# Use of Lincomycin-Impregnated Demineralized Freeze-Dried Bone Allograft in the Periodontal Defect After Third Molar Surgery

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Purpose: The aim of the present study was to evaluate the periodontal regenerative capacity of demineralized freeze-dried bone allograft (DFDBA) alone or used with local lincomycin.

Materials and Methods: In the present single-blind, randomized, controlled clinical trial, 20 subjects 26 years old or older, requiring extraction of bilateral third molars (M3s), were included. Each subject was randomly assigned to receive either DFDBA or DFDBA plus lincomycin therapy. Within the subjects, 1 M3 site was randomly selected to be the experimental site and the contralateral served as the control and was permitted to heal without intervention. The primary variables were changes in the probing depth (PD), clinical alveolar bone levels (ABLs), and radiographic alveolar bone density (ABD) on the distal aspect of second molar between baseline (immediately postoperatively) and 26 weeks postoperatively ($T_{26}$). Appropriate sample sizes and descriptive, bivariate, and multivariate statistics were computed.

Results: For both treatment and control sites, between $T_0$ and $T_{26}$, statistically significant improvements were seen in the ABLs and ABD ($P < .05$). Within-subject comparisons showed no significant differences in PD, ABL, or ABD between the treatment and control M3 sites at $T_0$ or $T_{26}$ ($P > .05$). Also, no significant differences were found in the PD, ABL, or ABD between the 2 treatment M3 sites at $T_{26}$ ($P > .05$).

Conclusions: The results of the present study have revealed that the PD, ABL, and ABD improved after M3 removal in subjects 26 years old or older, irrespective of the treatment or control group. Reconstructive procedures (eg, DFDBA with or without lincomycin therapy) did not offer predictable benefits compared with a no-treatment protocol in patients younger than 30 years old.



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Surgical removal of impacted third molars (M3s), whether for prophylactic or symptomatic reasons, is a common procedure performed by oral and maxillofacial surgeons. Periodontal pocket formation on the distal aspect of the adjacent second molar (M2) is one of the postoperative outcomes of this procedure.[1,2]

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The risk factors associated with this iatrogenic sequela after M3 extraction include age (>25 years), direction of eruption (mesioangular or horizontal), preoperative bony defects, and resorption of the M2 root surface.[3] Controversy exists regarding the need for a reconstructive procedure to eliminate persistent, or prevent the development of new, periodontal defects on the distal aspect of the M2 after M3 removal.[4]

Many studies have evaluated the therapeutic effect of various reconstructive techniques, including bone substitutes such as demineralized bone matrix (DBM) or synthetic bone matrix, platelet-rich plasma, guided tissue regeneration (GTR), and soft tissue procedures, after M3 removal.[4-7] In contrast to a number of randomized clinical trials that have failed to show a clinically significant benefit from reconstructive procedures, some investigators have reported a significant improvement in the M2 periodontal parameters after M3 removal.[4,8]

A diminished bone growth and tissue repair response can result if infection of the bone graft materials occurs.[9] The treatment of infected bone grafts with systemic antibiotics alone has had several drawbacks, including poor penetration into the ischemic tissue at the wound site and the potential for systemic toxicity. Various local antibiotic delivery methods have been developed to circumvent the accessibility and toxicity issues associated with systemic antibiotic treatment.[10]

Studies have shown that lincomycin is effective against gram-positive cocci, including staphylococci, *Streptococcus viridians,* and b-hemolytic streptococci. Lincomycin has no effect on the cytotoxicity, proliferation, or metabolic activity of primary human osteoblasts, even at greater concentrations, and displays good activity against anaerobes.[11] The findings from Hnatko[12] in the treatment of osteomyelitis and soft tissue infections using lincomycin have important implications in the field of dentistry. Its effectiveness against bone infections has been attributed to its ability to penetrate bone and severely infected tissue. It has been shown that lincomycin applied to the alveolus on a tricalcium phosphate carrier can be used to accelerate wound healing and reduce complications such as alveolar periostitis, pain, trismus, and atrophy of the alveolar process after surgical extraction of M3s.[13] Lincomycin's mode of action is believed to result from inhibition of protein synthesis rather than interference with cell wall formation. The use of lincomycin has been associated with severe colitis, which can be fatal. The following adverse reactions have also been reported with the use of lincomycin: abdominal distress and persistent diarrhea, neutropenia, leukopenia, agranulocytosis, thrombocytopenic purpura, and hypersensitivity reactions such as angioneurotic edema. Because of its adverse effects and toxicity, it is rarely used today and has been reserved for patients allergic to penicillin or if bacteria have developed resistance.[14]

