to significant radiographic improvement as indicated by mean defect fill and defect angle changes, although the radiographic results were smaller in magnitude when compared with those of a previous study (Nibali et al. 2015). This aspect depends on the configuration of the intrabony defects enrolled in the present investigation, because the majority of the defects were shallow with wide a radiographic angle. At 6 months, the intrabony defects treated with the experimental procedure showed a statistically significant defect fill compared to sites provided with the control treatment (2.1 vs. 1.4 mm). The higher radiographic defect fill of sites treated with the experimental therapy may have been due to the fact that xHyA strongly induces the growth of osteoprogenitor cells and maintains their stemness, thus suggesting a potential regulatory effect of HA on the balance between self-renewal and differentiation during bone healing (Asparuhova et al. 2020). Radiographic reduction of the vertical intrabony component was associated with an increase in the radiographic defect angle. This increase is an expression of the substantial bone fill, which occurs in the most apical part of the defect. At baseline, the sites treated with xHyA showed a mean of radiographic defect angle of $\geq 36^\circ$, which was associated with a CAL gain of ≤ 4 mm (i.e., 2.8 mm). This result agrees with the outcomes of a previous study (Tsitoura et al. 2004) reporting a significant association between the baseline radiographic defect angle and CAL gain. These authors (Tsitoura et al. 2004) demonstrated that the probability of obtaining a CAL gain of ≥ 4 mm is higher when the radiographic defect angle is $\leq 22^\circ$ than when it is $\geq 36^\circ$. The majority of the intrabony defects enrolled in the present study were shallow with a wide radiographic angle. Usually, wide defect angles are related to shallow intrabony defects (Kim et al. 2006) and these characteristics did not negatively influence the final results because the healing (i.e., percentage of CAL gain) of deep and shallow defects was reported to be similar after periodontal treatment (Cortellini et al. 1998). The lack of a customized bite to place the film holder and the relatively short time interval for the radiographic analysis are limitations of this study. Since the radiographs were not taken in a standardized way, the correction factor method (Tu et al. 2010) was used to correct the potential variation in radiographic images due to positioning. However, the primary objective of the present investigation was to evaluate the efficacy of xHyA as adjunct to MINST in PD reduction of intrabony defects during the second step of periodontal therapy. The radiographic examination can be considered as an adjunctive exam to verify

the healing pattern of intrabony defect and to confirm the efficacy of xHyA in the healing process. Certainly, studies with a longer follow-up are needed to confirm these results.

Usually, CAL gain is set as the primary outcome measure when a biological agent is tested in the treatment of intrabony defects. Although regenerative properties (Mendes et al. 2008) of xHyA were observed in previous studies, in the present investigation the CAL gain was not considered as the primary outcome. The aim of present trial was to evaluate the efficacy of xHyA as an adjunct to MINST in the treatment of intrabony defects during step 2 of periodontal therapy. In other words, this trial investigated the possibility to resolve periodontal pockets associated with intrabony defects by means of non-surgical periodontal treatment and to avoid adjunctive surgical therapies (i.e., step 3 of periodontal therapy). Hence, in agreement with a previous study (Loos and Needleman 2020), PD was set as the primary outcome measure. One intrabony defect was treated per patient. If multiple teeth presented pockets associated with an intrabony defect, only the site with the deepest PD was selected for the study. In case of the same PD in two or more intrabony defects per patient, the site with the deepest radiographic intrabony component was selected.

A sample size of 11 patients with one intrabony defect was required in each group to reject the null hypothesis and obtain a statistically significant difference between test and control procedures (Rajan et al. 2014) with respect to the primary outcome (i.e., PD change). However, Rajan and co-workers evaluated the adjunctive effect of local application of HA gel following conventional subgingival instrumentation (SRP) in patients with periodontitis, and no previous studies compared the local adjunct of xHyA to MINST with respect to MINST alone in the treatment of intrabony defects. For these reasons, 42 patients with 42 intrabony defects were enrolled to avoid an underpowered sample size due to an overestimation of the expected difference between both procedures. This aspect may be considered a bias because a larger number of patients than indicated by the sample size calculation could increase the probability of obtaining a statistically significant difference. However, the recruitment of a larger sample increases the probability of achieving a statistical inference with the possibility to transfer the results to the population.

