A randomized controlled clinical trial evaluating the efficacy of zoledronate gel as a local drug delivery system in the treatment of chronic periodontitis: A clinical and radiological correlation

ABSTRACT

Background: This study aimed to evaluate the efficacy of local drug delivery system of zoledronate (ZLN) gel as an adjunct to scaling and root planing (SRP) for the treatment of human periodontal intrabony defects clinically and radiographically.

Materials and Methods: Forty intrabony defects (three walled and combined defects without involving furcation) in moderate to severely affected forty chronic periodontitis patients (range, 30–50 years) were randomly divided into two groups and treated either with 0.05% ZLN gel (ZLN n = 20; 1 dropout) or placebo gel (control group [CG] n = 20) after SRP. Clinical parameters such as plaque index (PI), gingival index (GI), tooth-specific pocket probing depth (T<sub>s</sub> PPD), and clinical attachment levels (T<sub>s</sub>CAL) were assessed at baseline and at 3 and 6 months using occlusal acrylic stent. Radiographic parameters were assessed at baseline and 6 months, utilizing “ONIS 2.5 PROFESSIONAL” and “SYNGO” software compatible with DentaScan to measure the volumetric bone changes in intrabony defects.

Results: In intragroup comparisons, both groups showed significant PI and GI reduction (P < 0.001) after treatment at 3 and 6 months. In intergroup comparisons, T<sub>s</sub>PPD reduction and T<sub>s</sub>CAL gain were significant only in ZLN at 6 months from both baseline and 3 months. Radiographically, significant reduction in defect depth and buccolingual width with volumetric defect gain of 40.24% ± 7.44% in ZLN compared to insignificant gain of 1.60% ± 4.06% in CG was observed at 6 months.

Conclusion: ZLN gel applied subgingivally in intrabony defects resulted in significant improvements both clinically and radiographically.

Keywords: Bisphosphonate, DentaScan, intrabony defects, local drug delivery, volumetric bone gain, zoledronate

INTRODUCTION

Potential therapeutic agents such as nonsteroidal anti-inflammatory drugs, chemically modified tetracyclines, and bisphosphonates (BPs) to treat bone resorption are well documented in periodontal literature. However, their role as a local drug delivery (LDD) system needs exploration, hoping that this therapy can provide a new wave of adjuvant pharmacologic agents for periodontal regeneration and osteogenic induction.

BPs are inorganic pyrophosphate analogs that are widely utilized in the management of systemic metabolic bone diseases such as Paget’s disease, hypercalcemia of malignancy,
BPs in periodontics came into light for the first time when the role of locally delivered alendronate (ALN) (second-generation nitrogen-containing BP) was emphasized in reducing the mucoperiosteal flap-activated resorption during surgery.\[2\] Recent literature search highlights the use of 1% ALN gel as a LDD system for treating intrabony defects in not only systemically healthy chronic periodontitis\[3\] individuals, but also those with simultaneous diabetes\[4\] as well as smoking habit.\[5\] Their results showed a significant probing depth (PD) reduction, periodontal attachment level gain, and improved bone fill.

Zoledronate (ZLN), a third-generation BP, is the most potent amongst all BPs known so far with the highest bone affinities.\[2,7\] Studies have demonstrated that local and systemic treatments with ZLN can enhance the osseointegration and fixation of orthopedic implants\[8-10\] as well as dental implants in rats.\[11\]

In the present trial, for the first time, ZLN has been used as a LDD system for treating periodontal intrabony defects. It acts by inhibiting the key enzyme (farnesyl pyrophosphatase)\[12\] of mevalonate pathway that regulates many cellular activities in osteoclasts, consequently leading to its apoptosis and reduced bone resorption.\[13\] These unique pharmacokinetic characteristics can thus enable small doses of ZLN which can be utilized as a LDD system in osseous defects with minimal unwanted side effects. The present study aims at evaluating the efficacy of ZLN gel as a LDD system as an adjunct to scaling and root planing (SRP) for the treatment of intrabony defects in chronic periodontitis.