The use of DBM to repair osseous defects after M3 extraction has not been thoroughly studied. Osseous repair with demineralized bone is unique. An allogenic DBM does not contain living cells or provide scaffolds for osteoconduction; instead, it possesses osteoinductive properties and can serve as an ideal drug delivery device for prophylactic treatment in a variety of different anatomic locations. The use of a DBM combined with local antibiotics would allow the release of the entire quantity of antibiotic as the material is being remodeled.[15-17]

We have described a lincomycin-impregnated human DBM that could be used in patients at high risk of M2 periodontal defects after M3 removal for alveolar bone defect reconstruction. The specific aim of the present study was to measure the periodontal parameters of M2 bone support (ie, probing depth [PD], alveolar bone level [ABL], and alveolar bone density [ABD]) before M3 extraction and compare them with the periodontal parameters measured 26 weeks after extraction to determine whether reconstruction procedures would be needed in subjects 26 years old or older.

## Materials and Methods

The original cohort included 20 subjects recruited from November 2011 to July 2012, in a split-mouth, randomized, single-blind, controlled clinical trial, at the maxillofacial surgery department (Shiraz University of Medical Science School of Dentistry, Shiraz, Iran). The inclusion criteria consisted of bilateral mandibular impacted M3s and risk factors for M2 periodontal defects after M3 removal: age 26 years or older and a fully impacted mesioangular or horizontal M3 position.[8]

The present study followed the Declaration of Helsinki for the medical protocol and ethics, and the regional ethical review board of Shiraz University of Medical Science School of Dentistry approved the study. All subjects provided written informed consent before the surgical procedure.

The subjects were randomly assigned to 1 of the 2 active treatment groups (DFDBA with lincomycin or DFDBA alone), and the 2 M3 extraction sites were randomly assigned to either the treatment or control group.

In the DFDBA group, the experimental M3 extraction site was grafted with demineralized DFDBA (particle size 500 to 1,000 mm; CenoBone, Tissue Regeneration Corporation, Kish, Iran) alone. In the DFDBA with lincomycin group, the experimental wound site was grafted with DFDBA plus 2 mL of sterile solution containing 600 mg of lincomycin (Upjohn SA, Puurs, Belgium).

The control extraction sites were closed primarily and allowed to heal spontaneously without any graft material. In the present study, when comparing the active treatments groups (DFDBA with or without lincomycin therapy) versus the control group, the subjects served as their own controls.

The same surgeon performed all the procedures with the patient under local anesthesia (lidocaine in a 4% solution with epinephrine at 1:100,000) through an envelope mucoperiosteal flap. The M3s were extracted using ostectomy and teeth sectioning with burs, as indicated. Postoperatively, the subjects were prescribed oral antibiotics for 7 days (penicillin 500 mg 4 times daily or clindamycin 150 mg 4 times daily for penicillin-allergic patients), oral nonsteroidal anti-inflammatory drugs for 4 days (ibuprofen 400 mg 4 times daily), and a chlorhexidine 0.12% rinse (30 mL, swish and spit, twice daily). Regardless of the group assignment, the postoperative care was the same.

Data on the following variables were collected by another surgeon who was unaware of the study groups: age, gender, PD, clinical ABL, and radiographic bone density. The PD was measured preoperatively, in millimeters, on the distobuccal and distolingual aspects of the adjacent M2 using a Michigan OW round dental periodontal probe (Hu-Friedy, Chicago, IL).

The clinical ABL was determined immediately after M3 removal by sounding the most apical extent of the created bony defect adjacent to the distal root of M2 using a calibrated Williams periodontal probe. A surgical stent was used as a fixed and repeatable occlusal reference point. These measurements were taken again after 26 weeks.

