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ORIGINAL ARTICLE

Combining autologous particulate dentin, L-PRF, and fibrinogen to create a matrix for predictable ridge preservation: a pilot clinical study

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Abstract

Objectives The aim of this study was to describe the histological and clinical outcome of "**d**entin block" (a mixture of autologous particulate dentin, leukocyte**-** and platelet**-**rich fibrin (L-PRF)**,** and liquid fibrinogen) in alveolar ridge preservation.

Material and methods **Ten** extraction sockets were grafted with "**d**entin **b**lock**,**" a mixture of particulate autologous dentin with chopped leukocyte-platelet**-**rich fibrin (L-PRF) membranes at a 1:1 ratio**,** and liquid fibrinogen as a binder. **Two** grafted sites were followed at 4 and 5 months, and 6 sites at 6 months. Biopsies were taken from the core of the grafted site for histologic and histomorphometric analysis.

Results All patients completed the study without any adverse event. The vertical and horizontal dimensions of the alveolar ridge were preserved or even increased after 4, 5**,** or 6 months and remained stable after 6 months of the implant placement. The histological examination revealed a median relative percentage of bone, dentin, and connective tissue of 57.0, 0.9, and 39.3%, respectively. A comparison of samples at different time points (4, 5, and 6 months) showed a progressive increase in the proportion of bone with a decrease in the proportion of dentin. The bone was compact with normal osteocytes and moderate osteoblastic activity. In **4 out of** 10 samples**,** no dentin was observed**;** in the other samples**,** it represented 1–5% (with geometric fragments).

Conclusions Dentin block showed to be a suitable bone substitute in an alveolar ridges preservation model.

Clinical relevance The promising results of dentin block as a bone substitute in alveolar ridge preservation could have an important clinical impact considering this biomaterial brings together the regenerative potential of three autologous products with excellent biological and clinical behavior, low risk of adverse effects, and feasible acquisition.

Keywords Bone substitute · Bone regeneration · Dentin · Dentin block · GBR · Ridge preservation

Introduction

The alveolar process is prone to major resorption in a vertical and horizontal dimension after tooth extraction [1, 2], primarily due to the loss of bundle bone. This may jeopardize future implant therapy [3], which requires an adequate three-dimensional osseous volume of the alveolar ridge, accompanied by good soft tissue architecture to provide an optimal esthetic, phonetic, and long-term functional result [4].

Various surgical techniques have been proposed to compensate for this resorption. Alveolar ridge preservation (ARP) techniques are widely used to compensate for this resorption. The application of grafting biomaterials into a fresh extraction socket has been thoroughly investigated in both animal and clinical studies [5–7]. These materials are used because they can possess one or more of these biological properties: osteogenesis, osteoinduction, and/or osteoconduction [8]. A systematic review from Vignoletti and co-workers [9] revealed that there are no clear guidelines on which biomaterial to select for this purpose. A recent meta-

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analysis highlighted that the resorption of the alveolar ridge cannot be "completely" avoided by ARP [10]. Therefore, further development in the regenerative field remains fundamental to provide new alternatives for biomaterials.

So far, autogenous bone is the only graft material with osteogenic properties, and therefore, it is considered the gold standard for bone grafting [11, 12]. Unfortunately, autogenous bone has limitations related to availability, morbidity, and risks during the graft harvest [13, 14]. Bone substitutes (allografts, xenografts, alloplastic materials, etc.) have variable rates of resorption and bone formation and are associated with a high financial cost [14]. Moreover, graft remnants may decrease the final bone-to-implant contact (BIC) [15].

Since the 1960s, dentin has been evaluated as a biomaterial to induce bone formation [16]. To date, several in vitro, in vivo, and clinical studies have reported promising results, proposing dentin as an adequate bone substitute [8, 17–20]. The fact that dentin shares the same embryologic origin as alveolar bone might explain its capacity for bone formation. Moreover, dentin and bone composition are very similar [21]. Dentin organic composition is mainly collagen I (90%), and it contains various growth factors such as bone morphogenetic proteins (BMPs), recognized as a promoter of bone formation [21, 22]. Dentin, as a bone substitute, has also the advantage that it does not cause host tissue reaction or heterotopic bone formation, which is an important safety aspect to consider when a graft is selected [23, 24].