To avoid bias related to the operator, all patients received the same therapy (i.e., MINST) and the randomization was done after completion of subgingival professional mechanical plaque removal of intrabony defects. Unfortunately, a placebo gel was not used to treat the sites of the control group. This fact could be considered a limitation of the present study because the operator and patients were not masked with respect to the test and control procedures.

The reason for omitting the placebo was due to the fact that, since the study was only supported by the institution of the authors, there was no possibility to obtain a suitable placebo gel. Although the lack of a placebo gel may be considered a bias, it has to be kept in mind that the randomization envelope was opened only when the operator completed subgingival instrumentation. Furthermore, most studies evaluating the use of xHyA gel in various trials (Pilloni, Rojas, et al. 2021; Pilloni, Zeza, et al. 2021; Ramanauskaite, Machiulskiene, Shirakata, et al. 2023) did not use a placebo. Additionally, the lack of a placebo gel reflects

the clinical reality, thus making the results more relevant for the clinician. The relatively short follow-up time (i.e., 6 months) may be considered another limitation. The aim of the present study was to test the efficacy of MINST with xHyA gel as an adjunct in the treatment of intrabony defects during the second step of periodontal treatment prior to periodontal re-evaluation. Data from the systematic review by Suvan et al. (2020) indicated a mean PD reduction of 1.4 mm and a proportion of pocket closure of 74% after 6–8 months. Hence, a follow-up of 6 months can be considered acceptable prior to the periodontal re-evaluation in daily practice.

The lack of split mouth design was a limitation of this study. Since the experimental and control sites were treated as part of step 2 of periodontal therapy together with other sites, the healing of test sites may be attributed to the patient's general response and not exclusively to the adjunctive use of xHyA. In fact, the mean FMPD significantly improved in both groups after 6 months, and no statistically significant differences were found between the groups. However, adjunctive delivery of xHyA accelerated healing of the experimental sites in the first 3 months.

Since the intrabony defects were treated by means of a non-surgical approach, the morphology of the intrabony component (i.e., number of residual walls) and its impact on the final outcomes were not evaluated. However, a recent study (Nibali et al. 2023) reported no evidence for the associations between defects characteristics and healing of intrabony defects following MINST. One of the limitations of present trial was the lack of patient-reported outcomes (i.e., PROMs). Although all patients received a minimally invasive therapy, the additional use of xHyA may reduce the post-operative discomfort.

Another limitation of the present study was the sample population recruited in the trial. These outcomes are related to a sample enrolled following strict eligibility criteria; however, the majority of patients with severe periodontitis are smokers and may also suffer from various systemic disorders (i.e., diabetes). Hence, the true efficacy of the experimental procedure cannot be generalized to the large majority of the population suffering from periodontitis.

5 | Conclusion

Taken together, the present results indicate that (a) treatment of intrabony defects with MINST, with or without the application of xHyA gel, resulted in statistically significant improvements in the investigated clinical parameters at 3 and 6 months after therapy, and (b) although the adjunctive use of xHyA gel to MINST improved the clinical outcomes compared with MINST alone up to 3 months, statistically significant differences were not observed at 6 months.

Author Contributions

Vincenzo Iorio-Siciliano: conceptualization, investigation. Andrea Blasi: data acquisition, statistical analysis. Leopoldo Mauriello: patient enrollment and data acquisition. Giovanni E. Salvi: co-drafted the manuscript and data interpretation. Luca Ramaglia: co-drafted the protocol and

manuscript, project administration. Anton Sculean: co-drafted the manuscript and data interpretation. All authors approved the final version.

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Conflicts of Interest

The authors declare no conflicts of interest.

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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Supporting Information

Additional supporting information can be found online in the Supporting Information section.