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ORIGINAL ARTICLE

Influence of locally delivered doxycycline on the clinical and molecular inflammatory status of intrabony defects prior to periodontal regeneration: A double-blind randomized controlled trial

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Abstract

Objectives: To test the effect of locally delivered doxycycline (DOX) administered 2 weeks prior to minimally invasive periodontal regeneration in terms of presurgical inflammatory status and cytokine expression profile in the gingival crevicular fluid (GCF). Secondary aim was to assess the early wound healing index (EHI) at 2 weeks after surgery.

Background: It is hypothesized that healing after periodontal regeneration is dependent on preoperative soft tissue condition, and that local antibiotics may improve the site-specific inflammatory status at short time.

Methods: Sites associated with periodontal intrabony defects requiring regenerative surgery and showing bleeding on probing (BoP) were included. At T0, experimental sites were randomly treated with subgingival instrumentation with or without topic DOX application. After 2 weeks (T1), defects were approached by means of minimally invasive surgical technique. GCF was sampled at both T0 and T1 for inflammatory biomarker analysis. Two weeks after surgery, the EHI was evaluated (T2).

Results: Forty-four patients were included. At T1, the number of BoP+ sites was statistically significantly less in the test group (27.3% vs. 72.7%; $p < .01$). The total amount of interleukin (IL)-1β ($p < .001$), matrix-metalloproteinases (MMP)-8 ($p < .001$), and MMP-9 ($p = .010$) in the GCF significantly decreased in the test group at T1, with relevant differences compared to controls. At T2, the EHI had an average value of 1.45 ± 0.86 in the test group while in the control, it was 2.31 ± 1.43 ($p = .027$). A statistically significantly positive correlation was observed between the amount of IL-1β and MMP-9 and EHI scores.

Mario Aimetti and Giacomo Baima; Giovanni N. Berta and Federica Romano equally contributed to the study.

Conclusions: Within the limitations of this study, sites treated with DOX showed improved clinical and molecular inflammatory parameters before surgery, as well as soft tissue healing 2 weeks after surgery.

KEYWORDS
antimicrobials (local), cytokines, gingival crevicular fluid, inflammation, periodontal regeneration

1 | INTRODUCTION

According to the European Federation of Periodontology (EFP) clinical practice guideline, it is recommended to treat teeth with residual deep pockets associated with intrabony defects 3 mm or deeper by periodontal regeneration surgery.[1,2] Patient-, defect- and site-related prognostic factors may influence the outcomes of the regenerative therapy, including plaque/inflammatory control, local anatomical characteristics, surgical technique, and innate wound healing potential.[3,4] Indeed, the persistence of high bacterial loads together with bleeding on probing (BoP) has been associated with poor clinical outcomes after regenerative treatment.[3,5]

In order to better control site-specific infection/inflammation, cause-related therapy may be supported by the subgingival application of adjunctive local antimicrobials.[2,6] Compared to systemic prescription, the use of a local delivery system permits a low dose to reach the periodontal pocket and acts site specifically, with fewer side effects and chances of developing bacterial tolerance to medications.[7] A recent systematic review with meta-analysis including 50 studies showed that application of local antimicrobials provided added benefits to subgingival instrumentation in terms of BoP and probing pocket depth (PPD) reduction, but only on the short-term follow-up (6–9 months). However, high risks of bias and heterogeneity were detected in the majority of studies.[6] For these reasons, clear recommendations for the use of local antimicrobials in clinical practice are lacking.[2]

Between known antibiotics, 14% doxycycline hyclate carried with a biodegradable polylactic-polyglycolic acid gel (DOX) was traditionally used as adjuvant therapy in the treatment of residual/refractory pockets.[6,8,9] In addition to its antimicrobial capacity, DOX has an action on matrix metalloproteinases (MMP) levels (mainly MMP-8 and MMP-9) due to its capacity to bind zinc and calcium ions within their catalytic domain.[10,11] This inhibition of collagenase enzyme activity and inflammatory cytokines in gingival crevicular fluid (GCF) seems to consequently reduce connective tissue and bone resorption, and favor improved healing outcomes of periodontal therapy.[10]

In the absence of previous literature data, it was hypothesized that the subgingival application of local DOX prior to periodontal regeneration procedure could be beneficial in achieving better control of inflammation and better soft tissue healing after surgery due to the improvement of the presurgical soft tissue quality. Moreover, it was expected to observe a significant change in cytokines and MMPs expression in the GCF.[12]

Therefore, the primary aim of this study was to test the effect of DOX administered 2 weeks prior to minimally invasive periodontal regeneration in terms of presurgical clinical and molecular inflammatory status. The secondary aim was to assess the early wound healing index (EHI) at 2 weeks after surgery.

