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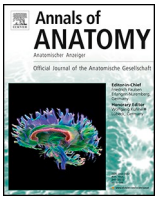

Alveolar ridge preservation with autogenous tooth graft: A histomorphometric analysis of 36 consecutive procedures

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ABSTRACT

Background: The aim of this study was to analyze the histomorphometric findings of autogenous tooth grafting (ATG) for alveolar ridge preservation (ARP), using graft material from extracted teeth. Variations by sex, age and location of extracted teeth, as well as any associated complications, were also assessed.
Materials and Methods: This prospective, single-cohort study was conducted using ATG placed in extraction sockets. After 5 months healing, bone biopsies were collected during implant placement and analyzed histomorphometrically to assess new vital bone, residual biomaterial, and connective tissue. The results underwent statistical analysis; non-parametric tests (Mann Whitney test for independent samples and the Kruskal-Wallis test) were applied.
Results: 27 patients (16 females, 11 males) underwent 36 ARP procedures. Histomorphometric analysis revealed a mean percentage of new vital bone of 29.14 % (± 10.86), residual tooth graft of 10.84 % (± 6.82), and intertrabecular connective tissue of 59.87 % (± 10.56). No significant differences were found in relation to age, sex or location.
Conclusions: ATG appears to be a promising material for ARP, without significant complications. Further comparative studies are needed to better understand this material's behavior.

1. Introduction

Alveolar ridge preservation (ARP) is defined as any procedure undertaken simultaneous to or following an extraction, designed to minimize external resorption of the ridge and maximize bone formation within the socket (De Risi et al., 2015). This will facilitate future prosthodontic treatment, including dental implant placement. Different bone substitute materials can be used for this procedure, with or without membranes (Willenbacher et al., 2016), such as autografts, allografts, xenografts and alloplastic materials (Willenbacher et al., 2016; MacBeth et al., 2017).

Although these bone substitutes are able to maintain the tissue contours in extraction sites, some differences in the quantity and quality of the regenerated tissue have been reported. In addition, the bone density of the maxilla, which is lower than that of the mandible, can present challenges for alveolar regeneration as its structural characteristics exert a negative influence on healing and graft integration (Majzoub et al., 2019). This difference in bone density, especially in the alveolar process, is particularly important in the selection of the most suitable bone substitute, as it affects the amount of new bone formed and the rate of resorption during the healing process (Chu et al., 2023; MacBeth et al., 2017); this may be attributed to the different

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physicochemical properties of the different biomaterials. Nevertheless, some authors claim that different graft materials do not have statistically significant effects on new bone formation (Canellas et al., 2020).

Other authors consider that xenografts and alloplasts, although effective in preserving ridge dimensions, have a slower bone turnover than autografts, which are considered more suitable for regions such as the mandible, where bone density is higher (Majzoub et al., 2019; MacBeth et al., 2017).

Despite these limitations, xenografts continue to be highly valued for ARP procedures due to their positive clinical results in maintaining alveolar ridge volume (Canellas et al., 2021; Canullo et al., 2022).

Kim et al., (2010) were the first to describe using autogenous dentin for guided bone regeneration. Since then, various authors have investigated the use of this tooth-derivate material in different clinical procedures, including the regeneration of defects after lower third molar extraction (Kuperschlag et al., 2020; Mazzucchi et al., 2022; Sánchez-Labrador et al., 2024), sinus lift augmentation (Jun et al., 2014; Minetti et al., 2019a,2019b), and ARP procedures (Minetti et al., 2019a, b; Joshi et al., 2016; Jung et al., 2018).

A recent systematic review by Sánchez-Labrador et al., (2023) analyzed studies of ARP procedures using autogenous tooth graft (ATG), concluding that this type of material achieved higher percentages of new bone formation than other bone substitutes. However, the review suffered several limitations including the different re-entry times for implant placement and different particulated ATG preparation methods. Nevertheless, the review concluded that ATGs are highly effective for post-extraction bone preservation, showing less resorption compared to xenografts, alloplasts, and allografts. They also exhibited high biocompatibility, as well as osteoinductive and osteoinductive properties. Nevertheless, the authors stressed the need for more homogeneous and longer-term comparative studies of ATG.

In this context, the present prospective clinical study set out to apply a strict protocol regarding reentry times and preparation methods, performing all ARP procedures consecutively and subjecting the outcomes to histomorphometric analysis. The primary objective was to evaluate percentages of new formed bone, residual graft, and intertrabecular connective tissue resulting from ARP procedures using ATG. Secondary objectives were to analyze potential differences in outcomes in relation to sex, age, and location, as well as any associated complications.

