# Homogenous Demineralized Dentin Matrix for Application in Cranioplasty of Rabbits with Alloxan-Induced Diabetes: Histomorphometric Analysis

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***Purpose:*** *The aim of this work was to evaluate the bone-repair process after implantation of homogenous demineralized dentin matrix (HDDM) slices in surgical defects created in the parietal bones of rabbits with alloxan-induced diabetes.* ***Materials and Methods:*** *Forty-eight rabbits were selected and divided into 4 groups of 12 rabbits: the control group, diabetic rabbits (D), diabetic rabbits with a PTFE barrier (D-PTFE), and diabetic rabbits with a PTFE barrier and with slices of homogenous demineralized dentin matrix (D-PTFE+HDDM). The diabetic animals received a single dose of alloxan monohydrate (90 mg/kg) intravenously on the marginal ear vein, and their blood glucose was verified daily. The rabbits were sacrificed after 15, 30, 60, and 90 days. The histologic findings show both better bone structure and significantly greater bone density, as determined by histomorphometric analysis, for the D-PTFE + HDDM group than for the other 3 groups (P < .01). It was also observed that the mean bone density increased gradually from 15 to 90 days (except in the D-PTFE group).* ***Conclusion:*** *It was concluded that the HDDM was biocompatible with the bone repair of diabetic rabbits and that HDDM slices stimulated bone tissue formation. Facilitation of bone repair with HDDM could be useful in diabetic patients.* Int J Oral Maxillofac Implants 2007;22:939–947

**Key words:** *bone repair, diabetes mellitus, homogenous demineralized dentin matrix, monohydrated alloxan*

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For bone tissue regeneration at least 3 components are necessary: osteoprogenitor and osteoconductive cells, growth factors, and absence of local infection. It is assumed that the osteoinductive cascade begins with chemotaxis of bone progenitor cells, mitogenesis, angiogenesis, and bone cell differentiation. The cell recruitment, division rate, and differentiation of these cell lines is under the direct control of growth factors, including bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β).[1–4] Homogenous demineralized dentin matrix (HDDM),[3,5,6] is a biologically active tissue capable of delivering BMP. Recent studies have shown that HDDM slices stimulate new bone tissue formation and that they are being resorbed during the bone remodeling process.[3,5–9]

For the present research, it was decided to induce diabetes in rabbits using alloxan, a well-known inducer of diabetes and hyperglycemia,[10–12] for use as an animal model. According to Fiorellini et al,[13] diabetes mellitus is included in a group of metabolic

diseases characterized by hyperglycemia. As a result of this metabolic disease, the level of glucose in the blood becomes elevated, a condition referred to as "high blood sugar." Glucose metabolites form after many cell biochemical processes and during tissue formation. They are a class of irreversible molecules that have been termed *advanced glycosylation end-products* (AGEs). These irreversible metabolites accumulate over a period of years in the extracellular and/or intracellular macromolecules, such as proteins and lipids. AGEs cause qualitative and quantitative changes in extracellular matrix components such as collagen, proteoglycans, laminin, and vitronectin. These changes in the extracellular matrix cause specific alterations in bone formation and bone remodeling. Bone tissue turnover has been shown to be suppressed; studies have shown decreases in the percentage of osteoclasts and osteoblasts as well as a decrease in osteocalcin synthesis.[13–15]

According to Nevins et al,[14] the streptozotocin-induced diabetic model produced altered blood glucose levels to allow the study of the effects of diabetes on the osseointegration of titanium implants. Diabetes was predictably induced and maintained, and osseointegration was consistently found in diabetic and control specimens. The rate of new bone formation in a 250-µm zone circumscribing the implants was similar for diabetic and control animals. However, bone-implant contact was significantly reduced for the diabetic group compared to the control group. It is important to recognize that the model used in this study was a model of uncontrolled diabetes. The results imply that patients with elevated glucose levels should not be treated with dental implants. Future investigations should aim to better understand the role of insulin control, the molecular mechanisms involved in diabetic bone wound healing, and endosseous implant osseointegration.