MATERIALS AND METHODS

Study population
In this 6-month follow-up, interventional, double-blinded, randomized controlled clinical study, a total of 113 chronic periodontitis patients (age range, 30–50 years) were screened. Only forty patients after screening fulfilled the inclusion criteria and were enrolled in the study. Each patient signed an informed consent form prior to study enrollment. The study was approved by the institutional ethical committee and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Selection criteria

Exclusion criteria

(1) Systemically healthy chronic periodontitis patients (age range, 30–50 years) having at least one intrabony defect with pocket PDs ≥ 5 mm or clinical attachment loss (CALs) ≥ 4 as measured by UNC-15 calibrated periodontal probe and vertical bone loss ≥ 3 mm on intraoral periapical radiographs or orthopantomogram [Figure 1a and c] and (2) Only those patients who on DentaScan showed three walled or combined defects without involving the furcation with radiographic defect angle ≤ 45\[14\] were included in the study by an independent blinded investigator [Figure 1b].

Exclusion criteria

(1) Patients allergic to BPs or on systemic BP therapy, tobacco and alcohol users, (2) immunocompromised patients or having taken antibiotics within preceding 3 months, (3) those who have undergone periodontal surgical treatment within the last 6 months, (4) pregnant or lactating females, and (5) serum creatinine clearance < 35 mL/min or in patients with evidence of acute renal impairment (patient’s medical history) since BP is eliminated from the body through renal excretion were excluded from the study.

Randomization

After enrollment, the patients were randomly assigned (by a computer-generated system using Excel 2013 v 15.0 for Microsoft windows) either to the ZLN group (n = 20; 8 females and 12 males; 1 dropout that failed to undergo reevaluation after 6 months) or control group (CG) (n = 20; 9 females and 11 males). All the bony defects included in the study were either three walled or combined defects at the interproximal sites of the adjoining teeth. In patients with more than one intrabony defects, the defect measuring greatest intrabony component depth was included in the study.\[15\] The study design is depicted in the flowchart [Figure 2].

Treatment procedure

SRP was performed at baseline until a smooth, hard, and clean surface was obtained as speculated by the investigator.
in both the groups. After 4 weeks of reevaluation of the periodontal status of the chosen sites in both the groups, if there was persistence of periodontal pockets, only then the LDD procedure was carried out.

The ZLN group sites received LDD of 0.05% ZLN gel (20 µl), whereas the CG sites received the same amount of placebo gel. Patients as well as the investigator “A” performing SRP both were masked for allocation into the ZLN or placebo group. The clinical parameters including full mouth plaque index (PI), gingival index (GI), tooth-specific PPD (TₚPPD), and clinical attachment level (TₚCAL) were recorded at baseline and at 3 and 6 months. TₚPPD and TₚCAL were measured to the nearest millimeter with the help of a UNC 15 periodontal probe (Hu-Friedy Mfg.Co., LLC, Chicago, United States) by another investigator “B” (masked to the treatment received) interproximally in all the chosen sites. A custom-made acrylic stent was fabricated for each patient to standardize the measurement of clinical parameters at all follow-up periods [Figure 1a].

Radiographic assessment of intrabony defects
Bone defect morphology was assessed using DentaScan, a high-resolution three-dimensional (3D) computed tomography (CT) scanner equipped with 3D image reconstruction software (SYNGO FAST VIEW, Siemens Medicals IKM/Germany 2004–2009). Slices with the thickness of 0.75 mm were made and spiral CT images of the maxilla and mandible were recorded, from which sections were made to record the respective measurements.

The radiographic parameters were measured on the panoramic view, cross-sectional view/sagittal view, and axial sections of the respective sites using the caliper provided with the SYNGO software, with accuracy to the nearest 0.1 mm. For calculation purpose, a fixed reference point cementoenamel junction with two auxiliary lines (AUX₁: auxillary line along the long axis of the tooth and AUX₂: auxillary line perpendicular to the AUX₁ through the most coronal extension of the lateral wall of the infrabony defect) were taken. “Onis 2.5” professional software, Digital Core, Co.Ltd., Tokiyo, Japan, was used to measure the volumetric variations in the intrabony defects with time. The volume of intrabony defect was calculated according to the formula following Cavalieri’s principle as given below:

\[
\text{Defect volume} = (\text{Summation of area of defect in subsequent sections}) \times (\text{width of axial section}) \text{ mm}^3, \text{i.e.}, (m_1 + m_2 + m_3 + m_4 + m_5 + m_6) \times 0.75 \text{ mm}^3
\]
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The obtained section surface area values were written down on the study pages created in Microsoft Office Excel 2007, version 1, Microsoft Corporation, America, United States, and the volume estimations were automatically conducted. Representative software photographs of radiological parametric calculation are represented [Figures 3 and 4] individually. Customized bite blocks were used to obtain films as reproducible as possible at baseline and after 6 months. All radiographs were reviewed by investigator “C” masked to the treatment received.