In ARP, dentin has shown able to preserve the dimensions of the alveolar ridge due to osteo-conductive property and its favorable rate of resorption and new bone formation [25]. Dentin showed already to be an effective bone substitute for vertical augmentation in extraction sockets with results comparable to the inorganic bovine bone, with for example similar implant stability [26].

To enhance wound healing, the use of biological additives, which regulate inflammation and angiogenesis, could be very beneficial. Choukroun and co-workers [27] introduced a second-generation platelet concentrate (the leucocyte and platelet-rich fibrin (L-PRF)), which is simple in preparation and does not require a biochemical blood handling. The fibrin network has many similarities with the one formed during natural healing. Moreover, the release of high quantities of transforming growth factor Beta-1 (TGF beta-1), platelet-derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF), and thrombospondin-1 from L-PRF [28] stimulates biological functions, such as chemotaxis, angiogenesis, proliferation, and differentiation [29–31].

Several medical disciplines have used L-PRF in different types of surgery, including periodontology, implant dentistry, and maxillofacial surgery, reporting a positive effect for the wound healing process [32–41].

Recently, a split-mouth, randomized clinical trial reported significant benefits in the preservation of vertical and horizontal ridge dimensions with the use of leukocyte-platelet-rich fibrin (L-PRF) as "sole" grafting material. It showed similar results in ARP as other bone substitutes, with several advantages such as simplified procedures, less post-operatory pain and inflammation, the absence of residual graft particles, and the benefit of its low cost [42]. These benefits of L-PRF in the healing time are confirmed by a systematic review showing that ARP improves when bone substitutes are combined with L-PRF [8].

On the other hand, a new concept, "liquid fibrinogen," has been introduced as a binder to particulate bone graft, allowing improving its stabilization in the defect and adding a potential biological effect by their cellular content, which could accelerate the healing process and optimize the new bone formation [43].

The aim of the present pilot study was to describe, for the first time as far as the authors know, the clinical and histological outcome of a "dentin block" (a mixture of 50% autogenous dentin, 50% L-PRF membranes chopped into small pieces, and liquid fibrinogen to agglutinate all together for better handling) as a bone substitute using alveolar ridge preservation as test model.

Material and methods

Patient selection

Four patients with a total of 10 extraction sockets at locations scheduled for future implant rehabilitation were enrolled in this pilot study. The patients met the following inclusion criteria: older than 18 years, no medical history that contraindicates the surgical procedure, at least one tooth that had to be extracted but without endodontic treatment, post-extraction socket with four bone walls, request for replacement by an implant. The exclusion criteria were systemic disease that might impair bone metabolism, antiresorptive therapy (as bisphosphonates), pregnancy, psychiatric conditions, and heavy smokers (> 10 cigarettes). Each patient agreed to participate in the study, providing written informed consent. The Ethics Committee of Universidad de Los Andes approved the study protocol by the Revised Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013).

Surgical approach

Preparation of dentin, L-PRF membrane, and liquid fibrinogen mix is shown in Fig. 1. Each tooth was extracted carefully, preserving the integrity of the four bony socket walls. A high-speed tungsten carbide bur was used to clean the tooth and to remove any decay, restoration, or foreign materials. The tooth was dried by air and ground with the Smart Dentin Grinder