Among the complications associated with type-1 diabetes in humans is inadequate bone formation, which can result in osteopenia and delayed fracture healing. To investigate the mechanisms by which diabetes affects bone formation, a bone marrow ablation model has been used. Mice were made diabetic by administration of multiple low doses of streptozotocin. The animals were killed after 0, 2, 4, 6, 10, and 16 days of bone marrow ablation. Histologic analysis demonstrated that the amount of immature mesenchymal tissue was equivalent in both the experimental and control animals on day 4. On day 6 a burst of bone formation occurred in the control group; bone formation in these mice was significantly greater than in the day-6 diabetic mice. This deficit was evident at the molecular level, as shown by diminished expression of osteocalcin and collagen Type I. When transcription factors were examined, core-binding factors alfa 1 (Cbfa1), runt domain factor 2 (Runx2), and human homolog of the drosophila distal-less gene (Dlx5) expression were substantially reduced in the diabetic mice compared with the control group on days 4 and 6. C-fos but not c-jun expression was also suppressed in the diabetic group, although it is not closely linked to bone formation. Insulin treatment substantially reversed the effect of diabetes on the expression of bone matrix osteocalcin, collagen Type I, and transcription factors Cbfa, Runx2, and Dlx5. These results indicate that diabetic animals produce sufficient amounts of immature mesenchymal tissue but fail to adequately express genes that regulate osteoblast differentiation, which in turn leads to decreased bone formation.[15]

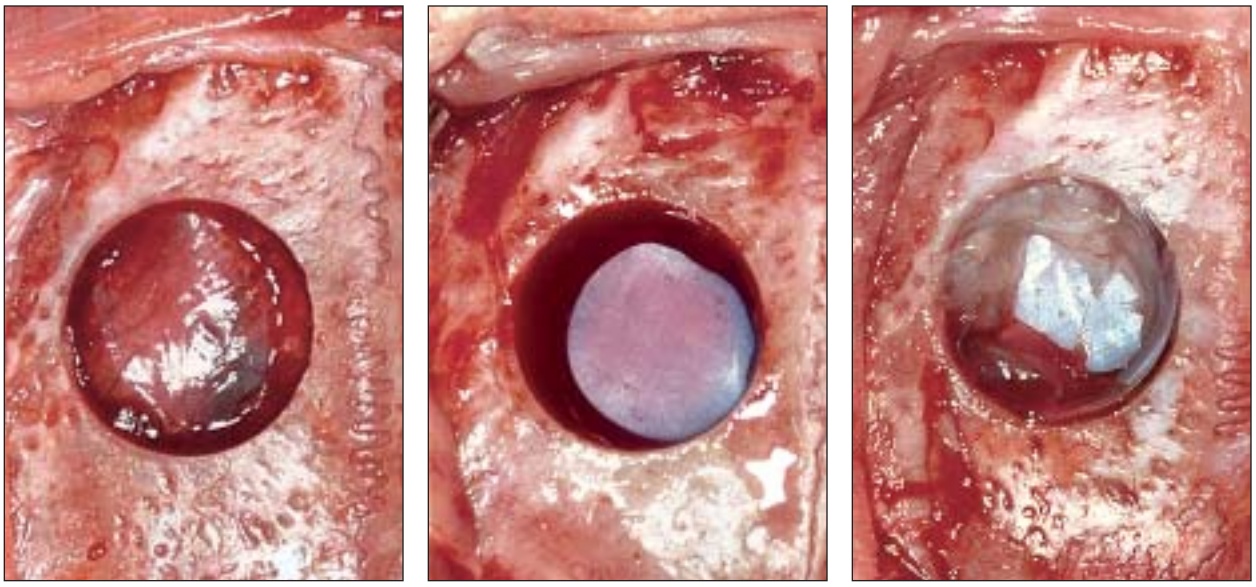
The aim of this work was to evaluate the bone repair process after implantation of HDDM slices in surgical defects created in the parietal bones of rabbits with alloxan-induced diabetes. Polytetrafluoroethylene (PTFE) barriers were used in some experimental groups to accomplish a guided bone regeneration technique. A secondary purpose was to verify whether HDDM can be helpful for bone consolidation and repair in fractures in patients with diabetes.

## MATERIALS AND METHODS

Forty-eight New Zealand adult rabbits with an average weight of 3.5 kg were divided into 4 groups: the control group, diabetic rabbits (D), diabetic rabbits with a PTFE barrier (D-PTFE), and diabetic rabbits with a PTFE barrier and with slices of homogenous demineralized dentin matrix (D-PTFE+HDDM). All animals received humane care as defined by the criteria of the National Research Council, and the study protocol was approved by the Committee for Animal Use of the São José dos Campos School of Dentistry of the São Paulo State University (UNESP).

### Induction of Diabetes Mellitus and Blood Glucose Measurement

Diabetes mellitus was induced in 36 of the rabbits by a single intravenous injection of the toxic monohydrate alloxan at a dose of 90 mg/kg (Sigma-Aldrich, St Louis, MO). The drug was dissolved in 5 mL of NaCl (0.15 mol/L) and immediately administered to the anesthetized rabbit via the marginal ear vein. Animals received 10% glucose in drinking water for the first 24 hours for the transient hypoglycemia developed after alloxan treatment and were then placed on regular rabbit chow and water. In this study, a blood glucose level greater than 200 mg/dL was considered to indicate hyperglycemia. NPH Human



**Figs 1a to 1c** Bone defect accomplished in the parietal bone in *(a)* the D group, *(b)* the D-PTFE group, and *(c)* the D-PTFE+HDDM group.