Formulation of gel

0.05% ZLN gel was prepared using commercially available drug (5 mg ZLN per 100 ml). Weighed quantity of carbopol 934 P was added to distilled water to produce a 2% weight/weight solution. It was then allowed to soak for 2 h by continually stirring the solution. Commercially available ZLN solution was added followed by 1% triethanolamine to neutralize the carbopol solution to form a gel of pH 6.8. Finally, preservatives such as methyl paraben and propyl paraben solutions in ethanol were incorporated in the gel. The placebo gel was prepared by the above mentioned procedure without adding active ingredient, i.e., ZLN.[20]

After formulation, characterization of ZLN and placebo gels was done.

1. Organoleptic rating [Table 1]
2. Determination of viscosity of both ZLN and placebo gel was done by Brooke’s field viscometer (BV) [Tables 2 and 3].[21]

As per the readings of Tables 2 and 3, placebo gel was found to be a little more viscous than ZLN gel since the active drug was a liquid formulation which was absent in the placebo. The sole purpose of this determination was to justify that both gels formulated were of almost similar viscosity so that they remain indifferent in their physical nature for easy masking to the investigator as well as viscous enough to be retained in the intrabony defect.

Local drug delivery procedure

Twenty microliter of gel measured by micropipette was delivered at the experimental (0.05% ZLN) and the CG sites (placebo gel) with a syringe having blunt cannula passively into the periodontal pocket associated with the intrabony defect followed by COE-PAK dressing at the treated site for 2 days.

Gingival crevicular fluid collection

Gingival crevicular fluid (GCF) was collected from the experimental sites in any two of the randomly selected patients on 2nd, 7th, and 15th day of the delivery of the ZLN gel with the objective of determining the concentration of ZLN that remains at the site. The sites were isolated with cotton rolls to prevent contamination with saliva. GCF samples were collected with the help of glass microcapillaries[15] from the base of the vertical defect after 2 days of delivering the gel. The microcapillaries were used vigilantly until slight resistance was felt and then left there for 30 s and collected in Eppendorf tubes with no transport media [Figure 5]. The GCF rises due to the capillary action in the glass microcapillaries. In case of contamination with blood, the samples were discarded. Following collection, the sample containing Eppendorf tubes was kept in an ice box and immediately transported to the laboratory where they were stored at a temperature of 4°C until its analysis by ultraviolet (UV) spectrophotometer.

Figure 3: Calculation of radiological parameters: (a) Defect depth (D) (mm): the distance from cementoenamel junction to apical extension of the bony defect at baseline; D1 (at 6 months), (b) mesiodistal width of defect MDW (mm): the horizontal distance on auxiliary line (AUX2) from alveolar crest to point on root surface involving the defect at baseline; MDW1 (at 6 months), (c) buccolingual width (BLW) (mm): the horizontal distance from the most coronal point of the buccal crestal bone to lingual crestal bone on cross-sectional view; BLW1 (at 6 months)

Figure 4: (a) Area calculation of the defect on axial section by ONIS 2.5 Professional software, (b) defect volume = (area of defect in subsequent sections) mm² × (width of axial section) mm, i.e., (m1 + m2 + m3 + m4 + m5 + m6) × 0.75 mm³
Analysis of zoledronate concentration in gingival crevicular fluid samples by ultraviolet-spectrophotometry

Double-beam UV spectrophotometry (UV-1700 Pharma Spec, Shimadzu) is a combination of a tungsten halogen and deuterium lamp, filters, windows, mirrors, a photomultiplier, and data station with a software. In this type of instrument, the monochromatic light (range, 200–400 nm) is split by a rapidly rotating beam chopper into two beams which are directed alternately in rapid succession through a cell containing the sample and one containing the solvent only (blank).\[21\]

Determination of absorption maxima (lambda maximum)

A standard solution (10 µg/ml) of ZLN was prepared in ethanol and scanned by UV-VIS spectrophotometer (UV-1700 Pharma Spec, SHIMADZU) between 200 nm and 400 nm. The lambda maximum was found to be 272.5 nm\[21\] [Figure 6].