2. Material and methods

2.1. Study design and approval

The present study was designed as a single-cohort clinical study and included a total of 36 consecutive ARP procedures performed with ATG from simultaneously extracted teeth. The study was conducted at the Postgraduate Oral Surgery Clinic, Faculty of Dentistry, Complutense University of Madrid, Spain, between September 2022 and September 2023. All patients were provided with full information about the purpose of the study and the procedures involved and gave their informed consent to take part.

The study was conducted following STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Cuschieri, 2019). All procedures involving human participants fulfilled ethical standards established by institutional and/or national research committees in accordance with the 1964 Helsinki declaration and subsequent amendments. The study protocol was assessed and approved by the Research Ethics Committee at the San Carlos Hospital of Madrid, Spain in July 2022 (Registration Code Nº 22/464-EC_X).

2.2. Participants

Selection criteria are listed below.

Inclusion criteria

- Aged $\geq$ 21 years.
- Anterior or posterior teeth with a diagnosis of at least one non-restorable tooth.
- No active periodontal disease.
- No relevant systemic diseases (American Society of Anesthesiologists classification ASA I or ASA II).
- Able to understand and carry out instructions given by the researchers.

Exclusion criteria

- Refusal to participate in the study after explanation.
- Inability to attend follow-up visits 48 h and one week after the procedures.
- Smoking $\geq$ 10 cigarettes/day.
- Being immunosuppressed or having systemic diseases related to poorer tissue healing, such as type I and II diabetes, or hemostasis disorders.
- Undergoing treatment with antibiotics, anticoagulants, and/or anti-inflammatory drugs within 4 days prior to the procedure.
- Need for antibiotic prophylaxis.
- Pregnancy or breastfeeding.

2.3. Intervention

All maxillary and mandibular extractions were performed by the same surgeon (T.B.C). Extractions were performed atraumatically to preserve as much alveolar bone as possible, in order to improve the chances of successful ATG placement.

The reasons for the tooth extractions were as follows:

- Teeth with impossible periodontal prognosis to be maintained, due to loss of bone support.
- Tooth fractures that compromised the structural viability of the tooth.
- Leaking crowns that affected the integrity of the tooth and did not allow a functional restoration.
- Severe endo-periodontal lesions, affecting both pulp and periodontal support tissues.

Local anesthesia with 4 % articaine and epinephrine 1:100,000 (Ultracaín, Normon SL, Madrid, Spain) was used. After specific nerve blocking, an atraumatic extraction was performed. The extracted teeth were prepared for use by removing calculus, filling debris with a diamond turbine bur (Dentsply Maillefer, Ballaigues, Switzerland), and polishing root surfaces with diamond turbine burs with abundant irrigation in order to remove the periodontal ligament; in case of any endodontic filling, this was removed using Gates Glidden burs (Dentsply Sirona Inc, Delaware, USA). The tooth was cut into fragments $\leq$ 5 mm and placed dry inside the Tooth Transformer device grinder (S.R.L, Milan, Italy) following the manufacturer's instructions (Fig. 1). Different liquids were then added to the container to demineralize the dentin, hydrochloric acid (0.1 M), releasing BMP-2 and collagen type 1, and to eliminate any residual toxicity, hydrogen peroxide (H2O2 10 %). Four different phases of rinsing with demineralized water and mineralized water were used to neutralize acid residues (Inchingolo et al., 2023). The device was activated to grind the fragments down to the adequate particle size, which was checked using the sieve attached to the collecting container. In this way, the ATG was prepared in under 25 min. During this preparation time, the granulomatous tissue of the alveolus was removed.

Once the graft material was prepared, it was placed in the alveoli with a periosteal elevator, filling all the space left by the extraction, covered with a collagen sponge and closed with Supramid 4/0 suture (Aragó, Barcelona, Spain) (Fig. 2).

The patient was instructed in the postoperative measures to be

Fig. 1. Tooth preparation. (A): tooth extracted with root fracture; (B): tooth cleaned by a diamond turbine bur; (C): tooth cut into fragments; (D-E): Tooth Transformer device; (F): prepared ATG material.