Panoramic radiographs were taken using the same digital machine immediately and 26 weeks after surgery. All the digital panoramic radiographs were saved in a Tagged Image File Format, and then the bone density in the region of interest was measured using ImageJ software.[18]

Data analyses were performed using the Statistical Package for Social Sciences software, version 15 (SPSS, Chicago, IL). Statistical significance was set at $P < .05$.

## Results

The original cohort included 20 subjects, and a total of 40 M3 extraction sites were evaluated. The mean age ± standard deviation was 26.5 ± 1.9 years (range 25-30). Of the 20 subjects, 6 (30%) were men. The tooth-specific variables for the study groups (treatment and control) are summarized in Table 1. No statistically significant differences were found in the distribution of the study variables among the groups ($P > .05$). Clinical evaluation of the postoperative healing revealed an excellent soft tissue response to both treatment methods without any complications or reaction to the lincomycin. With respect to the PD at the distal aspect of M2, a considerable decrease was observed after 26 weeks at both the treatment and the control sites, but the difference within and between the groups (DFDBA and DFDBA plus lincomycin) was not statistically significant ($P > .05$; Table 2).

The changes in the ABL over time, stratified by treatment group, are summarized in Table 3. Immediately after surgery, a significant alveolar bone defect was present on the distal aspect of M2, shown by the alveolar bone height, which ranged from a mean of 12.05 mm (DFDBA-treated M3 sites) to 11.10 mm (DFDBA plus lincomycin-treated M3 sites). No statistically significant difference was found between the study groups in terms of the immediate postoperative ABL ($P > .05$).

In the DFDBA- and DFDBA plus lincomycin-treated and control (untreated) M3 sites, clinically and statistically significant improvements occurred in the ABLs during the 26-week study period. Within the treatment groups, the DFDBA-treated M3 sites improved their ABL by 4.3 mm ($P = .002$). The DFDBA plus lincomycin-treated M3 sites had an average ABL improvement of 3.30 mm ($P = .003$). The control M3 sites had an average ABL improvement of 3.75 mm for the DFDBA group and 4.65 mm for the DFDBA plus lincomycin group.

At the end of the follow-up period, the ABLs for the DFDBA-treated (7.75 ± 2.5 mm) and its control group (7.35 ± 1.8 mm) and the DFDBA plus lincomycin-treated (7.80 ± 1.3 mm) and its control group (7.50 ± 2.1 mm) were not significantly different ($P > .05$). Also, no statistically significant difference was found in the ABLs between the DFDBA- and DFDBA plus lincomycin-treated M3 sites ($P > .05$).

The changes in the ABD over time, stratified by treatment status, are summarized in Table 4. No statistically significant differences were found between the study

**Table 1. SUMMARY OF M3 VARIABLES STRATIFIED BY STUDY GROUPS**

| Variable | Group: Treatment Site | Group: Control Site | *P* Value |
|---|---|---|---|
| M3 Sample size (n) | 20 | 20 | NA |
| Right | 11 | 9 | .355* |
| Left | 9 | 11 | |
| M2 probing depth at baseline | | | |
| Distobuccal | 3.40 ± 1.74 | 3.35 ± 1.27 | .920[y] |
| Distolingual | 3.16 ± 1.11 | 3.21 ± 1.12 | .871[y] |

Data presented as mean ± standard deviation.
* Chi-square test.
*y* Independent samples *t* test.

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Table 2. CHANGES IN PROBING DEPTH DURING THE STUDY PERIOD STRATIFIED BY TREATMENT STATUS

| Study Variable | Preoperative PD (mm) | 26-wk Postoperative PD (mm) | *t* Test* | *P* Value* |
|---|---|---|---|---|
| DFDBA | | | | |
| Treatment site | 3.10 ±2.1 | 2.20 ±1.1 | 1.711 | .121 |
| Control site | 3.90 ± 1.4 | 2.30 ± 0.6 | 3.748 | .005 |
| *t* Test[y] | −0.981 | −0.239 | | |
| *P* value[y] | .339 | .813 | | |
| DFDBA plus lincomycin | | | | |
| Treatment site | 3.55 ±1.3 | 2.50 ±0.5 | 2.067 | .069 |
| Control site | 2.85 ± 0.9 | 2.70 ± 0.9 | 0.355 | .730 |
| *t* Test[y] | 1.349 | −0.583 | | |
| *P* value[y] | .194 | .567 | | |
| DFDBA | 3.10 ± 2.1 | 2.20 ± 1.1 | 1.711 | .121 |
| DFDBA plus lincomycin | 3.55 ± 1.3 | 2.50 ± 0.5 | 2.067 | .069 |
| *t* Test[y] | 0.565 | 0.535 | | |
| *P* value[y] | .579 | .600 | | |