Quantitative estimation of zoledronate: Preparation of calibration curve/standard curve of zoledronate in ethanol

Accurately measured 10 ml of the marketed formulation (5 mg of ZLN in 100 ml) was taken and diluted with 100 ml of ethanol to obtain a concentration of 5 µg/ml (stock solution). From this stock solution, various aliquots ranging from 1 to 10 µg/ml were prepared. The absorbance of these solutions was spectrophotometrically measured at 272.5 nm against reference blank solution.\[21\] Regressed curve of ZLN ethanol at 272.5 nm is summarized in Table 4 and shown graphically in Figure 7. The absorbance and concentration of ZLN in GCF samples at 2 h, 7th day, and 15th day are summarized in Table 5.

The data suggested that ZLN was detected in the GCF sample from 2 h till 15th day of its placement in the intrabony defect. The concentration percentage of the drug gets reduced from 23.8% to 12.65%. The drug was retained in the target defect site for 15 days, suggesting a controlled release.

Statistical analysis

Mean values and standard deviations (SDs) were calculated for all parameters for both groups at all time periods. Groups were compared by repeated measures with two-factor analysis of variance. The significance of intra- and inter-group mean difference was calculated by Tukey’s post hoc test. Groups were also compared by independent Student’s t-test or Mann–Whitney U-test. All statistical tests of hypothesis were two sided and employed a level of significance of <5% (P < 0.05), which was considered to be statistically significant. Considering an SD of 2.55 mm, it was calculated that twenty patients were needed in each group to provide 95% power with an alpha error of 0.05 and confidence interval of 95%. All analyses were performed on SPSS software (PSAW, windows version 18.0, IBM Corporation, America, United States).

RESULTS

Thirty-nine patients out of forty completed the study. Only one patient in ZLN group failed for follow-up reevaluation. All patients in the test group tolerated the drug well without any adverse drug reaction to the drug. Both the groups were statistically comparable for mean PI and mean GI scores at all time periods [Table 6]. In intragroup comparison, Tukey test revealed significant (P < 0.001) decrease in the above parameters of both the groups at 3 months and 6 months in comparison to baseline [Table 7].

Mean baseline data of tooth-specific pocket PPD (T_pPD) and T_CAL at baseline and 3 months were statistically comparable in
both groups, but significant reduction was seen in ZLN group at 6 months compared to CG \((P < 0.05)\) [Table 6]. The intragroup comparison revealed ZLN group to have significant reduction in both the above parameters \((P < 0.001)\) at 6 months from both baseline and 3 months as compared to CG [Table 8].

Radiographic parameters showed the following trends with significant reduction in mean defect depth (D) and mean buccolingual width of defect (BLW) in ZLN group compared to CG at the end of 6 months, while statistically insignificant mean mesiodistal defect width reduction was seen in both the groups at 6 months [Table 9]. The mean radiographic angle fill showed insignificant increase of \(0.29° \pm 1.67°\) and also insignificant mean volumetric bone gain percentage of \(1.60% \pm 4.06%\) at 6 months from baseline in CG. While in ZLN group, there was a statistically significant increase of \(14.45° \pm 1.67°\) and \(40.24% \pm 7.44%\), respectively, from baseline to 6 months [Tables 10 and 11].

**DISCUSSION**

Evaluation of the efficacy of 0.05% of ZLN gel as LDD was carried out in the present in vivo study because this delivery system offers advantages of adequate concentrations at the target site with a reduced dosage, fewer applications, high patient acceptability,[22] and has benefit of less adverse reactions compared to systemic regimen.

Till now, only a few studies have been documented using BPs (ALN) as a LDD system in periodontal defects.[4,5,23] Moreover, to the best of our knowledge, this is probably the first study in which ZLN has been utilized; therefore, a direct comparison with other studies was not possible.

**Clinical parameters**

PI and GI scored for both the groups showed statistically significant reduction from 0 to 6 months. Intergroup comparison showed no statistically significant difference at 0, 3, and 6 months in terms of PI and GI. Since SRP was performed in both the groups, a statistically significant reduction in PI and GI was found at the end of the study in both the groups.

ZLN group showed statistically significant \(T_{PPD}\) reduction and \(T_{CAL}\) gain in contrast to CG with insignificant results,
respectively, at 6 months from baseline. The significant findings in mean PI, GI, T_PPD, and T_CAL were in accordance with the previous studies, in which 1% ALN gel was utilized as a LDD for the intrabony defects in chronic periodontitis patients in their 6-month follow-up study. However, in contrast, another study did not observe any improvement in PD measurements in periodontitis of monkey model treated with systemic ALN for a duration of 10 weeks. The lack of such effects can be explained by the short duration of treatment and mode of administration of drug.