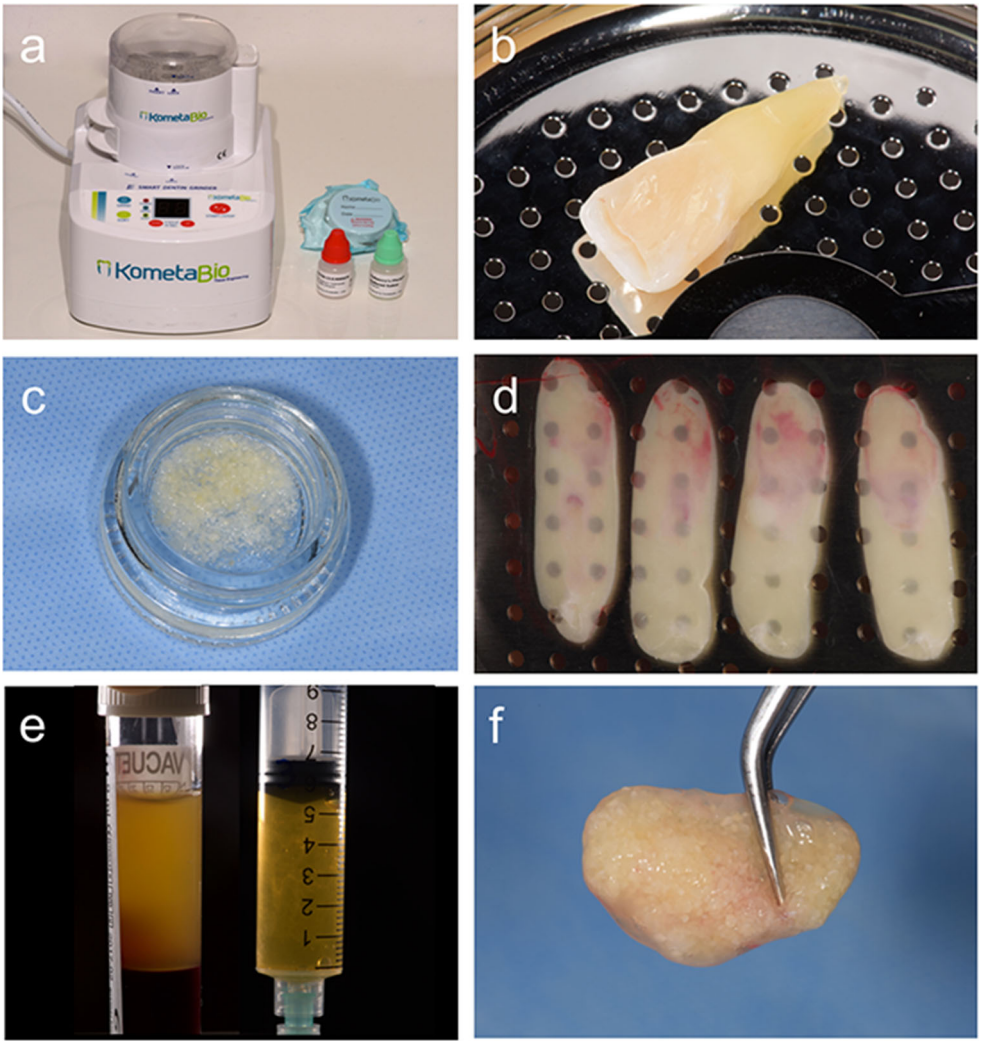

Fig. 1 Preparation and application of dentin block. **a** Smart Dentin Grinder TM (Kometa Bio, Holon, Israel). **b** Extracted central incisor after cleaning. **c** Particulate dentin as obtained at the end of the process following a specific protocol (organic rest free) (particles 300 μm–1200 μm in size). **d** Five L-PRF membranes after compression of the cloths in Xpression box (IntraSpin, Intra-Lock, Florida, USA). **e** Liquide fibrinogen, aspirated from white cap tubes, after 3 min of centrifugation. **f** Dentin block obtained after mixing 0.5 g dentin particles with 2 chopped L-PRF membranes and afterwards adding the liquid fibrinogen

TM (Kometa Bio, Holon, Israel), according to the protocol of the manufacturer (Fig. 1a–c). Dentin particles were obtained with a dimension of 300–1200 μm [44].

At the same time, L-PRF membranes were prepared. Four to six 10-cc blood samples were collected in vacutainer tubes without anticoagulant (red cap, glass coating) and immediately centrifuged at 2700 rpm (408*g*) for 12 min (IntraSpin, Intra-Lock, Florida, USA). Two extra blood samples were collected in 9-cc non-coated vacutainer tubes also without anticoagulants (white cap). These last samples were centrifuged at 2700 rpm for 3 min only. The yellow fluid (liquid fibrinogen) at the top of the white cap tubes was aspirated with a sterile syringe, avoiding red blood cells (Fig. 1d). The L-PRF clots obtained after 12 min of centrifugation were placed in the Xpression box (IntraSpin, Intra-Lock, Florida, USA) for 5 min to gently compress (by gravity) into membranes (Fig. 1e).

To prepare the L-PRF block (Fig. 1f–g), the L-PRF membranes are cut into small pieces and mixed with the dentin particles at a ratio of 2 membranes/0.5 g dentin (which provides a 1:1 volume ratio). The liquid fibrinogen is added to the homogeneous mix and stirred gently for ± 10 s while shaping the mixture to the desired form. The fibrinogen will be converted into fibrin (primarily from activated blood platelets from the chopped L-PRF membranes) within a few minutes and traps the biomaterial and the L-PRF pieces to form a strong block, resulting in a handy and compact graft ("dentin block") (Fig. 1g).