2 | MATERIALS AND METHODS

The protocol was approved by the Institutional Ethical Committee (protocol number 00309/2021). The study complies with the principles of the Declaration of Helsinki and it is reported according to the CONSORT statement. All patients provided signed informed consent before enrollment.

2.1 | Study design and population

The study was designed as a double-blinded, randomized clinical trial with two parallel groups and a 1:1 allocation ratio. Consecutive patients who completed steps I–II of periodontal treatment at the Section of Periodontology, C.I.R. Dental School, University of Turin were screened for inclusion between January to November 2022 by two calibrated examiners. The following eligibility criteria were considered: (1) diagnosis of stage III or IV periodontitis[13]; (2) full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) < 15% at the time of enrollment; (3) steps I–II of periodontal treatment (etiological therapy) completed at least 2 months prior to screening; and (4) the presence of one natural tooth with residual PPD ≥6 mm, BoP+, and a radiographic intrabony component ≥3 mm, without furcation involvement, suitable for a minimally invasive surgical technique (MIST)[14] procedure.

Exclusion criteria were: (1) age < 18 years; (2) smokers; (3) contraindications for periodontal surgery; (4) systemic diseases affecting periodontal healing (i.e., diabetes mellitus); (5) pregnancy and lactation; (6) history of periodontal surgery at the experimental teeth; (7) allergies to doxycycline and tetracyclines; and (8) assumption of antimicrobials in the last 3 months.

2.2 | Sample size and randomization

The difference in presurgical BoP between test and control groups was set as the primary outcome of the study. A previous report showed that out of the total number of sites with BoP receiving debridement alone as retreatment, BoP was effectively solved in 25% of the treated sites.[5] Considering a further decrease up to 65% clinically relevant after local application of DOX, a sample size of 44 patients (22 per group) was needed at 0.05 two-sided alpha error and 80% power.

A random permuted block randomization list with a 1:1 allocation ratio was generated on a computer using statistical software (SPSS, IBM version 28) by an independent researcher not involved in the selection, treatment, outcome assessment, and statistical analysis. To conceal assignment, forms with the treatment modality were put into opaque and sealed envelopes with the patient number on the outside. The envelopes were placed into the custody of the study coordinator, who opened them prior to DOX application and informed the clinician. The patient, the surgeon, and the examiner who performed the measurements were blinded to treatment assignment. Code breaking was performed after statistical analysis.

2.3 | Intervention

2.3.1 | Presurgical procedures

Two weeks before surgery, each experimental site received gentle subgingival debridement alone (control) or in combination with a single local administration of DOX (test; Ligosan). Debridement was performed under local anesthesia (mepivacain 2% 1:1000) for a time of 5 min by means of ultrasonic devices with ultrathin tips by the same experienced clinician. Great attention was paid to avoid marginal and interproximal soft tissue damage. In the test group, local DOX was administered according to the manufacturer's instruction. Briefly, the plastic needle of the syringe was inserted into the sulcus and the product was progressively released in the periodontal pocket up to the gingival margin. The syringe was then removed and a cotton pellet was used to compact the gel into the sulcus. In the group receiving gentle debridement alone, the administration of local DOX was simulated in all the steps without injecting the antibiotic. Patients were then instructed to use properly floss or interdental brushes in that area for the following 10 days.

2.3.2 | Surgical procedure

The experimental sites were later approached by means of a MIST[14] under magnification loops. Briefly, vertical-releasing incisions were avoided, and the full-thickness flap was minimally raised. Granulation tissue was removed from the defects. The root was thoroughly scaled using mini curettes and ultrasonic device with specific tips and chemically treated by EDTA (PrefGel, Institut Straumann AG). Regenerative procedure was carried out using a combination of enamel matrix derivatives (Emdogain, Institut Straumann AG) and bone xenograft (Bio-Oss, Geistlich Pharma AG).[1,2,4] The flaps were repositioned and sutured (Gore-tex, WL Gore & Associated) in order to obtain passive primary closure.