Radiographic parameters

Radiographic studies have reported that nitrogen-containing BP such as ALN when given as a systemic treatment and mode of administration of drug.
or topical application resulted in reduction of alveolar bone resorption after mucoperiosteal flap surgery. Although the studies involving the utilization of local application of the potential BP ZLN are few in the dental literature, worth mentioning is the study of Adam et al., 2012, highlighting through histological and microcomputed tomographic analysis that a single low-dose (16 μg) local application of the ZLN provides maximum anchorage by preventing bone resorption during orthodontic treatment. The authors have also mentioned that it might be achieved with local application of ZLN at as low quantity as 0.2 μg.[26]

A review study has shown the occurrence of osteonecrosis in patients suffering from multiple myeloma or metastatic bone disease as a side effect when high dosage of ZLN was administered systemically for the treatment (4 mg/100 ml intravenous every 6 months).[27] In the present study, no such necrosis was observed, it could be because of low dosage, i.e., 20 μl of 0.05% of ZLN that was delivered.

Pre- and post-LDD of the gel, depth of defect radiologically showed reduction in both control and ZLN groups, with results statistically significant only in ZLN group ($P < 0.05$) at 6 months [Figure 8]. It can be explained by periodontal healing in the form of bone fill in the deepest part of the intrabony defects.[28] Further, these results were in accordance with the study conducted with ALN gel as a LDD system for intrabony defects that also showed a significant mean reduction in intrabony depth of defect and significant vertical defect fill at the end of 6 months.[4]

Statistically nonsignificant reduction was noticed in terms of mean mesiodistal width in both the groups. It suggested that the height of interdental alveolar crest associated with the intrabony defect from which the mesiodistal measurements were taken on DentaScan images was almost static with minimal remodeling in the form of bone formation or resorption through the time period of 6 months.

Table 9: Inter- and intra-group comparison ($P$) of mean difference in defect depth, mesiodistal width, and buccolingual width between control and zoledronate groups at different time periods

| Periods             | Mean defect depth (mm) | Mean MDW (mm) | Mean BLW (mm) |
|---------------------|------------------------|---------------|---------------|
|                     | Control ($n = 20$)     | ZLN ($n = 19$) | Control ($n = 20$) | ZLN ($n = 19$) | Control ($n = 20$) | ZLN ($n = 19$) |
| 0 month versus 3 months | 9.51±3.04              | 8.25±2.66     | 0.559         | 2.71±0.73      | 2.77±0.67         | 0.998         | 9.54±2        | 9.58±1.87     | 1.000         |
| 0 month versus 6 months | 9.42±3.05              | 7.00±2.40     | 0.045         | 2.69±0.88      | 2.58±0.91         | 0.975         | 9.50±2.31     | 8.81±1.85     | 0.775         |

MDW: Mesiodistal width, BLW: Buccolingual width, ZLN: Zoledronate

Table 10: Intergroup comparison ($P$) of mean radiographic angle fill and volumetric bone gain of both groups at 6 months

|                  | Mean radiographic angle fill (°) | Mean volumetric bone gain (%) |
|------------------|----------------------------------|-------------------------------|
|                  | Control ($n = 20$)               | ZLN ($n = 19$)               | Control ($n = 20$) | ZLN ($n = 19$) |
| 0.29±1.67        | 14.45±9.44                       | 5.53                          | <0.001           | 1.60±4.06      | 40.24±7.44      | 17.63          | <0.001         |

ZLN: Zoledronate

Table 11: Inter- and intra-group comparison ($P$) of mean difference in bone defect angle and volume of intrabony defect between control and zoledronate groups at different time periods

| Periods             | Mean BDA (°) | Periods             | Mean volume of intrabony defect (mm$^3$) |
|---------------------|--------------|---------------------|-----------------------------------------|
|                     | Control ($n = 20$) | ZLN ($n = 19$) | Control ($n = 20$) | ZLN ($n = 19$) |
| 0 month versus 3 months | 33.30±11.21  | 34.79±9.96          | 0.984        | 0 month         | 144.64±148.23   | 100.44±94.47   | 0.678         |
| 0 month versus 6 months | 33.58±10.97  | 49.24±14.91         | 0.003        | 6 months        | 144.87±154.97   | 59.48±52.47    | 0.015         |

BDA: Bone defect angle, ZLN: Zoledronate

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in mean BLW of defect after 6 months can be explained by the fact that the majority of included intrabony defects had intact buccal and lingual cortical plates and the healing of such lesions takes place by some resorptive changes at the peak of crests and filling at the base of the lesion.\[30\]

