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Original Article

Evaluation of the effect of allograft with doxycycline versus the allograft alone in the treatment of infrabony defects: A controlled clinical and radiographical study

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ABSTRACT

Background: Successful prevention and treatment of periodontal disease are contingent on effective control of periodontopathic microbiota based on the premise of periodontal disease being infectious disorders. An anti-microbial agent, i.e., doxycycline has been incorporated into the allograft to control infection and facilitate healing during and after periodontal therapy.

Materials and Methods: Using a split-mouth design, 15 patients showing clinical evidence of almost identical bilateral infrabony defects requiring bone grafting procedures were randomly selected. In each patient, infrabony defects on one side were designated as Group A (control group) and infrabony defects of the contralateral side of the same arch were designated as Group B (test group). Clinical assessment of probing pocket depth and attachment level and radiographic evaluation of the defect depth was done pre-operatively and at 12-week and 24-week post-operatively. The relative efficacy of the two treatment modalities was evaluated using paired Student's *t*-test and the comparative evaluation between the two groups over the 3 time intervals was done using independent Student's *t*-test.

Results: Both the groups exhibited a highly significant reduction in probing depth and gain in clinical attachment level (CAL) and a linear bone fill at the end of 12 and 24 weeks. Comparative evaluation showed a statistically significant gain in bone fill in Group B as compared to Group A, whereas a non-significant reduction in probing depth and gain in CALs between the two groups at the end of 24 weeks (whereas mean reduction in probing depth and gain in CAL were also greater in Group B but the difference was statistically non-significant).

Conclusion: The increase in linear bone fill in Group B signifies the role of doxycycline in augmenting regenerative potential of allograft by combating residual infection and through host modulation.

Key Words: Allograft, anti-microbial agent, host modulation, infection, regeneration

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INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the investing and supporting tissues of the teeth. Its major etiological factor is the dental plaque, which harbors a multitude of periodontal pathogens resulting in the destruction of periodontium.[1] It involves the annihilation of the gingival and periodontal ligament fibers, the apical proliferation of the junctional epithelium, and the resorption of the alveolar process, leading to the formation of uneven defects in the interdental and marginal bone.

Histological evidence in humans indicates that bone grafting is the only treatment that leads to regeneration

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of bone, cementum, and functionally oriented new periodontal ligament coronal to the base of previous osseous defects.[2] Osseous grafts including autograft, allograft, and xenograft have often been applied for periodontal regeneration with or without guided tissue regeneration.

Among allografts, the most commonly used bone graft is demineralized freeze-dried bone allograft (DFDBA)[2] and is known to stimulate bone formation through osteoinduction.[3,4] This osteoinductive effect is predominantly due to the demineralization of FDBA exposing and activating a variety of bone morphogenetic proteins (BMPs) located in the bone matrix.

Successful prevention and treatment of periodontal diseases are, however, contingent on effective control of periodontopathic microbiota. So, the various anti-microbial agents have been incorporated into the graft materials to control infection and facilitate healing during and after periodontal therapy. It is based on the premise of periodontal disease being infectious disorders.[5]

The concept of local drug delivery was pioneered by Goodson, *et al*.[6,7] with the aim of achieving a high concentration of an antibiotic directly at the site of periodontal infection in order to inhibit the target pathogens, with minimal side effects and lesser reliance on patient's compliance for taking medication.

Among the wide variety of anti-microbials that have been tried to control infections following periodontal therapy, doxycycline has exhibited promising results. It is potentially a valuable antibiotic with a broad spectrum of its activity against the numerous periodontal pathogens. It has the ability to concentrate in the gingival crevicular fluid at levels, substantially greater than that in the serum[7-10] and also binds to the tooth surface to be slowly released in active form, thereby prolonging therapeutic effects.[11] It demonstrates anti-collagenolytic and anti-proteolytic properties that aid osseous regeneration and also helps in reducing periodontal disease progression.[12-15] Doxycycline has also been shown to initiate demineralization on the bone surface layer that results in the release of osteogenic factors such as transforming growth factor (TGF)-β, insulin-like growth factors, or BMPs that trigger bone induction.[16,17]

Taking a clue from the above observations, an attempt has been made in this study to clinically and radiographically evaluate the regenerative potential of doxycycline loaded allograft vis-a-vis the allograft alone in the treatment of human periodontal infrabony defects.