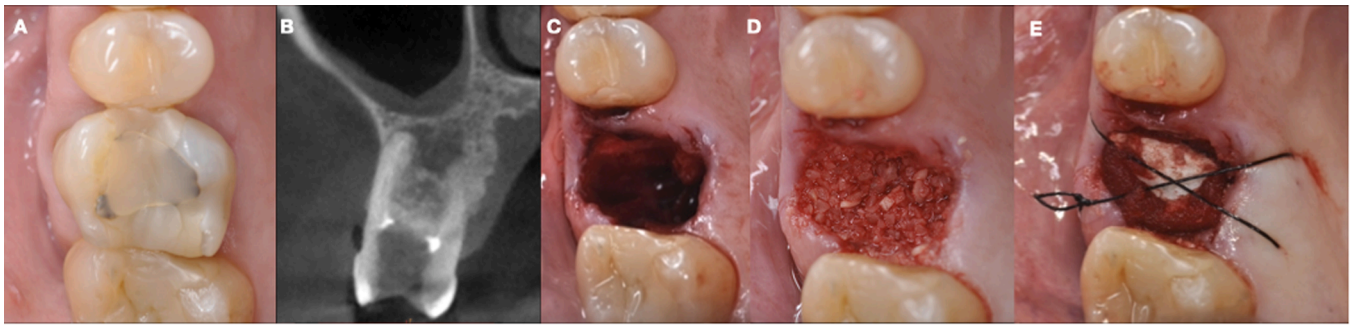

Fig. 2. ARP procedure. (A): pre-operative situation; (B): pre-operative CBCT; (C): alveolus without interradicular septum; (D): ATG placed in the alveolus filling it completely; (E): collagen sponge and suture.

followed (no rinsing or spitting for 24 h, soft, cold diet, and local cold application) and prescribed the following medication: 600 mg ibuprofen every 8 h for 3 days, and 650 mg paracetamol every 8 h as rescue analgesic if pain occurred 4 h after taking ibuprofen. Antibiotics were administered in case of infection, consisting of 750 mg amoxicillin, three per day for 5 days.

Sutures were removed one week after the procedure. Each patient was evaluated one month and 4 months after the ARP procedure, when CBCTs were performed to quantify bone width and height.

Five months after ARP, re-entry for implant placement was performed, and a bone biopsy was harvested. A bone level Naturactis Euroteknika implant (Lyra Etk, Sallanches, France) was placed in the tooth/graft-regenerated area (Fig. 3), keeping it submerged for three months, when second-stage surgery was performed to restore the patient with an implant-supported fixed prosthesis.

Following the biopsy, histopathological and histomorphometric analysis followed a protocol similar to that used by other authors (Zellner et al., 2023). Bone samples obtained with a trephine were fixed in 10 % buffered formaldehyde for at least 48 h and then carefully decalcified with Histofix decalcifier 3 (PanReac AppliChem ITW Reagents, Monza, Italy).

Subsequently, the samples were gradually dehydrated in 96 % and 100 % ethanol, followed by immersion in xylene. Eight representative 5 µm sections were obtained from each case, mounted on slides, and stained using the Hematoxylin & Eosin technique (Sigma Aldrich, USA). The slides were mounted with permanent mounting medium Eukitt (PanReac AppliChem ITW Reagents, Monza, Italy).

The slides were evaluated using an Olympus BX51 microscope (Tokyo, Japan). Firstly, a descriptive report of the histological characteristics of all bone biopsies was produced. The Cell^A software package (Olympus) with an Olympus DP20 digital camera was used to perform histomorphometric analysis. Three representative areas of interest (AOI) were defined for each case, including the central part and the length of the bone samples at 20× magnification.

Histomorphometric evaluation in the three AOIs assessed the presence of vital bone, graft material, and connective tissue. Vital bone was identified by the presence of osteocytes in osteoid lacunae; the graft material was identified as basophilic fragments of acellular foreign material with a tubular dentin morphology; and the remainder consisted of connective tissue (blood vessels, fibrous tissue). Using the software's point-counting system, the number of pixels corresponding to the three tissue types in each area was quantified and divided by the total number of pixels for that area. In addition, the number of osteocompetent bone cells—osteocytes, osteoclasts, and osteoblasts—was counted in each AOI. Finally, the average percentage of each tissue type (vital bone, graft material, and connective tissue) across the three AOIs was calculated to obtain the final percentage for each tissue type in the bone sample, as well as the total number of bone cells (osteoclasts, osteocytes, and osteoblasts) (Fig. 4).