The mean radiographic BDA and volumetric defect gain percentage showed statistically insignificant increase of \(0.29^\circ \pm 1.67^\circ\) and \(1.60\% \pm 4.06\%\) in CG and statistically significant increase of \(14.45^\circ \pm 1.67^\circ\) and \(40.24\% \pm 7.44\%\) in ZLN group from baseline to 6 months \[Figure 8\]. It has been documented that intrabony defects with small angles (0°–45°) show greater potential for bone fill in comparison to wide angles (45°–90°) defects.\[14\] Moreover, since in our study, narrow angular defects with radiographic defect angle \(\leq 45^\circ\) were selected, both groups showed potentiality for bone fill in the deepest part of the defect. The significant increase only in the ZLN group might be due to the drug ZLN that have inhibited bone resorption by osteoclastic apoptosis and in turn promoted bone formation by remodeling. Further, the increment in BDA associated with experimental group was suggestive of bone formation not only in the base of the defect, but also on its slopes. The results of our study were in accordance with the previous studies,\[15,20\] in which vertical defect fill of 42.85% and 40.4%, respectively, was seen with topically delivered ALN in intrabony defects. However, a direct comparison of the present study could not be drawn because none of the studies till now have calculated the volumetric changes associated with intrabony defect in chronic periodontitis patients.

The overall favorable results can be attributed to the adequate concentration of ZLN which was maintained in the intrabony defect and consequently detected in GCF for 15 days. It can be attributed to the polymer polyacrylic acid (PAA) that was used in the preparation of gel. PAA is proposed to improve the intimacy of contact and increase the residence time of a dosage form in the periodontal pocket.\[30‑33\] These findings suggested that 0.05% ZLN delivered subgingivally in the intrabony defects resulted in favorable clinical and radiological findings. Moreover, no patient had reported the incidence of osteonecrosis in experimental group upon follow-up evaluation.

CONCLUSION

The present study showed that the local delivery of 0.05% ZLN into periodontal pockets associated with intrabony defects resulted in significant T:\text{PPD} reduction, T:\text{CAL} gain, radiological defect depth reduction, radiographic defect angle fill, and volumetric defect gain at the end of 6 months. For assessing the bone defect morphological changes in response to ZLN gel, the high-resolution 3D CT scanner with image reconstruction software was used. “ONIS 2.5 Professional” and “SYNGO Fast View, Siemens AG 2004–2009” software were used to calculate the volumetric changes of intrabony defect to the nearest accuracy for the first time in periodontal research with the help of Cavalieri’s principle-based formula.

Periodontal vertical osseous defects have a complex anatomy that can be best visualized only after mucoperiosteal flap reflection. Since crestal bone loss accompanies after surgical procedure, the noninvasive LDD system was preferred for treating such bony defects. Compelling data have already suggested that BPs such as ALN are efficacious in the matter of osseous regeneration in intrabony defects when used as LDD system. However, clinical studies utilizing ZLN, which is the most potent BP till date, remained unexplored and hence the reason for selection. It has twenty times greater relative potency for osteoclast inhibition than ALN. ZLN selectively binds to the osseous surface undergoing resorption (high turnover rate) and, when multinuclear osteoclast approaches the surface, the BP molecule gets endocytosed inhibiting its machinery that regulates cellular activities. It has
been shown that there is no prolonged antiresorptive effect with time, suggesting that BP buried deep inside the bone remains inactive at least as long as it is buried there. Hence, bone formation is expected by the physiological osseous remodeling process once all osteoclasts near the resorptive bone surface get apoptosed by the ZLN.

Radiological assessment through high-resolution 3D CT scan images has helped in better understanding of healing of periodontal osseous defects, especially three-walled and combined intrabony defects. Thus, subgingivally delivered 0.05% ZLN gel resulted not only in improvement of clinical parameters, but also in a volumetric bone gain at 6 months. Therefore, it proves to be a better noninvasive approach for the periodontal regeneration of intrabony defects in patients with chronic periodontitis.

Further studies with larger sample size are required to evaluate the clinical efficacy of ZLN as a LDD system in chronic periodontitis patients. In addition, research needs to be directed toward assessing the drug release profile of ZLN in GCF to understand in depth at molecular and pharmacological level its mechanism of action in bone regeneration of intrabony defects when used as a LDD system.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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