MATERIALS AND METHODS

Study population

A controlled, single blind study was designed with 15 patients (10 males and 5 females) in the age group of 30-50 years. Patients older than 50 years were not included in the study due to the reported decreased regenerative potential in these patients.[18] The patients were selected from among those reporting at the Department of Periodontics, Punjab Government Dental College and Hospital, Amritsar. The patients selected were suffering from chronic generalized periodontitis (based on the criterion established by American Academy of Periodontology, 1999),[19] having two or more bilateral two-walled or three-walled infrabony defects, with a pocket depth > 7 mm and radiographic evidence of infrabony (vertical) defect. The exclusion criteria for selection of the patients were as follows:

1. Smokers and alcoholic patients.
2. Patients with any clinical sign and symptoms of trauma from occlusion.
3. Patients suffering from any systemic disease.
4. Patients with a known history of allergy.

All patients meeting the selection criteria were consecutively enrolled from February 2005 to January 2006.

Before surgery, all these patients received supragingival scaling and oral hygiene instructions. Patient oral hygiene status was evaluated after a period of 2 weeks by the O'Leary plaque index[20] and considered satisfactory when the plaque score of < 10% was present. For each patient, two interproximal sites, one in each quadrant of the same arch were selected based on periodontal pocket, measuring about 5-7 mm and with a radiographic evidence of infrabony (vertical) defects. Sites were selected by the simple random sampling technique and assigned as control site (Group A) and test site (Group B). In each patient, the infrabony defect of one side of the arch was designated as Group A and the infrabony defect of the contralateral side of the same arch was designated as Group B. Group A was treated by the placement of demineralized freeze-dried bone matrix (DFDBM) alone and Group B was test side where defects were treated by the placement of DFDBM loaded with

doxycycline. Patients were subjected to occlusal equilibration if required and routine laboratory investigations were performed.

Parameters

Clinical parameters assessed were probing depth and clinical attachment level (CAL) (to ascertain clinical attachment loss). The clinical measurements were obtained by one examiner using the Goldman-Fox/ Williams color-coded probe. Pre-fabricated acrylic occlusal stents were fabricated so that measurements made post-surgically could be at the same position and angulation as that made pre-surgically. The stents were stored on the cast to minimize distortion [Figure 1].

Radiographic assessment was made to measure the amount of linear bone fill using intraoral periapical films (size 32 mm × 41 mm) Kodak dental intraoral E-Speed Film, Carestrean, Inc. 150 Verona Street, Rochester, NY 14608 USA. Radiographically, the infrabony defect depth was ascertained using a standardized radiographic technique and by measuring from a fixed reference point (the adjacent cuspal tip) to the most apical point of the base of the defect. A grid was used as an adjunct to the X-ray film to ensure accuracy in the measurements.

Materials

The DFDBM used in this study was prepared from the bones obtained from the Department of Orthopedics, Government Medical College, Amritsar and consisted of parts of long bones, which were stored in 10% formaldehyde solution till the time of preparation, in the manner described by Urist.[21]

Steps in the preparation of demineralized freeze-dried bone matrix Cortical bone was harvested and cut into small pieces.

1. The bone pieces were immersed in 100% ethyl alcohol for 1 h.
2. The bone was frozen at −80°C for 1-2 weeks.
3. Later on, the bone pieces were grounded and severed to minute particle size.
4. The graft material was again immersed in 100% ethyl alcohol and washed repeatedly with distilled water to remove chemicals used in the processing.
5. Decalcification of graft material was done with 0.6 N HCl for 24 h.
6. The graft material was then washed in a sodium phosphate buffer for 72 h to remove residual acid.
7. Graft material was packed in sterile ampoules in an ultraviolet laminar unit.
8. After packing, the graft material was refreeze dried at −80°C for 1 week.

The DFDBM used in this study was prepared from the cortical bone as they contain more BMPs than the cancellous bone.[22]

Doxycycline hyclate (100 mg) (an antibiotic) in powdered form was added to the DFDBA at a 4:1 DFDBA to doxycycline powder ratio for the preparation of the drug loaded graft. Both the DFDBM powder and doxycycline were measured by digital weighing machine (Sigma Balances, Model No. SG200/B®) [Figure 2]. Pre-measured amount of these two powders was then taken and was mixed in normal saline at a 4:1 DFDBA to doxycycline powder ratio.[23] The required amount of this drug loaded graft was then placed in the designated infrabony defects.