2.3.3 | Postoperative and maintenance care

All patients received antibacterial treatment (amoxicillin and clavulanic acid 1 g bid for 6 days), analgesic medication (ibuprofen 600 mg, every 8 h for 3 days), and 0.20% chlorhexidine digluconate mouthrinse for 1 min (2 times/day for 2 weeks). Clinical check and wound detersion was performed 1 week later. Sutures were removed 14 days after surgery. During the postoperative period, patients were prescribed to avoid toothbrushing and flossing in the treated area.

2.4 | Clinical measurements

Clinical measurements were taken at the deepest point of the selected defects by using a manual 1-mm graduated periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL) at baseline (14 days before surgical treatment, T0), and at the day of surgery (T1) by the same blinded examiner. To perform the intra-examiner calibration, 10 non-study patients presenting with intrabony defects were evaluated by the examiner on two separate occasions within 48 h. The percentage of agreement within 1 mm between repeated measurements of PPD and clinical attachment level (CAL) was ≥92% and ≥94%, respectively. The following clinical parameters were assessed at T0 and T1: presence/absence of bacterial plaque (PI), presence/absence of BoP, PPD, gingival recession (REC), CAL, and the width of keratinized tissue (KT). The number of bony walls was registered intrasurgically. Two weeks after surgery, the early wound-healing index (EHI)[15] was assessed by a second blinded assessor.

2.5 | GCF sampling

GCF samples were collected from the experimental sites at T0 and T1 before clinical examination to prevent contamination with blood. Sites were isolated with cotton rolls and supragingival plaque was carefully removed. After the sites were gently dried with air syringe, GCF samples were collected with paper strips (PerioPaper Strips, Oraflow Inc.) that were inserted into the pocket until mild resistance was felt, and then allowed to remain there for 30 s. Strips contaminated by blood were discarded. The amount of collected GCF was measured with a Periotron 8000 (Oraflow Inc.). All strips with GCF were placed separately into coded sealed Eppendorf microcentrifuge tubes containing 100 μL of sterile phosphate-buffered saline (PBS) and stored at −80°C until processing.

2.6 | Multiplex bead immunoassay

IL-1β, IL-6, IL-12, MMP-8, and MMP-9 total amount were detected in biological samples by means of a high-sensibility Bio-Plex Suspension Array System (Bio-Rad Laboratories S.r.l.) according to the manufacturer's instructions. Briefly, opportune anticytokine antibody-conjugated beads were loaded into individual wells of a 96-well plate. After washing, standards and undiluted GCF samples were added to respective wells and incubated for 30 min. Plates were thus washed and biotin-conjugated detection antibody was added. After another 30 min of incubation and consequent washing, streptavidin-conjugated PE was added for 10 min. Therefore, the complex was solubilized by adding the Bio-Plex assay buffer to each well and analyzed with the Bio-Plex Suspension Array System. Total amounts of each molecule were determined.

2.7 | Statistical analysis

Statistical analyses were conducted using commercially available software (SPSS, IBM version 28). The statistical unit was the patient. To test whether the data were normally distributed, the Shapiro–Wilk test was done. Between groups differences in quantitative variables were tested using the independent *t*-test and the Mann–Whitney *U*-test, as appropriate. Differences in the frequency of the categorical variables were verified using the chi-square test or the Fisher exact test. Paired t-test and Wilcoxon test were used to detect intragroup differences in quantitative variables over time. Correlations between cytokines levels in GCF at T1 and EHI scores were assessed with the Spearman correlation analysis. Finally, the EHI scores were dichotomized into two categories, one representing favorable wound healing (EHI degrees 1 and 2) and the other one representing unfavorable healing due to incomplete flap closure and/or necrosis (EHI degrees 3, 4, and 5). A multiple exploratory stepwise backward logistic regression model was built to identify predictors of favorable EHI. Data were presented as odds ratio (OR) and 95% confidence intervals (CI). *p* Values <.05 were considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Figure 1 summarizes the flow chart of the study. Forty-four patients were consecutively enrolled in the study and randomly assigned to the test or control experimental procedures. All participants, 22 in the test group (12 females; mean age: 58.9 ± 8.5 years) and 22 in the control group (14 females, mean age: 55.7 ± 9.6 years), received the allocated procedure and were included in the statistical analyses. At baseline (T0), there were no statistically significant differences ($p > .05$) between the groups for any of the variables at patient-level (Table 1). The distributions of intrabony defects according to teeth were 31.8% anterior, 45.5% premolar, and 22.7% molar for the test group and 36.4% anterior, 31.8% premolar, and 31.8% molar, for the control group. No statistically significant difference was detected for any of the baseline defect characteristics between test and control defect sites.