2.4. Data collection

The following data were recorded:

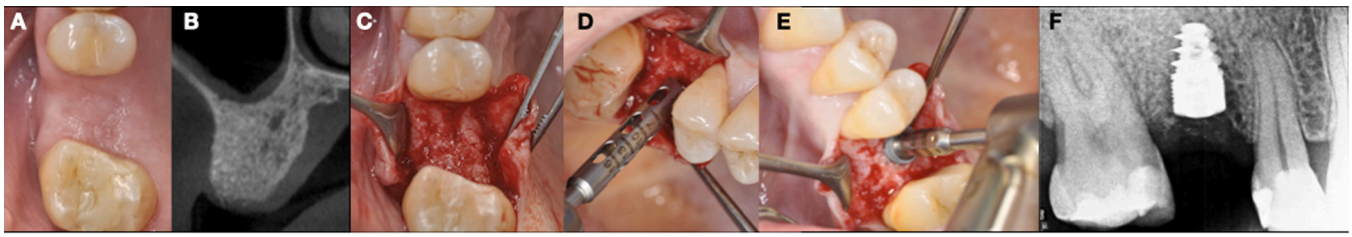

Fig. 3. Implant placement. (A): clinical aspect 5 months after ARP; (B): postoperative CBCT at 5 months; (C): bone aspect at re-entry; (D): trephine bur to harvest bone biopsy; (E): implant placement; (F): periapical x-ray to confirm correct implant position.

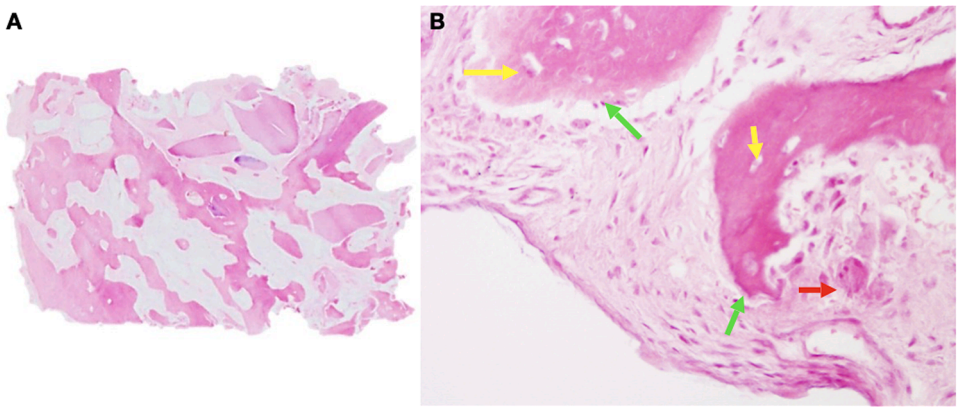

Fig. 4. Histomorphometric slide. (A): complete histological section. No modifications were made to the image after capture; (B): histological section with the presence of osteocytes (yellow arrows), osteoblasts (green arrows) and osteoclasts (red arrow).

a) Pre-operative variables

- Patient characteristics (demographic data and medical history): age (≤ 50, 51–60, ≥61), sex, general health conditions, pharmacological treatments, and tobacco and alcohol consumption.
- Tooth characteristics: reason for extraction (pain, caries, periodontal status), tooth type (incisor, canine, bicuspid or molar) and location (maxillary or mandibular).

b) Intra-operative variables

- Tooth preparation: type of tissue removed.
- Complications.
- Insertion torque.

c) Post-operative variables

- Histomorphometric data: 5 months post-operatively, a bone biopsy was harvested in order to quantify new bone formation, percentages of residual biomaterial and connective tissue.
- Complications after ARP procedures with ATG.

2.5. Calibration

Before conducting the study, intra-examiner reproducibility (of the anatomical pathologist) was established, calibrating the main variable (new bone formation) with 10 histomorphometric analyses conducted in previous studies. As this was a quantitative variable, the intraclass correlation coefficient (ICC) was calculated: 0.994 (CI 95 %: 0.985–0.998) indicating excellent agreement.

2.6. Statistical analysis

Statistical analysis was conducted at the Data Processing Center of the Complutense University of Madrid by an independent statistician. Data were analyzed with SPSS* Statistics 29.0 software (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY: IBM Corp). Firstly, a descriptive study of frequencies was made, calculating means, median values, standard deviations, and ranges. Secondly, data were analyzed with inferential statistics with a 95 % Confidence Interval, and so a significance level of $p < 0.05$.