Surgical procedure

This study was conducted in accordance with the principles laid down by the Declaration of Helsinki.[24]

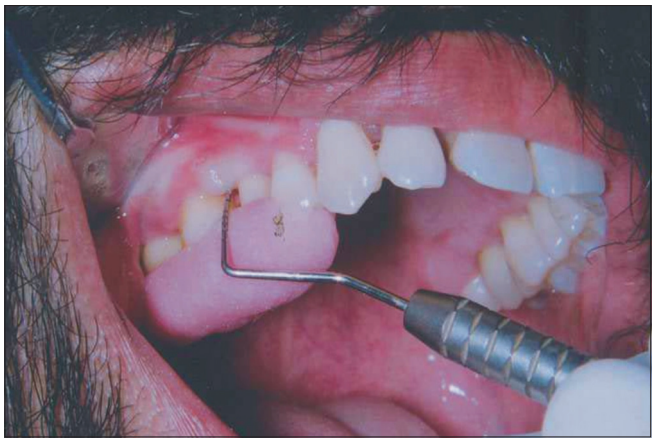

Figure 1: Probing pocket depth measurement by William's calibrated periodontal probe using customized acrylic stent

Figure 2: Weighing machine

After an explanation of the proposed study criterion, including treatments and the potential risks and benefits, the participants were asked to sign a written consent form prior to periodontal surgery. The study was conducted after receiving clearance from the ethical clearance committee of the institute. Each patient was pre-medicated using 10 mg diazepam and 0.3 mg glycopyrrolate intramuscularly 45 min before the surgical procedure.

The area to undergo surgery was anesthetized with lignocaine hydrochloride 2% with adrenaline 1:200,000. Full-thickness gingival flaps were prepared. Initial incision was made in such a manner as to prepare as much attached gingiva as possible. Full-thickness flaps [Figure 3] were elevated to expose the osseous defect. Granulation tissue was removed and the area was flushed copiously with normal saline so that the interproximal defects (infrabony defects) are clear [Figure 4] and prepared prior to the placement of graft either alone [Figure 5] or in combination with doxycycline [Figures 6 and 7]. The defects were slightly overfilled and the soft tissue pulled snugly over the wound and sutured to approximate primary closure[22] with interrupted interdental sutures using 3-0 black braided silk [Figure 8].

Antibiotic therapy (amoxicillin 250 mg + cloxacillin 250 mg + 60 million lactobacillus spores, 3 times a day) for 5 days along with an anti-inflammatory agent for 3 days was prescribed post-operatively. The patients were asked to follow diet instructions strictly and perform adequate plaque control by rinsing with 15 ml of 0.12% chlorhexidine gluconate for 30 s twice daily for 2-week post-operatively. Sutures were removed 1 week after surgery.

OBSERVATION AND RESULTS

All subjects tolerated the surgical procedure well, experienced no post-operative complications,

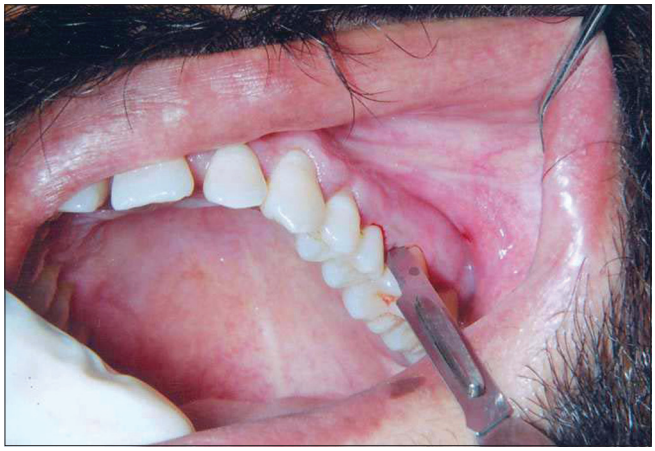

Figure 3: The crevicular incision being given for exposing the site of infrabony defect