Insulin (Humulin N-100; Eli Lilly do Brasil, São Paulo, Brazil) and Regular Human Insulin (Biohulin R-100; BioBRÁS, Minas Gerais, Brazil) were administered subcutaneously to maintain a stable serum glucose level between 200 mg/dL and 350 mg/dL. The blood glucose level was monitored with Onetouch Ultra (LifeScan/Johnson & Johnson, Milpitas, CA) 24, 48, and 72 hours after alloxan treatment and 3 times a day thereafter until sacrifice.

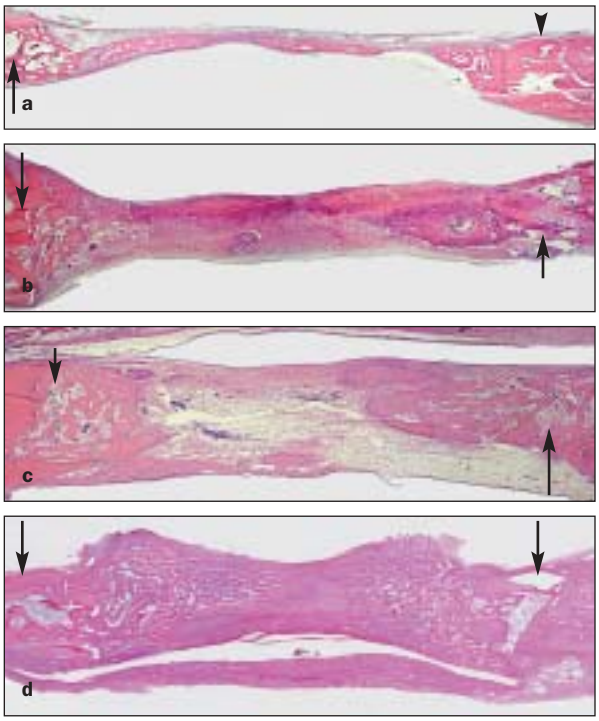
### HDDM

The HDDM was obtained by extraction of the central incisor of the rabbits of control group. HDDM was then prepared in slices as previously described.[5,6] The pulp tissue was totally removed using a retrograde technique. Tooth root was planed and washed with sterile physiologic serum at 2°C. The teeth were immersed in the 0.6N-hydrochloric acid solution at 2°C until complete demineralization. The specimens were then washed for 3 hours in distilled water under constant agitation for total acid removal. After this process, the HDDM was cut into slices of approximately 8 µm thickness with frozen microtomy (Model CTD; International-Harris Cryostat/International Equipment, Needham Heights, MA). These slices were immersed in a box filled with ethyl alcohol 70° ethyl alcohol/gentamicin (5 mL alcohol/0.2 mL of gentamicin) and stored at 2°C until implantation.

### Bone Defect Creation and Postoperative Phase

The animals were anesthetized intramuscularly with Rompum (0.1 mg/dL; Bayer, São Paulo, Brazil) as a preanesthetic solution and Ketalar (0.25 mg/dL; Holliday-Scott, São Paulo, Brazil) for complete anesthesia. An incision was made in the sagittal plane of the head, followed by muscular dissection, plane to plane. An incision was then made in the periosteum. Subsequently, a circular bone defect was surgically created in each parietal bone with the aid of a 8.0-mm trephine and irrigated with 0.9% sterile saline solution. The depth of the bone defect was equal to the thickness of the removed cortical bone. HDDM was placed in slices within the bone defect around the periphery of each defect. In the D-PTFE+HDDM group, the PTFE barrier was positioned on the bone defect floor, and HDDM was then placed. The external surface of the bone defect was completely recovered by the PTFE barrier. In the D-PTFE group, the PTFE barrier was placed on the floor of the defect and on the surface of the surgical bone defect. In the control and diabetic groups, the bone defects were quickly filled with blood clot. Subsequently, the periosteum, muscle, and skin were closed with a suture (Fig 1).