Alveolar ridge preservation

After exhaustive cleaning, each socket was filled with the "dentin block" shaped according to the size and shape of the socket. After the adaptation of the graft to the socket, the graft was covered with two layers of L-PRF membranes extending 2 mm in the envelope prepared between the periosteum and the bony borders of the socket (360° around). The wound was secured with a silk suture, not with the intention to close the wound, but simply to keep the graft and membranes stable in position (Fig. 2).

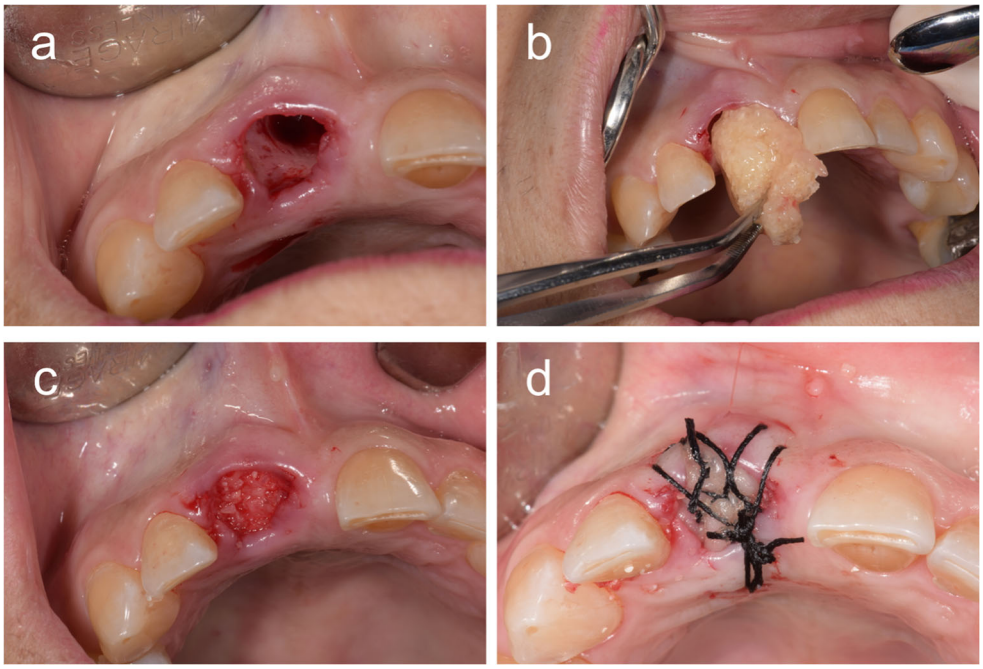

Fig. 2 Ridge preservation via the use of a dentin block. **a** Extraction socket (4-wall defect) of the central upper incisor (tooth 1.1). **b** Dentin block graft insertion. **c** Relative position of dentin block towards the marginal bone. **d** Dentin block graft is covered by two layers of L-PRF membranes secured with a silk suture. **e** Bone healing after 5 months, at the moment of biopsy and implant placement

Dimensional changes evaluation by CBCT

Horizontal and vertical measurements of the alveolar ridge were evaluated by CBCT at the pre-extraction socket, after 4 months of alveolar ridge preservation and 6 months later of implant placement.

Biopsy collection

After a 4- to 6-month healing period, at the moment of the implant placement, a biopsy from the core of the grafted site was obtained using a 2-mm trephine bur. The biopsy was immediately fixed in 10% neutral buffered formalin and then dehydrated through baths of progressively more concentrated (from 50 to 100%) alcohol and subsequently embedded in paraffin. Finally, a tissue section of 4-μm thick was prepared and stained with hematoxylin-eosin for histological analysis.

Histological and histo-morphometric analysis

Two independent professionals analyzed the specimens at × 10 magnification using ImageJ software. All specimens were digitized at the same magnification using a Leica DM500 microscope (Leica Microsystems, Wetzlar, Germany) and a digital camera (ICC 50 HD, Leica, Wetzlar, Germany) to measure the proportional areas of the new bone, residual dentin graft particles, and connective tissue. The spatial scale of the active image was defined so measurements could be performed in calibrated units (μm^2). These measurements were expressed as a mean percentage of the total surface area of the section. One reviewer made a detailed histological evaluation of the image.