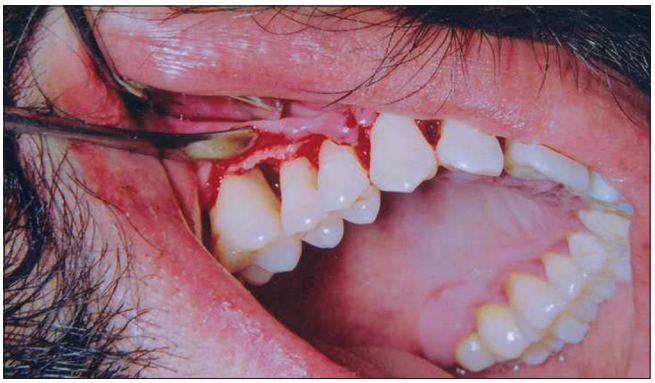

Figure 4: The designated site with exposed infrabony defect (Group A)

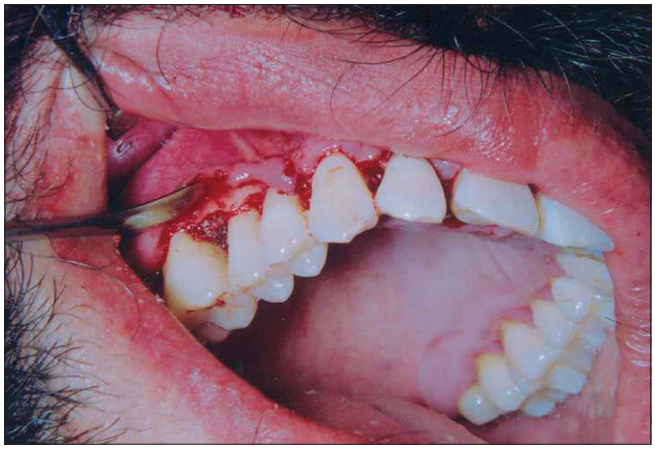

Figure 5: The infrabony defect loaded with demineralized freeze-dried bone matrix graft (Group A)

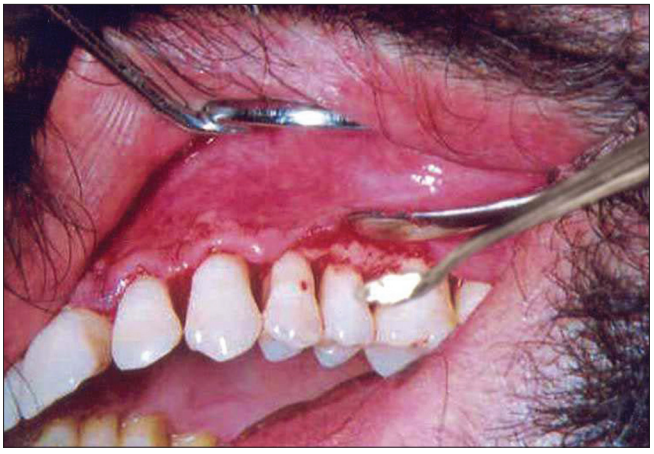

Figure 6: The doxycycline loaded demineralized freeze-dried bone matrix being placed in infrabony defect

complied with the study protocol, and completed the 24-week follow-up. The post-operative assessments for the parameters were done at 12 weeks and at 24 weeks. The observations recorded were subjected to statistical analysis. No difference in results was found between male and female patients. Similarly, with regard to age, periodontal regenerative capacity was found to be similar in all patients (30-50 years). The mean values of probing depth, CAL, and infrabony defect depth [Table 1] at the three points in time were evaluated. The efficacy of two treatment modalities at 12-weeks and 24-week post-operatively was evaluated using the paired Student's *t*-test because the observations at the two points in time were expected to be closely related to each other. The two Groups A (DFDBM) and B (DFDBM with doxycycline) were then comparatively evaluated over the 3 time intervals using independent Student's *t*-test.

On analyzing the clinical criterion of reduction in probing depth of the two groups, DFDBM loaded with doxycycline exhibited a statistically highly significant reduction in probing pocket depth (PPD) for all the three points of time. DFDBM used alone exhibited a statistically highly significant reduction in PPD at 12-week and 24-week post-operatively, but a non-significant reduction in the PPDs between 12-week and 24-week post-operatively [Table 2].

Regarding the gain in CAL, it was seen that both DFDBM loaded with doxycycline and DFDBM used alone exhibited a highly significant gain in clinical attachment at all three points of time viz. 12 weeks, 12-24 weeks, and 24 weeks post-operatively [Table 2].

The linear bone fill ascertained radiographically for both the groups was statistically highly significant for all the three points of time [Table 2].