The animals were treated with antibiotics (benzylpenicillin [Pentabiótico]; 0.1 mL/kg; Fort Dodge Saúde Animal, São Paulo, Brazil) 24 hours before and 1 hour after the surgery. Five days after surgery an anti-inflammatory medication was administered (2.8 mg/kg; celecoxib [Celebra], Pfizer Pharmacia, São Paulo, Brazil). These drugs were injected intramuscularly in all animals. Animals were sacrificed 15, 30, 60, and 90 days after surgery; 3 animals of each group were sacrificed each time. The bone content of the




**Figs 2a to 2d** The surgical defect region after 15 days. The limits of the surgical bone defect are indicated by arrows. The bone defect was filled by new bone formation in the *(a)* control group, *(b)* D group, *(c)* D-PTFE group, and *(d)* D-PTFE+HDDM group (hematoxylin-eosin; original magnification ×50).

created defect was removed in bloc, fixed in 10% formalin for 72 hours, decalcified in Plank-Rychlo solution ($AlCl_26H_2O$ [126.10 g], HCl [85 mL], HCOOH 88% [52 mL], and distilled water [500 mL]), and embedded in paraffin. The histologic sections were cut with a thickness of approximately 6 µm and were stained with hematoxylin-eosin and Schmorl's stain.

### Histomorphometric Analysis

Randomization, as previously described,[5,6] was used for the selection of sections for histomorphometric analysis to eliminate sampling bias. A Zeiss II reticule was placed over a compensation microscope (10×; W-PI; Carl Zeiss, Oberkochen Germany) to evaluate the bone density. The reticule image was superimposed on the desired histologic fields. The reticule points (Ni) and the total number of points over the bone defect (N) were counted. The bone density was evaluated with the following formula: bone density = Ni/N. Approximately 100 sections were obtained from each bone defect selected for examination. Of these sections, 4 were randomly chosen for histomorphometric analysis. Subsequently, 8 histologic fields from the surgical bone defect region were analyzed. At this step, a 20× objective (A-Plan, Carl Zeiss) and an ocular 10× (W-PI, Carl Zeiss, Germany) of an optical microscope (Axioskop 40, Carl Zeiss, Germany) were used. The objective showed a 100-point reticule encompassing a bone-tissue area of 7,840 $\mu m^2$.

### Statistical Analysis

The histomorphometric results were submitted to analysis of variance (ANOVA) and to the Tukey test with the aid of the GraphPad InStat software version 3.00 for Windows 95 (GraphPad Software, San Diego, CA). The level of significance used was $P < .05$.

## RESULTS

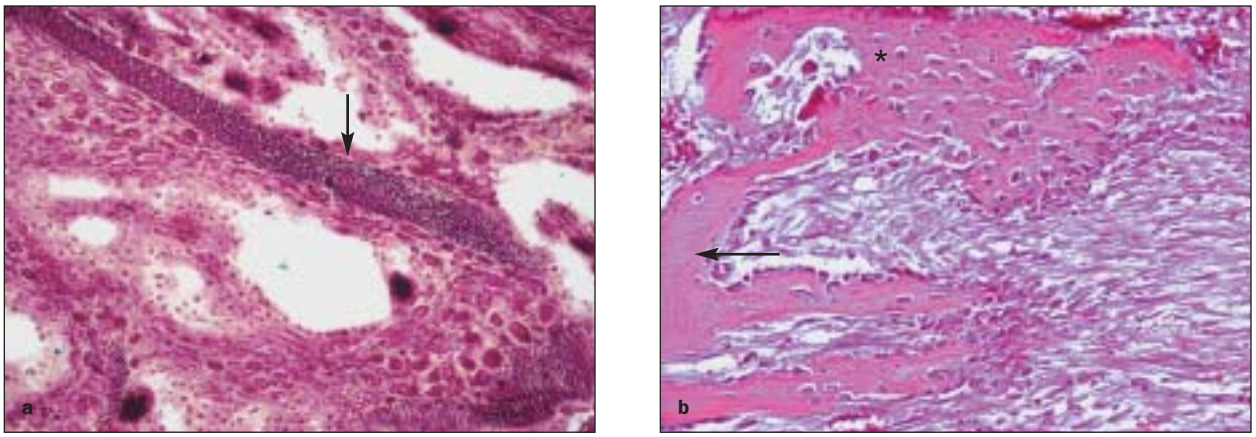
### Microscopic Features

*15 Days.* The histologic sections of the control group showed the bone defect filled with osteogenic connective tissue and with discrete and immature bone trabeculae. The connective tissue consisted mainly of osteoprogenitor cells and collagen fibers, which were parallel to each other, as well as a few mononuclear inflammatory cells. The immature bone trabeculae were delicate and irregular. Many cells were localized in the periphery of bone defect, growing toward the central portion of it. Some histologic sections also showed muscle and adipose tissue in the bone defect area (Fig 2a).