3.2 | Clinical outcomes

3.2.1 | BoP reduction

No adverse outcomes were observed in any of the patients. At T1, there were no intragroup changes in any of the clinical periodontal parameters compared to T0, except for BoP (Table S1). At T0, all the sites presented with BoP in both groups. At T1, in the test group, the BoP decreased to 27.3% while in the control group, it decreased to 72.7%. The number of sites with persistent bleeding was statistically significantly less in the test group ($p < .01$).

3.2.2 | EHI

At T2, the EHI had an average value of 1.45 ± 0.86 in the test group while in the control group, the average value was 2.31 ± 1.43. The difference between the two groups was statistically significant, favoring the test group ($p = .027$). In order to explore the predictors of a better or worse EHI, a logistic regression model was implemented, showing how the presence of a noncontained defect (OR = 0.13; 95%CI: 0.02–0.77) and the presence of BoP at T1 (OR = 0.11; 95%CI: 0.02–0.65) decreased the probability of obtaining a better EHI (Table 2).

3.3 | Inflammatory molecules analysis

At T0, no significant differences were present in terms of GCF volume or in any of the analyzed inflammatory mediators between the test and control groups. At T1, GCF volume decreased more in the test (from 1.5 ± 0.5 μL to 1.2 ± 0.5 μL) than in the control group (from 1.7 ± 0.6 μL to 1.6 ± 0.5 μL) with statistically significant difference between each other ($p = .015$). At the same time point, the total amount of IL-1β ($p < .001$), MMP-8 ($p < .001$), and MMP-9 ($p = .010$) significantly decreased in the test group, whereas in the control group, only MMP-8 ($p = .001$) was reduced (Figures 2A and 3A,B). The difference between the two study groups was statistically significant for all the biomolecular markers, except for IL-12 (Figures 2 and 3). A statistically significantly positive correlation was observed between the amount of IL-1β ($r = .730$, $p < .001$) and MMP-9 ($r = .598$, $p < .001$) in the GCF at T1 and EHI scores.

4 | DISCUSSION

In the present RCT, the local DOX application led to a statistically significant decrease in BoP at the experimental sites, which