On comparative evaluation between the two groups, results indicated that DFDBM loaded with doxycycline exhibited greater reduction in probing depth (P = 0.81) [Table 3] and mean gain in the CAL (P = 0.30) [Table 4].

The mean values of the gain in linear bone fill (over both the groups) during the 3 time intervals were statistically significant at 1% level of significance [Figure 9]. DFDBM loaded with doxycycline, however, exhibited statistically significant linear bone fill as compared to DFDBM used alone (P = 0.034) [Table 5].

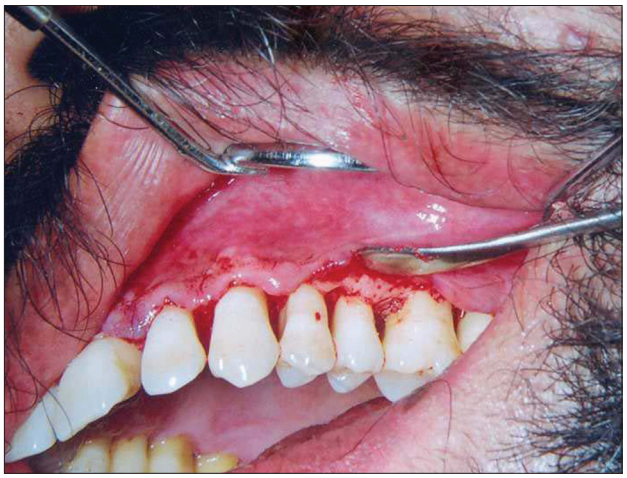

Figure 7: The demineralized freeze-dried bone matrix loaded doxycycline placed in infrabony defect (Group B)

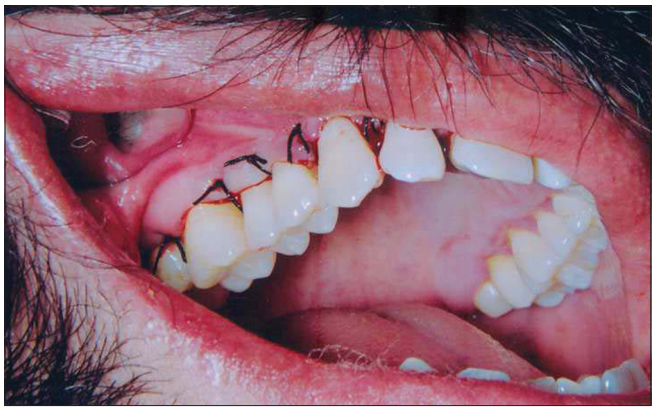

Figure 8: The operated site after suturing

Table 1: Measurement of PPD, CAL and BDD using DFDBM alone (Group A) and DFDBM loaded with doxycycline (Group B) (in Mm)

	Group A (Mean±SEm)			Group B (Mean±SEm)		
	Pre-operative	12-weeks post-operative	24-weeks post-operative	Pre-operative	12-weeks post-operative	24-weeks post-operative
PPD	7.00±0.47	3.93±0.42	3.80±0.53	6.60±0.45	3.93±0.37	3.07±0.23
CAL	5.67±0.72	3.37±0.61	2.67±0.61	5.20±0.60	2.73±0.41	2.00±0.34
BDD	7.53±0.67	5.73±0.69	4.53±0.57	7.87±0.48	5.27±0.56	3.40±0.41

PPD: Probing pocket depth; CAL: Clinical attachment level; BDD: Infrabony defect depth

Table 2: Change in PPD, CAL and BDD using DFDBM (Group A) and DFDBM loaded with doxycycline (Group B) (in Mm)

	Group A (Mean±SEm)			Group B (Mean±SEm)		
	Pre-operative to 12 weeks post-operative	Pre-operative to 24 weeks post-operative	12-24 weeks post-operative	Pre-operative to 12 weeks post-operative	Pre-operative to 24 weeks post-operative	12-24 weeks post-operative
PPD	3.07±0.52	3.20±0.63	0.13±0.39	2.67±0.41	3.53±0.39	0.87±0.34
CAL	2.40±0.66	3.00±0.67	0.60±0.19	2.47±0.51	3.20±0.55	0.74±0.20
LBF	1.80±0.20	3.00±0.29	1.20±0.24	2.60±0.23	4.47±0.29	1.87±0.26

PPD: Probing pocket depth; CAL: Clinical attachment level; LBF: Linear bone fill; SEm: Standard error of mean; BDD: Infrabony defect depth; DFDBM: Demineralized freeze-dried bone matrix