Applying the Shapiro Wilk test (less than 50 samples), it was found that data did not display normal distribution, and so non-parametric tests were used: the Mann Whitney test for independent samples (two comparative groups) and the Kruskal-Wallis test (more than two comparative groups). Whenever the Kruskal-Wallis test indicated significant differences, paired comparisons were made with Bonferroni corrections.

3. Results

3.1. Patients and teeth characteristics

A total of 27 patients were included in this study, 16 females (59.26 %) and 11 males (40.74 %), with ages ranging from 26 to 76 years old (mean 54.5 ± 12.78; Median 57 and interquartile ranges p25–75: 46–68). The total sample consisted of 36 teeth: 1 incisor (2.77 %), 13 bicuspids (36.11 %), and 22 molars (61.11 %). Patient characteristics, teeth locations, reasons for extraction, insertion torques, and histomorphometric data are presented in Table 1.

Data about tooth cleaning and the tissues removed are presented in Table 2. The crown and the root of each tooth were used, either the whole tooth or a part (cutting the tooth to avoid and eliminate fillings, infected tissue, or prosthetic elements).

3.2. Percentage of new vital bone

The percentage of new vital bone was 29.14 ± 10.86 % (CI 95 %: 25.46 %-32.81 %; Median 28.35 % and interquartile ranges p25–75: 21.30 %-35.85 %). There were no statistically significant differences in new vital bone percentages in relation to sex (p = 0.844), as is shown in Fig. 5.

No statistically significant differences were found between maxilla and mandible in relation to new vital bone formation (p = 0.616) (Table 3) (Fig. 6).

Nor were any statistically significant differences observed between different age groups, ≤ 50, 51–60, ≥ 61 in relation to new vital bone formation (p = 0.588) (Table 4) (Fig. 7).

Lastly, no statistically significant differences between locations (molar, bicuspid or incisors) were found in relation to percentages of new vital bone (p = 0.278).

3.3. Percentage of residual tooth-graft

Residual tooth-graft percentage was 10.84 ± 6.82 % (CI 95 %: 8.53 %-13.14 %; Median 9.98 % and interquartile ranges p25–75: 5.61 %-14.25 %). No statistically significant differences were observed in residual graft material between the sexes (p = 0.895).

There were no statistically significant differences between maxilla and mandible in relation to percentages of residual tooth-graft (p = 0.942).

Nor were statistically significant differences found between age groups, ≤ 50, 51–60, ≥ 61 in relation to percentages of residual tooth-graft (p = 0.687).

Table 1
Patient characteristics and histomorphometric data.

Patient	Age	Gender	Location	Extraction Reason	Torque (Ncm)	%Vital bone	% Residual tooth-graft	% Intratrabecular connective tissue
1	76	Female	16	Periodontal	35	61.25 %	9.49 %	29.26 %
1	76	Female	25	Periodontal	40	29.11 %	11.03 %	59.86 %
2	55	Male	47	Fracture/Decay	45	28.10 %	26.98 %	44.92 %
3	69	Male	45	Endo-Perio	30	36.54 %	2.97 %	60.49 %
4	69	Female	17	Fracture/Decay	30	18,13 %	0 %	81.87 %
5	47	Female	16	Endo-Perio	40	50.85 %	12.11 %	32.35 %
6	61	Female	15	Fracture	35	19.85 %	14.50 %	65.65 %
7	42	Male	36	Periodontal	35	29.28 %	15.47 %	55.35 %
8	76	Female	41	Periodontal	30	27.52 %	13.51 %	58.97 %
9	61	Female	15	Periodontal	40	9.07 %	11.78 %	75.25 %
10	57	Female	35	Periodontal	35	17.05 %	25.37 %	57.58 %
11	68	Female	24	Fracture	35	24.07 %	17.48 %	58.45 %
12	71	Female	26	Periodontal	40	35.73 %	17.74 %	46.53 %
12	71	Female	27	Periodontal	45	12.98 %	27.33 %	59.69 %
13	47	Male	14	Fracture/Decay	35	20.31 %	9.87 %	69.82 %
13	47	Male	35	Fracture/Decay	45	21.52 %	10.02 %	68.46 %
14	64	Female	16	Fracture	40	38.69 %	8.21 %	53.10 %
15	50	Male	27	Fracture	40	28.59 %	18.64 %	52.77 %
16	41	Female	16	Endo-Perio/ Decapitated crown	35	22.57 %	9.93 %	67.50 %
16	41	Female	25	Endo-Perio	40	20.88 %	9.07 %	70.05 %
17	53	Female	16	Endo-Perio	35	31.26 %	5.93 %	62.81 %
17	53	Female	26	Endo-Perio/ Decapitated crown	40	37.23 %	0 %	62.77 %
17	53	Female	27	Endo-Perio	35	26.68 %	4.29 %	69.03 %
17	53	Female	37	Endo-Perio	45	33.27 %	6.44 %	60.29 %
18	58	Male	34	Endo-Perio	30	35.95 %	2.08 %	61.97 %
18	58	Male	35	Filtered crown	40	43.88 %	3.54 %	52.58 %
19	44	Male	26	Endo-Perio	25	21.69 %	9.64 %	68.67 %
20	67	Male	37	Fracture	40	40.32 %	5.29 %	54.39 %
21	73	Female	36	Periodontal	45	13.26 %	14.39 %	72.35 %
22	37	Male	46	Endo-Perio/ Decapitated crown	45	21.07 %	12.39 %	66.54 %
23	59	Female	46	Fracture/Endo Perio	40	43.9 %	4.33 %	51.77 %
24	26	Female	26	Endo-Perio	35	35.29 %	4.06 %	60.65 %
25	39	Male	36	Endo-Perio	40	30.20 %	12.99 %	56.81 %
25	39	Male	37	Lower third molar donor	35	31.65 %	8.28 %	60.07 %
26	46	Male	24	Fracture	35	25.22 %	10.85 %	65.93 %
27	54	Female	15	Periodontal	35	25.99 %	14.11 %	59.90 %