In the D group, an area of bone defect was filled by osteogenic connective tissue, with few immature bone trabeculae. This connective tissue consisted of osteoprogenitor cells and collagen fibers parallel to each other, with some moderate inflammatory infiltrate of mononuclear cells. The immature bone trabeculae were mostly at the periphery of bone defect growing toward its center. The invasion of muscle fibers in the bone defect area during bone repair was also observed. The band of newly formed bone was thin compared to the original cortical bone (Fig 2b).

In the D-PTFE group, the histologic features were the same as those described for the D and C groups. However, there were numerous newly formed bone trabeculae compared with the D group. In some sections, the bone trabeculae showed various shapes in the central portion of the defect. In addition, there were areas of interstitial hemorrhage, and some mononuclear inflammatory cells were observed. Again there was a band of bone tissue in the defect, but unlike the band observed in the D group, this band was about the same thickness as the original cortical bone (Fig 2c).

In the D-PTFE+HDDM group, the bone defect was completely filled with bone tissue, although there were still some immature trabeculae. On a panoramic view of the defect, the newly formed bone appeared regular and exophytic (Fig 2d). In some areas, inti-




**Figs 3a and 3b** *(a)* Intimate contact of the osteoblastic cells with the HDDM slices (*arrow*), and *(b)* deposition of newly formed bone matrix *(asterisk)* on the surface of the HDDM *(arrow;* Schmorl's and hematoxylin-eosin; original magnification ×200).

mate contact between the osteoblastic cells and the HDDM slices and deposition of bone matrix on the surface of the HDDM slices was observed, as well as the incorporation of the HDDM slices with the newly formed bone trabeculae (Figs 3a and 3b). There was no sign of rejection of the HDDM slices.

*30 Days.* At this observation time, the bone defects of the control group were filled by immature bone tissue and osteogenic connective tissue. The bone trabeculae were mature and thick at the periphery and immature in the central portion of the defect; centripetal bone growth was observed. The osteogenic bone tissue was moderately infiltrated by mononuclear inflammatory cells, mostly in the central portion of the defect. The band of newly formed bone was thinner than the original cortical bone.

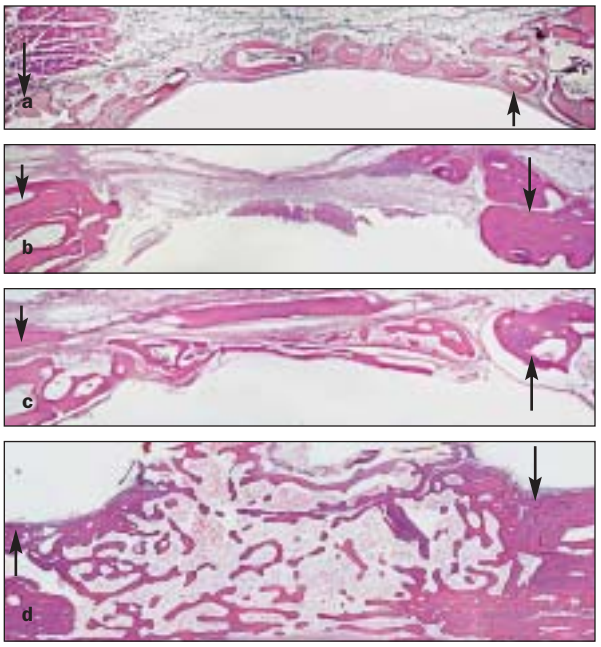
In the D group, the bone defect was filled by immature bone tissue and osteogenic connective tissue. The bone trabeculae were mature and thick at the periphery and immature in the central portion of the defect (ie, the histologic features were very similar to those of the control-group sections). In the D-PTFE group, the bone defect was filled by immature bone tissue and osteogenic connective tissue. The bone trabeculae were mature and thick at the periphery and immature in the central portion of the defect (again, as in the control group). Bone trabeculae grew toward the central portion of the defect, following the space left beneath the PTFE barrier. This demonstrates the osteoconductivity of the barrier.

More trabeculae were observed in the D-PTFE group than in the D or control groups. The osteogenic connective tissue was well organized, and the collagen fibers were parallel to each other. In addition, a moderate and diffuse infiltrate of mononuclear inflammatory cells was observed. In some histologic sections it was possible to verify the presence of bone marrow tissue, mainly in the periphery of the defect, and the band of newly formed bone tissue had about the same thickness as the original cortical bone.