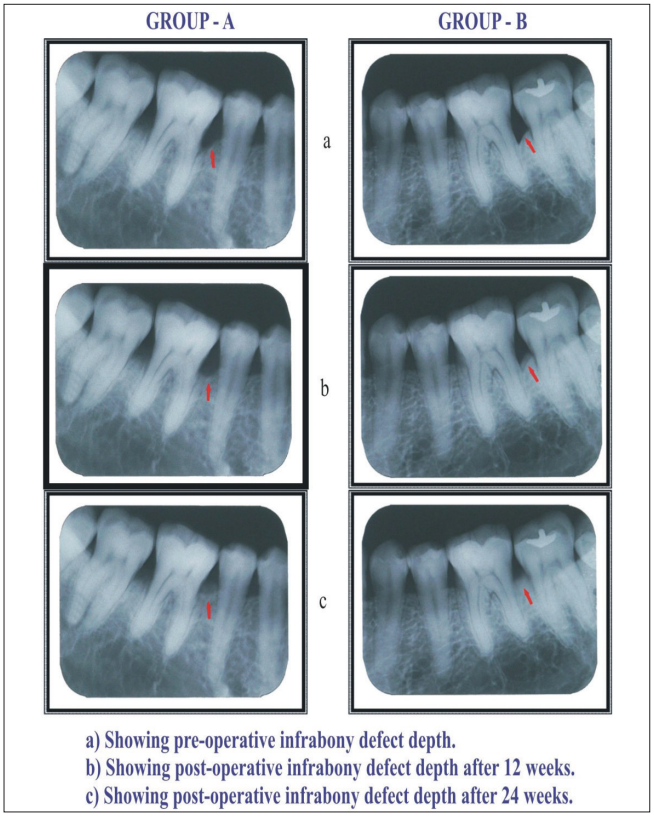

Figure 9: Comparison of radiographic bone fill of Group A versus Group B

Table 3: Comparative reduction in probing pocket depth between Group A and Group B

Group mean	Pre-operative to 12 weeks post-operative	Pre-operative to 24 weeks post-operative	12-24 weeks post-operative
Group A_{mean}	3.07±0.52	3.20±0.63	0.13±0.39
Group B_{mean}	2.67±0.41	3.53±0.39	0.87±0.34
P value	0.275^{NS}	0.329^{NS}	0.081^{NS}

P-level of significance; *Values are statistically significant at 5% probability level, i.e., *P*<0.05; **Values are statistically highly significant at 1% probability level, i.e., *P*<0.01; ^{NS}Values are statistically non-significant

Table 4: Comparative gain in clinical attachment level between Group A and Group B

Group mean	Pre-operative to 12 weeks post-operative	Pre-operative to 24 weeks post-operative	Post-operative from 12-24 weeks
Group A_{mean}	2.40±0.66	3.00±0.67	0.60±0.19
Group B_{mean}	2.47±0.51	3.20±0.55	0.74±0.20
P value	0.466^{NS}	0.409^{NS}	0.307^{NS}

P-level of significance; *Values are statistically significant at 5% probability level, i.e., *P*<0.05; **Values are statistically highly significant at 1% probability level, i.e., *P*<0.01; ^{NS}Values are statistically non-significant

Table 5: Comparative linear bone-fill (ascertained radiographically) between Group A and Group B

Group mean	Pre-operative to 12 weeks post-operative	Pre-operative to 24 weeks post-operative	Post-operative from 12-24 weeks
Group A_{mean}	1.80±0.20	3.00±0.29	1.20±0.24
Group B_{mean}	2.60±0.23	4.47±0.29	1.87±0.26
P value	0.006**	0.0006**	0.034*

P-level of significance; *Values are statistically significant at 5% probability level, i.e., *P*<0.05; **Values are statistically highly significant at 1% probability level, i.e., *P*<0.01; ^{NS}Values are statistically non-significant

DISCUSSION

Periodontal regeneration is now a major challenge in periodontal research and practice. It involves the use of regenerative therapy to restore the defects produced by the disease process. Bone grafting is known to be one such most important regenerative procedures.