Lastly, no statistically significant differences in percentages of residual tooth-graft were found between locations (molar, bicuspid or incisor) ($p = 0.919$).

3.4. Percentage of connective tissue

The percentage of connective tissue was 59.87 ± 10.56 % (CI 95 %: 56.30–63.25 %; Median 60.18 % and interquartile ranges p25–75: 54.87 %-67.08 %). There were no statistically significant differences between the sexes in relation to percentages of connective tissue ($p = 0.987$).

No statistically significant differences were found between maxilla and mandible in relation to percentages of connective tissue ($p = 0.625$).

Nor were statistically significant differences identified between different age groups, ≤ 50, 51–60, ≥ 61 in relation to percentages of connective tissue ($p = 0.828$).

Lastly, no statistically significant differences were found between locations (molar, bicuspid or incisors) in relation to percentages of connective tissue ($p = 0.284$).

3.5. Complications

No complications occurred after ARP procedures; no infections or healing complications were observed. After implant placement, one patient (patient number 4) presented a lack of implant osseointegration, which was removed and replaced after an additional 3-month healing period.

3.6. Insertion torque

Insertion torque ranged from 20 to 45 Ncm in all patients, as shown in Table 1.

4. Discussion

Alveolar ridge preservation (ARP) procedures aim to maximize new bone formation inside the alveoli and may be employ a range of different graft materials (autografts, xenografts, allografts, synthetic grafts). However, the varying chemical compositions of these materials, as well as the considerable differences observed in new bone formation, suggest a need to introduce new materials into this type of procedure.

In the present prospective study, 36 alveoli were preserved with autologous tooth graft (ATG), analyzing the outcomes. The percentages of new vital bone ranged from 9.07 % to 61.25 % (mean 29.14 % ± 10.86) after 5 months healing. These results are slightly higher than those obtained with xenografts in previously published studies. For example, Gholami et al., (2012) reported 18.76 % of newly-formed bone after 6 months, and Nart et al., (2017) documented 26.10 % after 4 months.

Comparing other bone substitutes, De Tullio et al., (2019) obtained mean percentages of vital bone after five months of ARP of 13.56 % ± 13.08 % with calcium sulfate, 17.84 % ± 7.32 % with sintered nano-hydroxyapatite, 58.72 % ± 8.77 % with a combination of both, and an even higher percentage of 80.68 % ± 21.8 % in the control group (blood clot) after six months. Avila-Ortiz et al., (2014) and Canullo et al., (2022) in their systematic reviews have also suggested that spontaneous socket healing without bone substitutes provides the worst outcomes in terms of bone dimension preservation, being associated with more bone resorption. Couso-Queiruga et al., (2021) in their systematic review and

Table 2
Cleaning method and tissue removal.