Statistical analysis

This paper only presents descriptive data. The histological data from both evaluators were averaged. These data are summarized in a table representing relative proportions of the different tissues (mean, median, and standard deviation).

Results

A total of 10 consecutive extraction sockets (4 incisors, 5 canines, and 1 premolars) in the maxilla of four female patients (mean age, 54 years; age range, 44–63 years) were included (Table 1). The reason for tooth extraction was root caries, endodontic pathology, and severe periodontitis. All patients completed the study.

Table 1 Demographic characteristic of patients

Variable	Tooth site ($N = 10$)	
Gender	Male	0
	Female	4
Age	Mean	54
	Range	44–63
Tooth site location	Incisor	4
	Canine	5
	Premolar	1

CBCT evaluation

Four months later of alveolar ridge preservation, the vertical and horizontal dimensions were preserved and even increased. Furthermore, these dimensional measurements remained stable after 6 months of implant placement (Fig. 3).

Clinical outcome

The healing was always without any sign of host tissue response (inflammation or rejection symptoms), and no postoperative complications were observed. At re-entry, the grafted sites presented a homogenous appearance without disaggregated graft particles, either in the flap or at the entrance of the previous socket. During the implant site preparation, the hardness of the newly formed tissue was comparable to dense type 2 bone.

Histological and histo-morphometric results

Table 2 shows the histo-morphometric analysis of the 10 sites (one per socket) carried out by two independent evaluators. The overall mean proportion of bone was 56.5% (S.D. 22.2%) and of the remaining dentin was 3.6% (S.D. 6.4%). In 4 of the 10 sites, all dentin seemed to have disappeared, and in another 2 sites its proportion was ≤ 1%.

When comparing the different time intervals, the relative proportion of bone increased (26.3% at 4 months, 56.5% at 5 months, and 66.5% at 6 months, respectively). The opposite was seen for the amount of remaining dentin (10.4% at 4 months, 4.8% at 5 months, and 0.9% at 6 months, respectively).

The samples in general showed the presence of compact bone tissue with normal osteocytes and with some geometric dentin fragments (5–10% of total mineralized tissue). The bone tissue showed moderate osteoblastic activity and a