In the D-PTFE+HDDM group, the bone defect was completely filled by bone tissue. A few areas of osteogenic connective tissue remained, but no inflammatory cells were observed. Mature and immature bone trabeculae of various thicknesses were observed. Bone marrow tissue was also observed; the marrow spaces were reduced. At the periphery of the defect, slices of HDDM had been incorporated into the newly formed bone matrix. The presence of some odontoblastic fibrils in the dentinal tubules of the slices of HDDM was verified with Schmorl's stain. Osteoblasts and osteoclasts populated the slices of HDDM; there was bone matrix over some HDDM slices.

*60 Days.* In the control group, the bone defect was filled with regularly distributed mature trabeculae, but some osteogenic connective tissue remained. The newly formed bone exhibited haversian canals, and bone growth inside the defect was still centripetal, with some osteoclastic cells starting bone remodeling. The band of newly formed bone tissue was similar in thickness to the original cortical bone.

In the D group, there was still osteogenic connective tissue. Discrete bone trabeculae were observed. This tissue was disorganized, and the collagen fibers were irregularly distributed. The bone trabeculae were mature at the periphery of the defect but immature in the central portion of the defect. Less bone tissue was observed in the D group than in the control or D-PTFE groups. In some sections, invasion of muscle and adipose tissue to the interior of the defect was observed.




**Figs 4a to 4d** The surgical defect region after 90 days. The limits of the surgical bone defect are indicated by arrows: *(a)* control group, *(b)* D group, *(c)* D-PTFE group, and *(d)* D-PTFE+HDDM group (hematoxylin-eosin; original magnification ×50).

In the D-PTFE group, the defect was filled with bone tissue and disorganized osteogenic connective tissue. The bone trabeculae were mature at the periphery but immature in the central portion of the defect. The trabeculae were homogenous and regularly distributed and were characterized by centripetal growth, which demonstrates the osteoconductivity of this barrier. The band of bone tissue at the periphery was about the same thickness as the original cortical bone.

In the D-PTFE+HDDM group, the bone defect was totally filled by bone tissue with mature trabeculae, well delimited and irregularly distributed, and with a large bone marrow space. The cortical bone was not well structured. Some slices of HDDM were still apparent. The bone marrow spaces were large and filled with yellow bone marrow. A panoramic view showed that the limits of the newly formed bone tissue were greater than the limits of the original cortical bone.

*90 Days.* In the control group, the bone defect was filled by mature bone tissue with regular and irregular bone trabeculae, and in some areas there was fibrous connective tissue that had not calcified, forming a relatively thick fibrous capsule, although an insignificant number of newly formed bone trabeculae permeated some areas (Fig 4a).

In the D group, the fibrous connective tissue in the bone defect was permeated by small, thin, irregular, mature bone trabeculae. At the periphery mature bone tissue grew toward the center of the bone defect. The amount of tissue in this group was very low compared to the other 3 groups. The D group revealed very few haversian systems. In all sections of the D group, there was undesirable tissue (Fig 4b).

In the D-PTFE group, the bone defects were filled by regular mature bone trabeculae, but there was also fibrous connective tissue as well as osteogenic connective tissue. The cortical bone was preserved in all sections observed in this group. The bone marrow spaces were very large, with red bone marrow. The PTFE barrier was dislocated in the histologic sections examined. The quality of bone tissue was well organized, with thick, regular bone trabeculae uniformly distributed within the bone defect (Fig 4c).

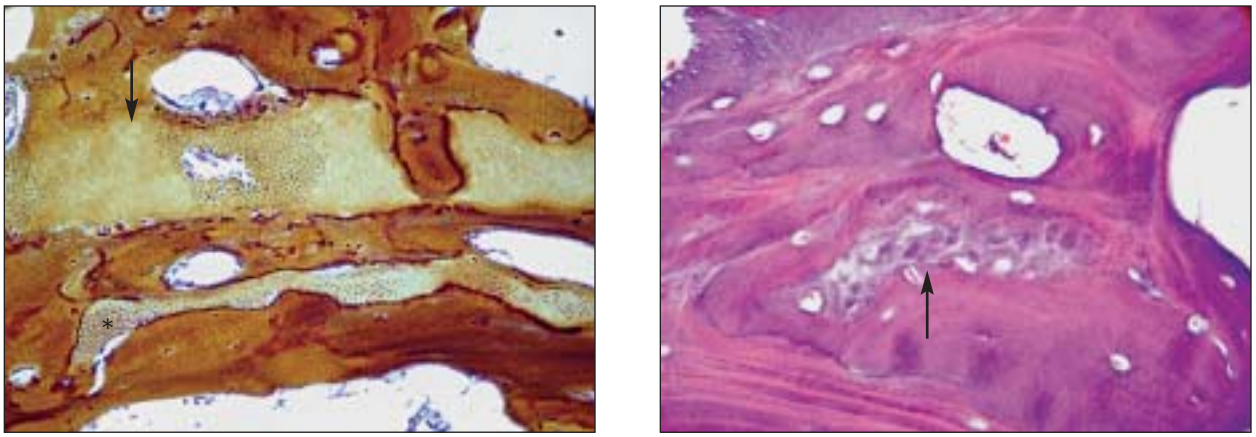
In the D-PTFE+HDDM group, the bone defect was completely filled by mature bone tissue, with regular bone marrow spaces, which were predominantly red. HDDM was well accepted by the host and was totally incorporated into the newly formed bone tissue (Fig 4d). Degradation of the HDDM slices during the bone remodeling process was still seen (Figs 5a and 5b). On a panoramic view, an area of exophytic bone tissue was observed within the bone defect in comparison with the adjacent areas. The structure of the bone tissue in this group was superior histologically.