Patient	Age	Gender	Location	Endodontic treatment	Crown	Root	Cleaning method
1	76	Female	16	NO	Whole	Whole	Diamond burr
1	76	Female	25	NO	Whole	Whole	Diamond burr
2	55	Male	47	YES	Partial (cut)	Whole	Diamond burr
3	69	Male	45	NO	Whole	Whole	Diamond burr
4	69	Female	17	NO	Partial	Whole	Diamond burr
5	47	Female	16	NO	No	Partial	Diamond burr
6	61	Female	15	NO	No	Whole	Diamond burr
7	42	Male	36	YES	Partial	Partial	Diamond burr
8	76	Female	41	NO	Whole	Whole	Diamond burr
9	61	Female	15	NO	Whole	Whole	Diamond burr
10	57	Female	35	NO	Whole	Whole	Diamond burr
11	68	Female	24	YES	Partial	Whole	Diamond burr
12	71	Female	26	NO	Partial (cut)	Whole	Diamond burr
12	71	Female	27	NO	Partial (cut)	Whole	Diamond burr
13	47	Male	14	YES	Partial	Whole	Diamond burr
13	47	Male	35	NO	Partial	Whole	Diamond burr
14	64	Female	16	YES	Partial	Whole	Diamond burr
15	50	Male	27	NO	Partial	Whole	Diamond burr
16	41	Female	16	YES	Whole (upper third molar)	Whole (upper third molar)	Diamond burr
16	41	Female	25	YES	Whole (upper third molar)	Whole (upper third molar)	Diamond burr
17	53	Female	16	YES	No	Partial	Diamond burr
17	53	Female	26	YES	No	Partial	Diamond burr
17	53	Female	27	YES	No	Partial	Diamond burr
17	53	Female	37	YES	Partial (cut)	Whole	Diamond burr
18	58	Male	34	NO	No	Whole	Diamond burr
18	58	Male	35	NO	No	Whole	Diamond burr
19	44	Male	26	YES	No	Whole	Diamond burr
20	67	Male	37	YES	Partial (cut)	Whole	Diamond burr
21	73	Female	36	NO	Partial (cut)	Whole	Diamond burr
22	37	Male	46	YES	Partial (cut)	Whole	Diamond burr
23	59	Female	46	NO	Whole	Whole	Diamond burr
24	26	Female	26	YES	Whole (upper third molar)	Whole (upper third molar)	Diamond burr
25	39	Male	36	YES	Partial	Whole	Diamond burr
25	39	Male	38	NO	Whole	Whole	Diamond burr
26	46	Male	24	NO	Whole	Whole	Diamond burr
27	54	Female	15	NO	Partial	Whole	Diamond burr

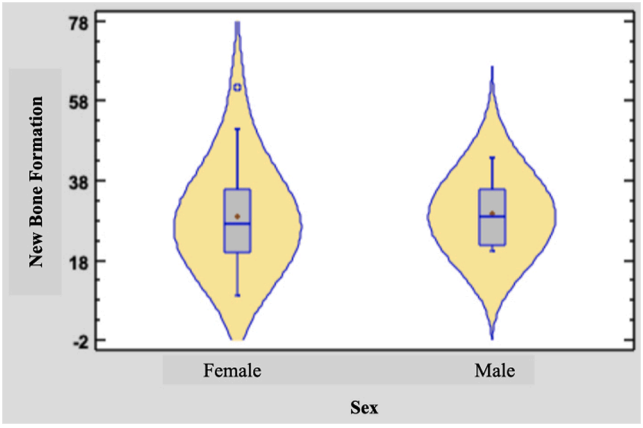

Fig. 5. Violin plot representing the differences in new bone formation between males and females.

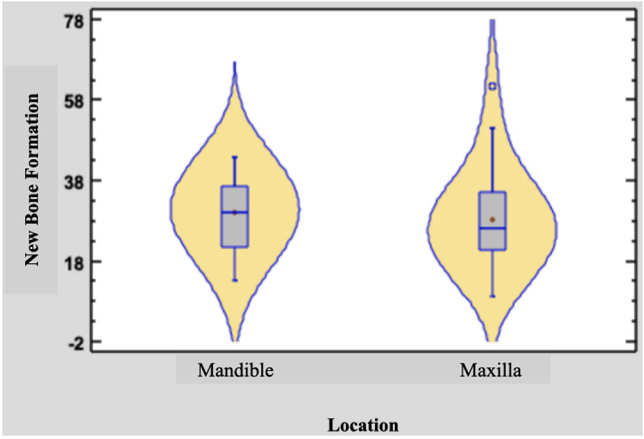

Fig. 6. Violin plot representing the differences in new bone formation between location (maxilla vs mandible).