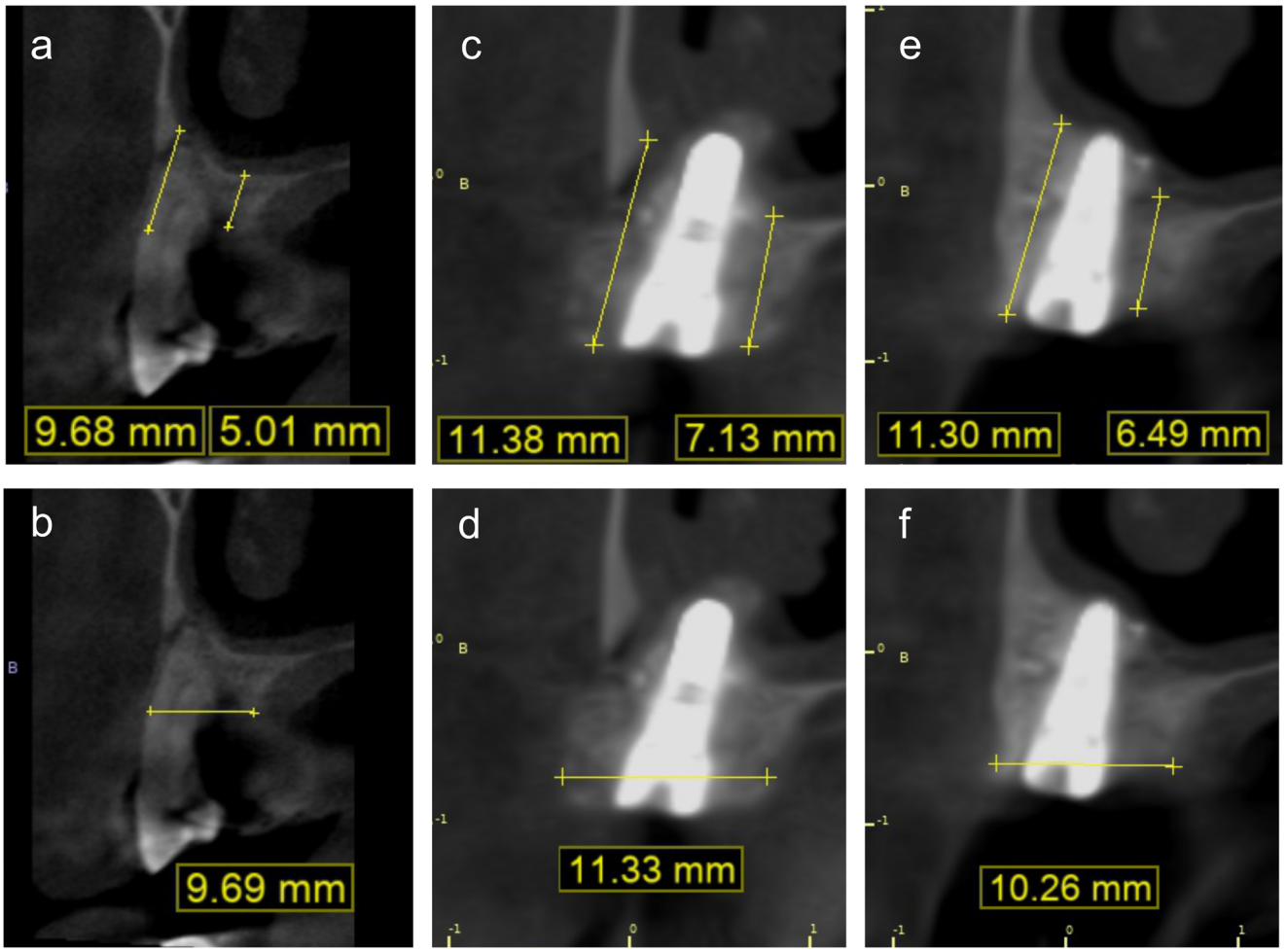

Fig. 3 CBCT comparative analyses between baseline and after 4 months. **a** Vertical dimension of alveolar ridge pre-tooth extraction (tooth 1.4). **b** Horizontal alveolar ridge measure pre-tooth extraction (tooth 1.4). Images **c** and **d** show the vertical and horizontal dimensions respectively of the alveolar ridge after 4 months of alveolar ridge preservation when the implant was placed. Images **e** and **f** show the vertical and horizontal dimensions respectively of the alveolar ridge after 6 months of implant placement

Table 2 Relative proportions in the 10 test sockets of bone, dentin, and connective tissue estimated via histo-morphometric analysis. Biopsies were taken after 4, 5, or 6 months of submucosal healing

Patient	Sample	Healing time (months)	Bone (%)	Dentin (%)	Connective tissue (%)
1	1	4	17.1	20.7	62.1
	2	4	35.5	0.0	64.5
2	3	5	60.6	4.9	34.5
	4	5	52.3	4.8	42.9
3	5	6	53.3	3.6	43.1
	6	6	80.2	0.9	18.9
	7	6	64.3	0.0	35.7
4	8	6	79.0	0.0	21.0
	9	6	86.0	0.0	14.0
	10	6	36.3	1.0	62.7
	Overall mean		56.5	3.6	39.9
	S.D.		*22.2*	*6.4*	*18.7*
	Median		57.0	0.9	39.3
	Mean/time point				
	4 months ($N = 2$)		26.3	10.4	63.3
	5 months ($N = 2$)		56.5	4.8	38.7
	6 months ($N = 6$)		66.5	0.9	32.6

S.D, standard deviation

medullary space filled with connective tissue showing a slight mononuclear infiltrate (Fig. 4).