### Histomorphometric Analysis

The histomorphometric analysis measured bone density in the area of newly formed bone tissue according to the aforementioned formula for bone density. Analysis of the results (Table 1 and Fig 6) demonstrated that the D-PTFE+HDDM group presented a significantly higher ($P < .01$) quantity of bone tissue compared to other groups at each of the 4 time points. At 15 days of observation, there was a statistically significant increase in the volume of bone matrix compared with the D group and D-PTFE groups ($P < .001$). After 90 days of observation, the D-PTFE+HDDM group showed significantly greater bone density compared with the D group ($P < .001$; Table 1).

## DISCUSSION

In the literature reviewed no work was found that utilized HDDM in diabetic patients. However, many of the chronic complications of diabetes mellitus are related to changes in bone metabolism, which may impair any healing process.[16,17] According to Lu et al,[15] who investigated the mechanisms that interfere



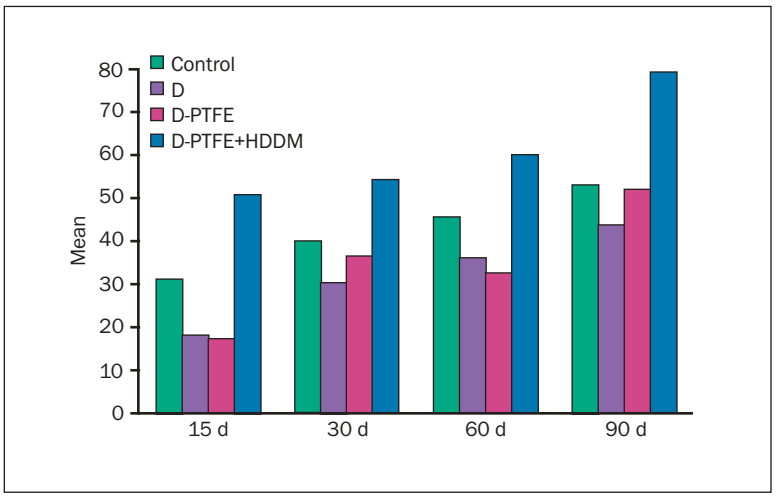
**Figs 5a and 5b** *(a)* Incorporation of immature bone matrix *(asterisk)* on the surface of an HDDM slice *(arrow)* and *(b)* degradation of the HDDM slices during bone remodeling in the center of the defect *(arrow;* Schmorl's and hematoxylin-eosin; original magnifications of ×200 and ×400, respectively).

**Table 1 Histomorphometric Analysis of Newly Formed Bone**

| Days after surgery | Control | D | D-PTFE | D-PTFE+HDDM |
|---|---|---|---|---|
| 15 | 31.66 ± 2.478 | 18.48 ± 4.843 | 17.60 ± 0.208 | 50.80 ± 1.528 |
| 30 | 39.96 ± 0.430 | 30.43 ± 15.06 | 36.70 ± 4.107 | 54.20 ± 1.239 |
| 60 | 45.60 ± 4.095 | 36.44 ± 7.937 | 32.74 ± 0.924 | 59.91 ± 3.322 |
| 90 | 53.24 ± 5.090 | 44.10 ± 17.52 | 51.93 ± 1.204 | 78.99 ± 1.343 |

Means ± SDs are shown.