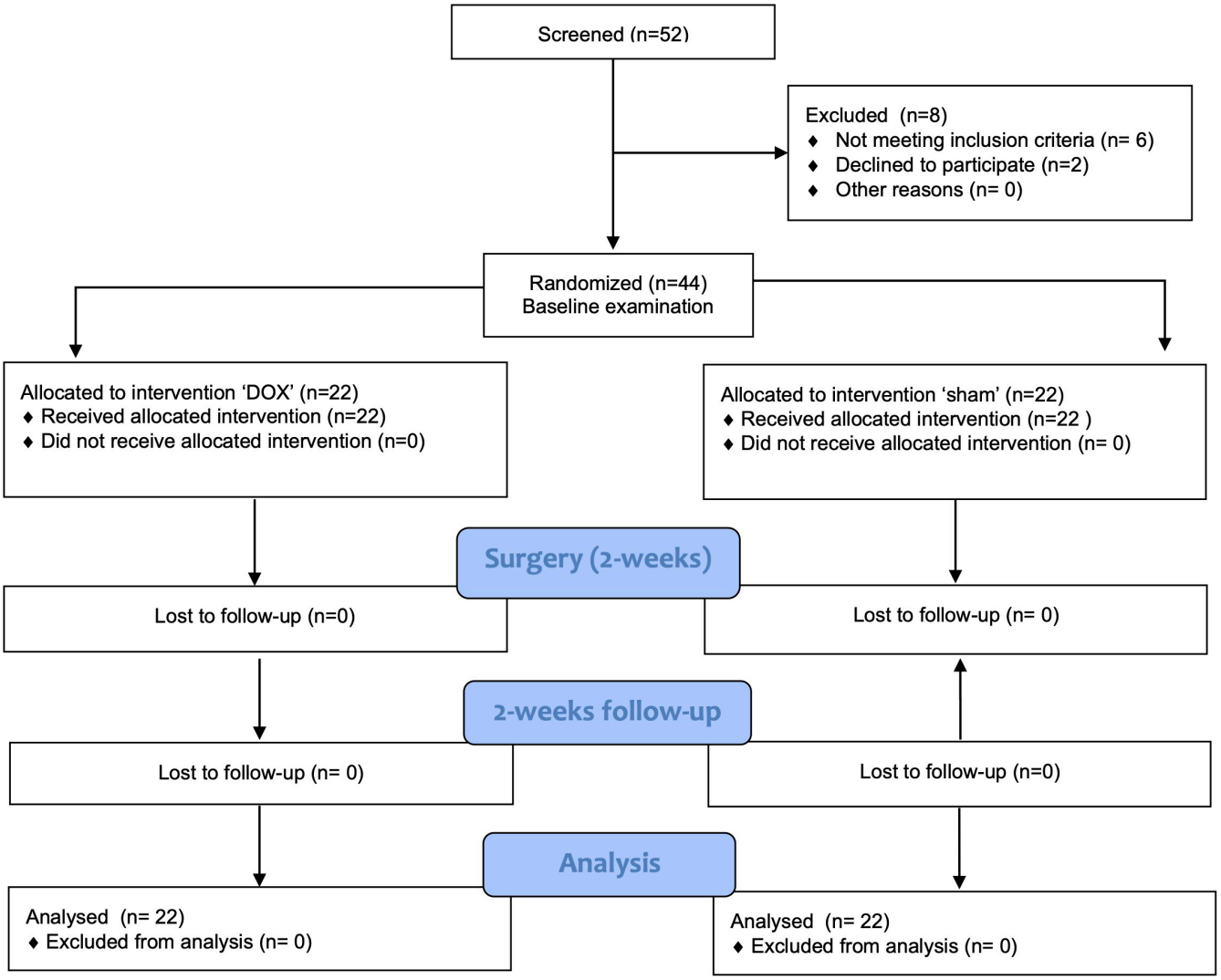

FIGURE 1 Flow-chart of the study design.

presented at the surgical appointment with less site inflammation. Indeed, persisting BoP sites decreased to one-fourth in the test group and three-fourths in the control group. These findings may have a relevant clinical implication for the site-specific modulation of the inflammatory state prior to minimally invasive periodontal regeneration using a locally delivered antimicrobial.

Previous studies have evaluated DOX use as an adjunctive during step II of therapy[8] or for persistent or recurrent pockets during supportive periodontal care.[11] Despite the initial clinical improvement, the benefit of local DOX seemed to fade over time, raising questions about the relevance of this option in the routinary clinical setting.[16] To the best of authors' knowledge, this is the first RCT that evaluated the adjunctive effect of local DOX in the site preparation before periodontal regeneration surgery. The biological rationale stems from the fact that, as suggested in literature by Heitz-Mayfield et al.,[5] the site-specific inflammatory burden has to be kept as low as possible prior to periodontal surgery. To this regard, DOX possesses both antimicrobial[17] and host-modulatory properties due to the inhibitory effect on collagenase activity,[10,18] allowing for better tissue quality and flap management. Moreover, it stimulated cell maturation and osteoblast differentiation, enhancing periodontal regeneration.[19] Lastly, compared to other formulations, DOX is delivered through a biodegradable gel, which combines easy application with the long-lasting release of the active principle, providing effective drug concentration at the site of infection with higher anticollagenolytic activity and low risk for the emergence of bacterial resistance.[20]

In this study, the molecular inflammatory profile in the GCF showed significant changes compared to baseline, with the application of DOX being associated with the highest reduction in MMPs and IL-1/IL-6 amounts. Proteins and metabolites in the GCF can be reliable markers of local inflammatory states and were associated with higher tissue catabolism.[21,22] In recent years, MMP-8 and 9 were widely investigated in GCF and saliva,[23] showing high accuracy for discriminating between periodontal health and disease, and longitudinally predicting disease progression.[24] Regarding the effect of local antimicrobials, a previous exploratory study found a decrease in MMP-8 levels at 7 days and 1 month after subgingival

TABLE 1 Characteristics of study subjects and experimental intrabony defects at baseline.