Data presented as mean ± standard deviation.
Abbreviations: DFDBA, demineralized freeze-dried bone allograft; PD, probing depth.
* Paired *t* test.
y Independent samples *t* test.

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groups in terms of the immediate postoperative ABD ($P > .05$). In the DFDBA-treated and its control group, a statistically significant increase was found in the ABD during the 26-week study period after M3 removal ($P < .05$). The DFDBA plus lincomycin-treated and its control group had an average ABD improvement of 398.16 and 525.35, respectively, with no statistically significant differences ($P > .05$). At the 26-week postoperative interval, the difference in the ABD between the treatment (DFDBA and DFDBA plus lincomycin) and control M3 sites was not statistically significant ($P > .05$). In addition, no statistically significant difference was found in the ABD between the DFDBA- and DFDBA plus lincomycin-treated M3 sites ($P > .05$).

Table 3. CHANGES IN CLINICAL ALVEOLAR BONE LEVEL DURING THE STUDY PERIOD STRATIFIED BY TREATMENT STATUS

| Study Variable | Immediate Postoperative ABL (mm) | 26-wk Postoperative ABL (mm) | *t* Test* | *P* Value* |
|---|---|---|---|---|
| DFDBA | | | | |
| Experimental site | 12.05 ±3.5 | 7.75 ±2.5 | 4.251 | .002 |
| Control site | 11.10 ±3.8 | 7.35 ±1.8 | 2.677 | .025 |
| *t* Test[y] | 0.576 | 0.405 | | |
| *P* value[y] | .572 | .690 | | |
| DFDBA plus lincomycin | | | | |
| Experimental site | 11.10 ±2.5 | 7.80 ±1.3 | 3.957 | .003 |
| Control site | 12.15 ±2.6 | 7.50 ±2.1 | 5.601 | .001 |
| *t* Test[y] | −0.906 | .372 | | |
| *P* value[y] | .377 | .714 | | |
| DFDBA | 12.05 ±3.5 | 7.75 ±2.5 | 4.251 | .002 |
| DFDBA plus lincomycin | 11.10 ±2.5 | 7.80 ±1.3 | 3.957 | .003 |
| *t* Test[y] | 0.500 | 0.524 | | |
| *P* value[y] | .889 | .603 | | |

Data presented as mean ± standard deviation.
Abbreviations: ABL, alveolar bone length; DFDBA, demineralized freeze-dried bone allograft.
* Paired *t* test.
y Independent samples *t* test.

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Table 4. CHANGES IN ALVEOLAR BONE DENSITY DURING THE STUDY PERIOD STRATIFIED BY TREATMENT STATUS

| Study Variable | Immediate Postoperative ABD | 26-wk Postoperative ABD | *t* Test* | *P* Value* |
|---|---|---|---|---|
| DFDBA | 485.57 ± 470 | 1178.53 ± 263 | −3.666 | .005 |
| Control site | 628.87 ± 494 | 1222.90 ± 215 | −3.926 | .003 |
| *t* Test[y] | −0.664 | −0.412 | | |
| *P* value[y] | .515 | .685 | | |
| DFDBA plus lincomycin | 773.98 ± 600 | 1172 ± 248 | −1.958 | .082 |
| Control site | 877.36 ± 559 | 1402.71 ± 340 | −2.127 | .062 |
| *t* Test[y] | −0.398 | −1.729 | | |
| *P* value[y] | .695 | .101 | | |
| DFDBA | 485.57 ± 470 | 1178.53 ± 263 | −3.666 | .005 |
| DFDBA plus lincomycin | 773.98 ± 600 | 1172.14 ± 248 | −1.958 | .082 |
| *t* Test[y] | 1.195 | −0.056 | | |
| *P* value[y] | .248 | .956 | | |

Data presented as mean ± standard deviation.
Abbreviations: ABD, alveolar bone density; DFDBA, demineralized freeze-dried bone allograft.
* Paired *t* test.
y Independent samples *t* test.