**Fig 6** Bone density at each of the 4 time points as determined by histomorphometric analysis.




with the bone repair process of diabetic patients, such patients are missing a certain gene that regulates osteoblastic differentiation and directly affects bone formation. Recently, researchers have associated growth factors, such as insulin growth factor, platelet-derived growth factors, BMPs, and other noncollagenous proteins,[6,8,18] and occlusive barriers with acceleration of the bone repair process. Some authors have reported the presence of these growth factors on bone matrix and dentin matrix.[1,5–7,18]

The search for an ideal osteoinductive material for implants has been the subject of many biomedical studies. Biocompatibility, storage requirements, facility of acquisition, cost in relation to benefits, and most importantly, potential for osteoinductivity have led researchers to consider biologic materials.[18] Osteoinductivity has been tested with bone marrow, fresh cancellous bone, fresh compact bone, dentin, and bone matrix. These elements have been applied in various conditions at bone defects in many contexts

(eg, in craniofacial deformities, in polytraumatized patients, and in oncologic, orthopedic, neurologic, and periodontal surgeries).[3,5–8,18–20] Researchers have turned to some of the same materials in their search for an ideal graft material, a material that would accelerate bone regeneration and also increase the volume of bone tissue to remodel the impaired area of bone to normal conditions. Bone graft materials can eliminate the necessity of a donor, which can be a tremendous advantage. However, it is important to emphasize the importance of the biocompatibility of those graft materials in order to avoid immune problems or immunologic incompatibility, which would also impair bone regeneration.[5–9]

In this present study, the results of the D-PTFE+HDDM group showed that HDDM was well accepted by the host and that HDDM was totally incorporated into the newly formed bone tissue. These results corroborate the findings obtained by Catanzaro-Guimarães et al,[7,18] Gonçalves,[2] Gomes et al,[5,6] Abreu et al,[9] and Carvalho et al.[8] The present study also verified that demineralization of HDDM slices did not destroy its osteopromotive properties, since it contributed to the acceleration of bone repair. The HDDM is a reservoir of biochemical factors that induce cellular proliferation as well as cell differentiation and chemotaxis (IGF-I and II, TBF-β, and BMPs).[1,3,8,18,19] The authors believe that demineralization of dentin matrix slices even facilitated the access of polypeptides to the area and stimulated the bone matrix molecules to help bone repair.

The results observed in all time periods of study, as in the control group, D group, D-PTFE+HDDM group, showed gradual and progressive increases in the average bone density, as determined by histomorphometric analysis, confirming results reported by Gomes et al,[5,6] Carvalho et al,[8] and Abreu et al.[9] However in D-PTFE group, the histomorphometric analysis showed a decrease in bone density between 30 and 60 days after surgery.

The PTFE barrier has been observed to have an osteoconductive property when well applied and maintained on bone defects in diabetic rabbits. However, its dislocation created difficulties for bone repair in some animals, which could justify the worst results at 15 and 60 days after surgery in histomorphometric analysis (Table 1). In the D-PTFE+HDDM group, significantly greater bone density was observed in comparison to the other groups (Fig 6 and Table 1).

Some authors believe that the use of slices of HDDM as a graft induces a neovascularization inside the bone defect and that undifferentiated mesenchymal cells in the perivascular region of the newly formed vessels could be induced to differentiate into osteoblasts by the induction of growth factors such as BMP from the HDDM.[1,5,6,8,19]

The presence of numerous osteogenic cells over the particles of HDDM after 15 and 30 days of observation corroborates results reported by Catanzaro-Guimarães et al,[7] Gomes et al,[5,6] Carvalho et al,[8] and Abreu et al,[9] whose analysis of particles and/or slices of HDDM suggested that this kind of material could be used as a graft as well as a suitable surface for fixation of undifferentiated mesenchymal cells, probably because of the larger contact surface for the deposition of bone matrix offered by demineralization of the dentin. After 60 days of observation, the bone defect in the D-PTFE+HDDM group, unlike the other 3 groups, was totally filled with bone tissue with the presence of bone marrow, which again demonstrates the osteopromotive property of the dentin matrix.

The absence of rejection of the HDDM corroborates the previously reported results of Carvalho et al.[8] This appears to be a consequence of the low antigenicity of HDDM.[5,6,9]

HDDM stimulated the proliferation and activity of osteoblastic and osteoclastic cells as late as 90 days postsurgery. The greater amount and the better quality of bone tissue in the group with HDDM compared with the other groups demonstrates the effects of HDDM. The bone tissue at this group was more regular and uniform in extension as well as in the central region of the bone defect. Thus, the results suggest that HDDM may be suitable as a graft material for humans.

## CONCLUSION

HDDM is biocompatible with the bone of diabetic rabbits. The HDDM slices showed osteopromotive properties. They were completely incorporated in the newly formed bone tissue and were resorbed during bone remodeling process. The D-PTFE+HDDM group showed significantly greater bone density than the other 3 groups at all time points examined ($P < .01$). In addition, the D-PTFE+HDDM group evidence better structural bone quality when compared with the other groups of this study. Facilitation of bone repair with HDDM could be useful in diabetic patients.

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