Variables	Test	Control	*p*-Value
Patient level			
Age (years; mean ± SD)	58.9 ± 8.5	55.7 ± 9.6	.259
Females/Males (*n*)	12/10	14/8	.540
FMPS (%; mean ± SD)	10.6 ± 1.8	11.5 ± 1.9	.101
FMBS (%; mean ± SD)	8.1 ± 1.7	8.9 ± 1.6	.160
Tooth level			
Tooth type (anterior/premolar/molar; %)	31.8/45.5/22.7	36.4/31.8/31.8	.628
Dental arch (maxilla, mandible; %)	72.7/27.3	50.0/50.0	.122
Site level			
Presence of plaque (%)	13.6	36.4	.162
BoP (%)	100	100	–
PPD (mm; mean ± SD)	8.2 ± 2.1	7.8 ± 2.7	.285
REC (mm; mean ± SD)	2.1 ± 1.6	1.9 ± 1.5	.700
CAL (mm; mean ± SD)	10.3 ± 2.9	9.7 ± 2.8	.493
KT (mm; mean ± SD)	5.2 ± 2.3	5.0 ± 1.6	.718

Abbreviations: BoP, bleeding on probing; CAL, clinical attachment level; FMBS, full-mouth bleeding score; FMPS, full-mouth plaque score; KT, keratinized tissue width; PPD, probing pocket depth; REC, gingival recession; SD, standard deviation.

TABLE 2 Logistic regression model to identify predictors of a favorable wound healing (EHI scores 1, 2, or 3) after periodontal regenerative surgery.

Variables	OR	95% (CI)	*p*-Value
Type of bony defects			
Contained	1		
Noncontained	0.128	0.021–0.768	.024
Presence of BoP at T1			
Yes	1		
No	0.110	0.019–0.654	.015

instrumentation in chronic and aggressive periodontitis patients, without any adjunctive effect of DOX.[25] However, patient allocation was not assigned randomly, and moderate PPDs were mostly included. Indeed, the highest DOX activity has been documented in sites with more severe PPDs.[11] MMP gene transcription is also stimulated by inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-alpha.[26] These proinflammatory molecules have been long explored as biomarkers for periodontitis, showing consistent decreases after treatment both in the present as well as in previous investigations.[27] Conversely, IL-12 values did not show any statistically significant changes after therapy in both study groups at short term. However, the exact role of this immune-modulatory cytokine in periodontitis pathogenesis and risk of progression needs to be further investigated.[28]

The reduced presurgical clinical and biochemical inflammatory status could have played a role for the better results of EHI in the test group (flaps closed for primary intention with the presence of small quantity of fibrin).[15] Consistently, a significant positive correlation was observed between the amount of IL-1β and MMP-9 in the GCF at T1 and EHI scores. These short-term results could express how a reduced collagenolytic activity and inflammatory local condition prior to surgical treatment could improve the healing of the site, which is important to maintain coagulum stability and prevent the contamination of the biomaterials used for reconstructing the intrabony component.[5,29]

This study was designed as a double-blinded RCT, limiting selection and confounding biases. Indeed, different factors may also affect the healing results after periodontal regeneration, including systemic modifying factors, flap design, defect anatomy, and reconstructive technologies.[3,30] In order to control for these variables, strict inclusion criteria for patient selection were applied. Moreover, potential variations in flap design were accounted by only selecting cases suitable for MIST and standardizing the biomaterial choice.[31] Also, from recent systematic reviews such as Stavropoulos et al.[32] and Nibali et al.,[29] it emerged that defect morphology was a pivotal determinant of coagulum stability and favorable healing outcomes. Indeed, in the exploratory logistic regression model, noncontained defects and persisting BoP at the day of surgery decreased the probability of obtaining a better EHI. This study also displays some limitations, mainly due to the short-term follow-up, which did not allow to make inferences on the clinical and radiographic outcomes of periodontal regeneration. Moreover, the generalizability of these findings is limited because of the exclusion of patients with risk factors associated to poorer healing outcomes, such as smokers and diabetic subjects.[3,4] To this regard, there is the potential to replicate this study design within these high-risk populations, which may benefit more from adjunctive DOX.[6,33,34]