Among all the grafts, the allograft (DFDBM) is the most widely used material because of the safety, ease of use, and purported osteoinductive and osteoconductive properties.[3,4] Demineralization of bone exposes the bone-inducing agent, i.e., BMPs which are multifunctional growth factors belonging to TGF-β, superfamily. Their hallmark is their ability to induce bone, cartilage, ligament, and tendon formation at both heterotopic and orthotopic sites.[25] These induce new bone formation during the healing process by irreversibly inducing differentiation of perivascular mesenchymal type cells into osteoprogenitor cells.[26,27]

Successful prevention and treatment of periodontitis are contingent upon effective control of the

periodontopathic bacteria residing in the various ecological niches of the oral cavity. Complementing mechanical therapy with local or systemic anti-microbial treatment modalities may enhance the therapeutic effect, thereby proving advantageous.[5,28,29]

The use of locally delivered anti-microbials, pioneered by Goodson, *et al*.[6,7] is a relatively new addition in the management of periodontal disease. All local drug delivery systems aim at achieving higher bactericidal concentrations of the antibiotic at the site of infection concomitantly lowering the total dose and thereby minimizing the risk of systemic side effects.

Tetracyclines constitute a family of antibiotics that have been found to be highly effective against putative periodontopathogens. Among them, doxycycline has exhibited encouraging results and has been used in various concentrations in conjunction with bone grafts and biodegradable membranes, hence proved to be beneficial.[16,17]

Doxycycline enhances regeneration by virtue of its various properties which are as follows:

1. Doxycycline enhances periodontal regeneration via its anti-microbial as well as host modulatory intrinsic activities by inhibiting the production as well as scavenging of reactive oxygen radicals generated by polymorphonuclear neutrophils.[30,31]
2. Doxycycline has the ability to condition dentin and create a surface morphology of exposed dentinal tubules and collagen fibrils that enable a fibrin linkage favoring the formation of new attachment.[8,32,33]
3. It exhibits a high degree of substantivity, by binding to the periodontally diseased root cementum and dentine, which serve as a reservoir and allow the antibiotic to be slowly released in a biologically active form at anti-bacterial levels into the adjacent environment for several days following its topical application.[34,11] The first such FDA-approved system for local application was, *Actisite*, which was developed by Dr. Max Goodson in 1983. A local concentration of 30 μg/mL has been found to eliminate most of the pathogenic bacteria associated with periodontal diseases, hence providing the local beneficial effects (especially anti-inflammatory) without the undue systemic side effects.[32]

Doxycycline and other tetracycline analogues have also been found to inhibit collagenases and other host-derived matrix metalloproteinases which are released as the periodontal disease progresses.[13,36] It also potentiates osseous regeneration defects when locally administered due to its anti-collagenolytic effect, which enhances the bone forming ability via osteoblast cell chemotaxis and reduced bone resorption.[16]

It also bears the capacity to protect alpha-1 proteinase inhibitor, from proteolytic inactivation in the gingival crevicular fluid (GCF).[14,37]

Routinely, various allografts and xenografts are being used for periodontal regenerative procedures. The advantage of this study is that augmentation of the regenerative potential of these grafts can be achieved by incorporating them with doxycycline. The results of this study are consistent with those of Al-Ali, *et al*.,[38] Pepelassi, *et al*.,[39] Drury, *et al*.,[17] and Chang *et al*.[16] who have demonstrated the beneficial effects of adjunctive use of Doxycycline in achieving bone regeneration. Wang and Cooke[40] and Gapski, *et al*.[41] demonstrated a new allograft of cancellous bone viz., human mineralized bone (Puros®) which is a solvent preserved bone allograft of cancellous bone and possesses the trabecular pattern and mineral structure better than freeze-dried bone. Another futuristic material can be the use of Grafton demineralized bone matrix which is cortical bone milled into elongated fibers of 0.5 mm in diameter and combined with glycerol carrier to stabilize the proteins and improve the graft handling.[42]

CONCLUSION

In conclusion, within the constraints of this study (short duration, small study sample size), doxycycline has been shown to potentiate regenerative capacity of allografts and hence can be successfully used in the treatment of periodontal infrabony defects. The results thus obtained, present a valid premise for further long-term studies to evaluate the regenerative potential of DFDBM loaded with doxycycline. From a futuristic viewpoint, attempts can be made to harness the potential additive effects of a sub-microbial dose doxycycline or a chemically modified tetracycline in conjunction with periodontal surgical procedures such as host modulatory therapy, to block the pathways of periodontal tissue destruction as well as to enhance wound healing and regeneration.

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