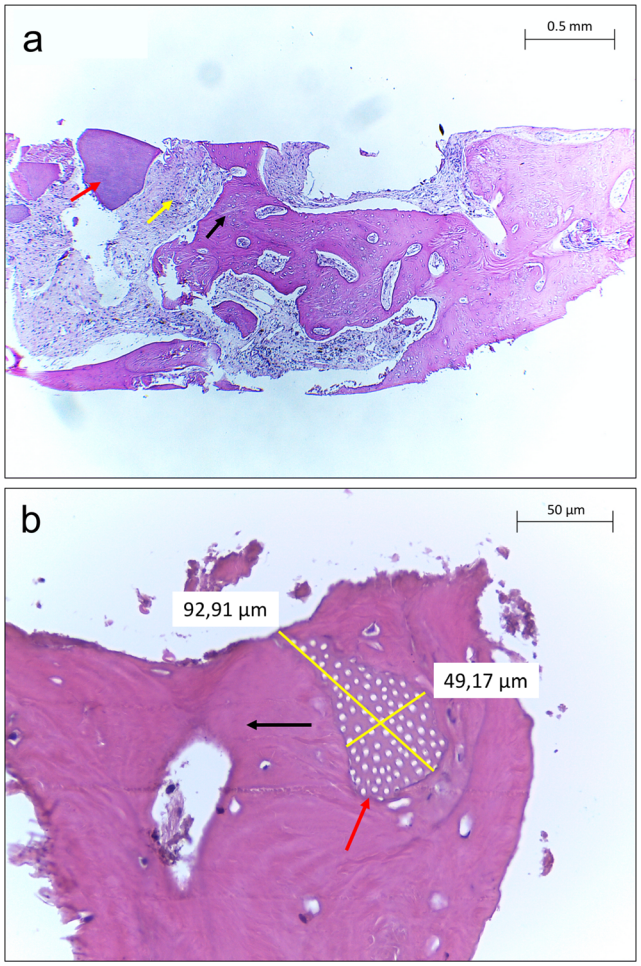

Fig. 4 Histological samples (hematoxylin-eosin staining). **a** Histologic section (× 10 magnification) from the core of grafted site showing compact bone (black arrow), connective tissue (yellow arrow), and a remaining dentin particle (red arrow). **b** Histologic section (× 100 magnification) showing newly formed lamellar bone (black arrow) embedding a dentin graft particle, with clear dentinal tubules (red arrow). The yellow lines indicate the width and length of dentin particle

Discussion

In the present study, the use of a "dentin block" in an alveolar ridge preservation model showed being able to preserve and even slightly increase the vertical and horizontal dimensions of the remaining stable after 6 months of implant placement. Additionally, an optimal bone formation without host tissue response to the graft and an appropriate rate of dentin resorption were observed.

Different ARP techniques have been used to prevent predictable horizontal and vertical bone resorption. Recently, Iocca and co-workers [45] presented a meta-analysis comparing different grafting materials for ridge preservation. The analysis showed that socket grafting was more clearly favorable than unassisted natural socket healing. The combination of freeze-dried bone grafts with a membrane appeared to be more efficient in the prevention of bone height resorption, whereas autologous bone marrow grafts resulted in the least resorption in ridge width [45]. However, these bone grafts have limitations related to availability and morbidity, uncertain resorption rate, cost-effectiveness, etc. [13, 14].

The "dentin block" is a mix of three autologous biomaterials: L-PRF, dentin, and liquid fibrinogen. The ability of dentin to promote bone formation in the alveolar ridge can be explained by its common embryological origin and similar composition [21, 22]. Dentin and bone also have non-collagenous proteins belonging to the SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) family, including dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), and osteopontin (OPN) [46]. Moreover, BMPs, transforming growth factor-beta (TGF-β), insulin growth factor I (IGF-I), and IGF-II have been detected in human dentin, and all are involved in the bone formation process [46]. Furthermore, Reis-Filho and co-workers [8] reported that demineralized human dentin matrix (DHDM) increased the expression of vascular endothelial growth factor (VEGF), the most important factor for angiogenesis and an essential element in every healing process. They also indicated that VEGF expression in the osteoblasts, stimulated by DHDM, could be necessary for the

communication between osteoblast and endothelial cells during bone repair [8]. de Oliveira and co-workers [47] evaluated histological and immunohistochemically rat alveolar wounds filled with demineralized human dentin matrix (DHDM). They observed in the experimental sites (DHDM) advanced healing compared with the control sites (blood clot) during all the observation period, even at 14 days. The dentin was not degraded but was incorporated into trabecular bone. They also reported a statistically significant ($p < 0.05$) increase in the number of osteoblasts positive for BMP-2 and BMP-4 at the experimental side, compared with controls at 10 days [47]. As such, dentin can be considered an absorbable matrix with osteoconductive and osteoinductive properties. As dentin is progressively degraded, BMPs are released in a controlled manner, stimulating bone formation [47].

Our histological analysis showed that autologous dentin was able to produce mature bone tissue. When the samples collected at different times were compared, one could observe an incremental increase in the bone and at the same time a decrease in the dentin percentage. Moreover, complete incorporation of the dentin particles into the new bone became visible with intimate contact without soft tissue in between, (resembling ankylosis). This observation is in accordance with the results observed by de Oliveira and co-workers and Reis-Filho and co-workers [8, 47]. This may confirm the biocompatibility of dentin in addition to its chemotactic characteristic for osteogenic cells due to the presence of bioactive molecules [47]. Bakhshalian and co-workers [23] reported an increased bone mass and superior bone quality in experimental defects in rabbits filled with allogenic demineralized dentin matrix (DDM) compared with control defects (empty).