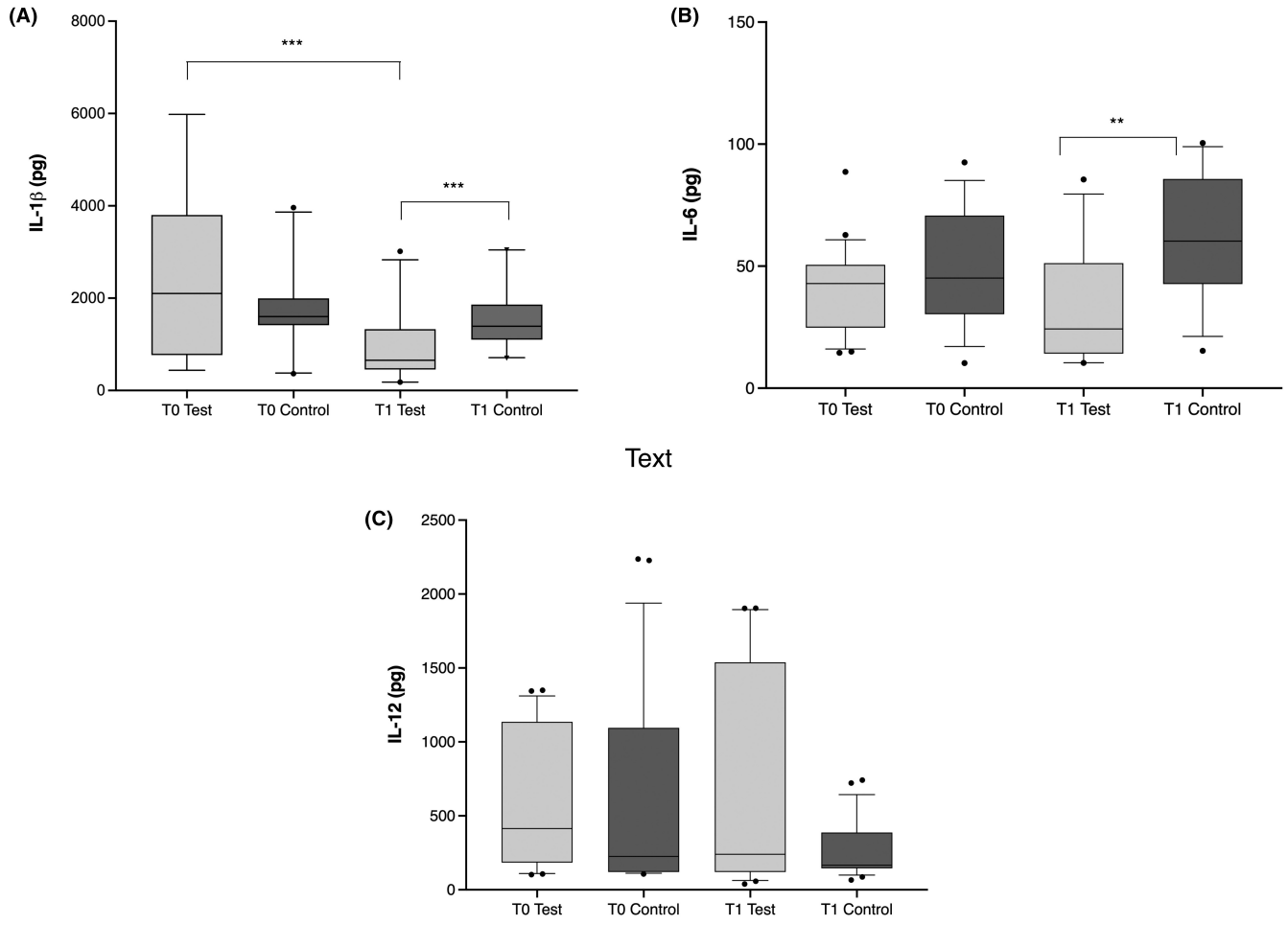

FIGURE 2 Box-and-whisker plots showing the total amount of IL-1β (A), IL-6 (B) and IL-12 (C) in gingival crevicular of the test and control groups before (T0) and after (T1) the subgingival instrumentation. The box represents median, 25% and 75% percentiles, the whiskers represents data within 10% and 90% percentiles.$^{**}p < .01$, $^{***}p < .001$.