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## Discussion

Extraction of lower M3s can result in a periodontal defect at the distal aspect of the lower M2, characterized by an increased PD (distance from the free gingival margin to the bottom of the gingival sulcus), an increased attachment level (distance from the free gingival margin to the cementoenamel junction), and a decreased alveolar bone height.[19]

In such cases, a predictable method to minimize the risk of M2 periodontal defects is desired. This issue has been studied using a variety of reconstructive techniques, including GTR therapy, bone substitutes, and flap design, applied in the setting of a randomized clinical trial.[4-7,19-21] Adding local antibiotics, for either prophylaxis or treatment to bone substitutes, has been a common practice in such clinical trials.[22]

Therefore, with a split-mouth technique, the periodontal regenerative capacity of a demineralized bone allograft alone or combined with local lincomycin versus untreated control M3 sites was evaluated in the present study after the extraction of deeply impacted lower M3s. During the follow-up period, a significant improvement in the periodontal parameters of all the participants (treatment and control groups) was observed. However, the differences in the periodontal parameters between the treatment (DFDBA and DFDBA plus lincomycin) and control groups were not statistically significant. In addition, no statistically significant differences were found in the periodontal parameters between the 2 treatment groups.

In previous studies, patient age older than 26 years was considered a risk factor for M2 periodontal defects after M3 removal.[8,23] In the present study, the mean age of the subjects was 26.5 years (range 25-30), and no significant differences were observed between the treatment and control groups in the periodontal parameters.

Dodson[4] compared placing demineralized bone powder or a resorbable membrane in 1 M3 site with no regeneration in the contralateral M3 site. The study included 24 patients undergoing bilateral M3 extraction. No statistically significant differences were found between the 2 techniques.[4]

However, when using the same study design in patients with a previous periodontal defect distal to M2, Dodson[23] showed a statistically significant reduction in the attachment level in the group with a bone graft compared with the control group. However, no statistically significant differences were found between the control group and the group with the resorbable membrane.[23]

Similarly, Karapataki et al[20] found no statistically significant differences in the postoperative PDs and attachment levels after placing resorbable or nonresorbable membranes in 19 patients. However, Pecora et al[8] demonstrated a clinically and statistically significant benefit with nonresorbable GTR therapy compared with no treatment in the setting of subjects with multiple risk factors for M2 periodontal defects after M3 removal. The risk factors included preexisting periodontal disease (attachment level > 3 mm), older subjects (age > 26 years), and close proximity of the M3 to the M2 (horizontal or mesioangular impaction).[8]

Sammartino et al,[24] in a study of 18 patients, found a significant reduction in the PD and attachment level in those patients treated with platelet-rich plasma gel

compared with the control group. Dodson[23] proposed bone regeneration techniques in patients older than 26 years with a horizontal or mesioangular impacted M3 and a distal periodontal defect, defined as more than 3 mm of an attachment level distal to the M2, before M3 extraction. Other investigators have suggested the use of these techniques when the PD is more than 7 mm and the attachment level is greater than 6 mm.[20,24]

Bone grafts have been considered a useful adjunctive therapy to gain blood clot stability in the periodontal defect.[23] Significantly less loss of the alveolar crest height, regeneration of new attachment apparatus, and new cementum occurred more frequently in the grafted than in the nongrafted defects.[25]

Autografts, allografts, xenografts, and alloplasts, with or without the use of a barrier membrane, have remained among the most widely used therapeutic strategies for the correction of periodontal osseous defects.[26]

Richardson et al[27] compared the bovine-derived xenograft Bio-Oss (Geistlich Pharma AG, Wolhusen, Switzerland) and DFDBA in a randomized clinical trial examining 30 human intrabony defects. Each material was used alone, without membranes, root conditioners, or antibiotics. The results demonstrated that compared with baseline, a significant improvement in the defect parameters was seen in both groups. However, no significant differences were found between the materials when compared with one another.[27]