An interesting clinical feature of our grafted sites was the bone quality with a hardness similar to that of dense trabecular (type 2). This could be related to results obtained by Kim and co-workers [48] from scanning electron microscope (SEM) analysis where the density, roughness, and homogeneity of autogenous tooth bone graft material were relatively similar to those of autogenous cortical bone.

The majority of dentin studies have evaluated demineralized dentin because this process could improve the collagen exposition and delivery of BMPs and other bioactive molecules [49]. However, our results showed that non-demineralized dentin also successfully promoted bone formation with an adequate dentin resorption/bone formation rate. It is possible to speculate that the dentin cleaner kit, based on alcohols and buffer solution to eliminate all organic remains, also improved the collagen fiber exposure and the release of growth factors.

In this study, the dentin had a particle size range of 300–1200 μm. The optimal particle size of a bone graft material depends on the type of bone substitute [50, 51]. The morphology and size of graft particles are important elements to consider because they determine the vascularization, cell migration, and cell attachment, which are essential conditions to induce the new bone formation [52]. Koga and co-workers [49] found better results with 70% dentin demineralization and large particle size (1000 μm) but proposed to analyze different degrees of dentin demineralization, for example, only surface demineralization and a mixture of particles with variable size, to obtain superior bone formation.

In the present study, the dentin graft was used in combination with L-PRF and liquid fibrinogen. L-PRF is the second generation of platelet concentrate obtained by a simple protocol of centrifugation (2700 rpm during 12 min) without additives. This straightforward and inexpensive technology has shown to be able to enhance the healing process and to promote tissue regeneration due to the presence of platelets and leukocytes which release growth factors and cytokines involved in the healing process.

The particular fibrin matrix with a specific tridimensional architecture also favors the different biological events during tissue regeneration [40, 53–58] and enhances graft stability. The incorporation of small L-PRF pieces in the dentin block to reduce the amount of particulate graft seems to maximize the regenerative process.

The slow release of growth factors from L-PRF and their fibrin mesh gives an excellent scaffold for the migration of stem cells and osteogenic cells, possibly improving the angiogenesis and the new bone formation. The 1:1 ratio of dentin and L-PRF in the dentin block produces a unique tridimensional distribution, generating spaces between the dentin particles so that the angiogenesis and cell migration can be facilitated. The liquid fibrinogen also releases growth factors and at the same time fixes all graft components together, allowing easy handling, avoiding the loss of graft particles, and stabilizing the regenerating tissues [43].

We chose different time points for sample collection due to the critical role of the resorption rate when evaluating a bone graft. Considering that most grafting techniques suggest 4- to 6-month period for implant placement, we adopted these intervals for sampling (4, 5, and 6 months) to get an overview of the resorption behavior of the dentin block graft.

The small sample size could be a limitation of our study. However, the primary objective was to obtain a preliminary view of the clinical and histological results from the use of new graft material as a starting point for future research. Our preliminary results show promising results with an autologous graft "dentin block" combining different regenerative properties originating from three biomaterials obtained from the patient himself with a low cost and a simple protocol, maximizing bone formation in a natural guided regeneration. It would be interesting in future studies to analyze the use of dentin with different degree of demineralization and to evaluate molecular aspects such as BMPs, growth factors, and cytokines released in the grafted sites in comparison with other bone substitutes. Another attractive issue would be a more detailed

clinical and imaging analysis with standardized measurement methods to quantify the degree of alveolar ridge resorption. Moreover, the use of dentin block should be evaluated in more demanding regenerative scenarios (sinus lift, horizontal or vertical bone augmentation, periodontal defects, sockets with bone dehiscence, etc.).

Within the limitations of the present descriptive study, it was concluded that dentin block was able to promote new bone formation, without host tissue reactions, and a favorable dentin resorption/bone formation rate. All patients demonstrated an adequate amount and quality of bone for implant placement. Although longer-term, multicenter, randomized, controlled clinical trials are required to confirm these observations, our finding suggests that a "dentin block" could be a promising new substitute for bone regeneration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the present study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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