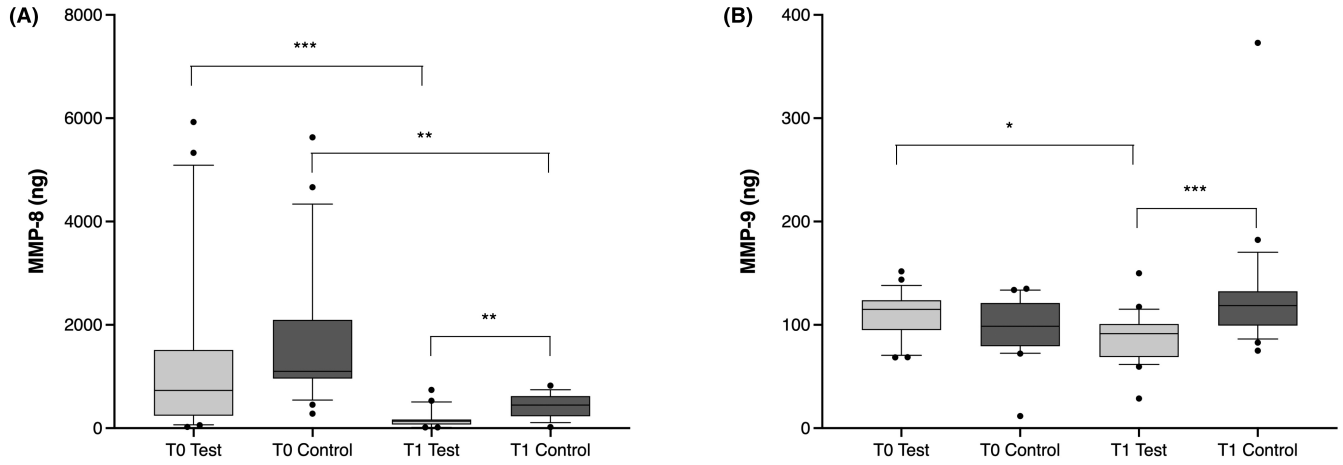

FIGURE 3 Box-and-whisker plots showing the total amount of MMP-8 (A) and MMP-9 (B) in gingival crevicular of the test and control groups before and after the subgingival instrumentation. The box represents median, 25% and 75% percentiles, the whiskers represents data within 10% and 90% percentiles. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

5 | CONCLUSION

Within the limitations of this study, it can be concluded that the sites treated with local DOX showed decreased inflammation at 2 weeks, displaying more BoP reduction and less total amount of molecular markers related to tissue catabolism compared to controls at the day of surgery. Moreover, the sites receiving local DOX showed a better early wound healing at 2 weeks after surgery. These observations may further corroborate the importance of keeping local

inflammation under control prior to periodontal regeneration techniques through a site-specific modulation of the collagenolytic activity. Therefore, local DOX may be incorporated into clinical treatment protocols for this specific use.

AUTHOR CONTRIBUTIONS

Mario Aimetti, Filippo Citterio, and Federica Romano made substantial contributions to conception of the study. Mario Aimetti, Giacomo Baima, Filippo Citterio, Giovanni N. Berta, and Federica Romano contributed to the study design. Giacomo Baima, Virginia Lorenzetti, and Nargiz Aliyeva performed clinical examinations and treatments. Giacomo Baima and Federica Romano performed data analysis and interpretation. Francesco Franco, Federica Di Scipio, and Giovanni N. Berta performed laboratory analysis. Giacomo Baima, Virginia Lorenzetti, Nargiz Aliyeva, and Federica Romano prepared the first draft of the manuscript. All authors have read, revised critically, and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any financial interests, either directly or indirectly, in the information listed in the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are listed in the main manuscript and Supporting Information.

CONSENT TO PARTICIPATE

All volunteers provided their free informed consent to participate.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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