Scabbia and Trombelli[28] evaluated the clinical outcome of deep intraosseous defects after reconstructive surgery with the use of a synthetic hydroxyapatite/equine type I collagen/chondroitin sulfate biomaterial (Biostite; Gaba Vebas srl, Rome, Italy) versus a bovine-derived hydroxyapatite xenograft (Bio-Oss, Geistlich Pharma AG). The results of their study indicated that both Biostite and Bio-Oss produce a statistically significant improvement in clinical attachment loss gain, probing pocket depth reduction, and radiographic depth gain when used in the treatment of deep intraosseous defects.[28]

In another study, Becker et al[29] tested different materials in postextraction sockets, including deproteinized bovine bone, demineralized freeze-dried bone, autogenous bone, and human bone morphogenetic proteins in an osteocalcein/osteonectin carrier. The results of their study indicated that bovine bone, DFDBA, and intraoral autologous bone do not promote healing in the extraction sites. The investigators also reported that intraoral autologous bone, xenogeneic bone, and DFDBA appeared to interfere with the normal healing process in the extraction sites.[29]

It has become common practice to empirically add antibiotics for either prophylaxis or treatment issues to autogenous, allograft, or xenograft bone grafting materials. The blood supply to a recent bone graft will be compromised; therefore, systemic use of antibiotics might be insufficient to provide adequate antibacterial concentrations. To obtain adequate antibiotic concentrations at the site of infection, high serum concentrations must be obtained, which carries the risk of systemic toxicity.[30]

Several attempts have been made to supplement allografts with antibiotics.[22,31-33] Such systems yield local antibiotic concentrations that are greater than those obtained with systemic antimicrobial administration and reduces the risk of systemic side effects.[22,31-34] High local antibiotic concentrations have been shown to be beneficial in the treatment of relatively avascular sites and organisms resistant to antibiotic concentrations obtained with systemic administration, including organisms in biofilms.[34-36]

Petri and Wilson,[37] using antibiotic-impregnated allogenic DBM, documented significantly improved bone healing in an M3 extraction surgical model compared with the untreated control sites. The age range in their study, however, was quite broad (18 to 43 years) and included patients at low risk (age # 25 years) of developing postoperative periodontal defects.[37]

Sanders et al,[38] in 1983, found that bone grafts containing antibiotics had greater predictability than those not containing antibiotics. Mabbry et al[39] reported that the combination of gentamycin and tetracycline mixed with freeze-dried bone allografts increased osseous regeneration. Other studies have reported a suppressive effect on bone formation.[40,41] These controversies might have resulted in part from the potential toxic effects of various antibiotics and their localized concentrations. Studies have demonstrated that some antibiotics are toxic to osteoblast-like cells, especially at greater concentrations.[40-42]

Knowing that high concentrations of antibiotics affect bone-forming cells and that anaerobes would most likely be the bacteria of concern, the clinician should consider these factors when choosing an antibiotic to add to the bone graft. Duewelhenke et al[11] investigated the effect of 20 antibiotics from different classes and antibacterial mechanisms in the cell cultures of primary human osteoblasts (PHOs). Of these, only lincomycin had no effect on cytotoxicity, proliferation, or the metabolic activity of PHOs, even at greater concentrations, and it exhibited good activity against anaerobes.[11] Systemic use of lincomycin has been known to cause pseudomembranous colitis and, as a result, has fallen into disuse. Local application at graft placement should circumvent this systemic toxic effect.

In conclusion, the results of the present study have revealed that the periodontal parameters improved after M3 removal in subjects aged 26 years or older, irrespective of the treatment or control group. Also, the reconstructive procedures did not offer predictable benefits compared with the no-treatment protocol in

our patients who were younger than 30 years. Therefore, we propose that age older than 26 years, as a risk factor of M2 periodontal defect formation after M3 surgical removal, should be further investigated. For a better evaluation of lincomycin's unique properties in bone grafting procedures, including no adverse effects on primary human osteoblasts, we would recommend using local lincomycin plus DFDBA in patients older than 30 years. More in vitro and in vivo studies with larger sample sizes are needed to provide complete guidance in this respect.

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