Table 3
Assessment of the variable location. Note there is nor significant differences between maxilla and mandible in any of the observed tissues.

Histomorphometric data location	Maxilla	Mandible	p-value
New Bone (%)	29.14 ± 10.86	28.12 ± 11.23	0.616
Residual Graft (%)	10.84 ± 6.82	10.75 ± 7.32	0.942
Connective Tissue (%)	59.87 ± 10.56	59.74 ± 10.64	0.625

meta-analysis, have also analyzed the dimensional changes of the alveolar ridge after unassisted alveolar healing, highlighting that spontaneous healing with a blood clot formation leads to significant bone resorption, suggesting that the blood clot alone may not provide adequate structural support or biochemical signals necessary to prevent bone resorption.

ATGs supply a matrix rich in minerals and bioactive proteins (such as BMP-2), which promote osteoinduction and enhance bone regeneration by providing a more favorable and structured environment for bone growth. Kim et al., (2010) demonstrated that the use of ATG provides effective bone regeneration with almost complete replacement of dentin

Table 4
Assessment of the variable age. No significant differences between the different age ranges in any of the tissues.

Histomorphometric data age group	≤ 50	51–60	≥ 61	p-value
New Bone (%)	31.05 ± 9.88	30.92 ± 10.75	27.12 ± 11.62	0.588
Residual Graft (%)	10.75 ± 6.91	10.74 ± 7.15	10.91 ± 7.02	0.687
Connective Tissue (%)	58.45 ± 11.20	59.23 ± 10.58	60.02 ± 10.34	0.828

to bone tissue within 5–10 months of graft maturation.

In a study similar to our own, Elfana et al., (2021) used ATG in ARP procedures, using whole or demineralized ATG to compare histomorphometric differences between these two options, obtaining a higher percentage of newly-formed bone in the demineralized group compared with the whole ATG group after six months maturation (48 % vs 37 %). The authors concluded that the dentin demineralization processes increased the bioavailability of BMP-2, which is directly involved in the osteoinduction process (Tanoue et al., 2018). Likewise, in the present study, the Tooth Transformer device was employed to demineralize ATG for the ARP procedure.

A recent systematic review by Sánchez Labrador et al. (2023) also observed high bone formation with ATG after maturation periods of 4–6 months, with percentages ranging between 20 % and 50 %. De Risi et al., (2015) found that allogeneic grafts achieved 54.4 % new bone formation at 3 months, and that xenografts obtained the lowest value at 5 months (23.6 %). Additionally, allografts presented the least amount of residual material (12.4 %-21.11 %), while xenografts and alloplastics showed over 35 % at 7 months.

Our findings are consistent with other published studies, suggesting that ATG may offer a competitive alternative to other graft materials, obtaining substantial bone formation and minimal residual material at re-entry.

In this regard, Minetti et al., (2022), using the same tooth processing device as the present study, obtained 37.9 % of vital bone and 7.7 % of residual tooth graft in maxilla and 38 % and 7 %, respectively, in mandible. These percentages are slightly different from those observed in the present investigation (29.14 % of vital bone and 10.84 % of residual dental graft in the maxilla and 28.12 % and 10.75 % in the mandible). These differences could be attributed to the wide re-entry time interval adopted in the study by Minetti et al. (3–12 months); in contrast, in the present investigation all biopsies were performed at 5 months. In any case, both studies confirm the biocompatibility and the osteoconductive and osteoinductive properties of ATG, which favors new bone formation without major complications.

Another factor to consider is the fact that the choice of graft material will also influence resorption of the alveolar process. A systematic review by Majzoub et al., (2019) showed that spontaneous healing (blood clot) of the alveolus leads to greater bone loss compared with the use of a bone substitute. Horizontal resorption of the alveolar ridge was found to be higher with blood clot formation (3.1 mm) compared with allografts (1.52 mm), xenografts (1.47 mm) and alloplasts (2.31 mm). Solyom et al., (2023) and Feng et al., (2023) in their respective systematic reviews found less width resorption when ATG was used compared with deproteinized bovine bone mineral (DBBM), beta-tricalcium phosphate (β-TCP), or blood clot formation.

In the present investigation, no significant differences in outcomes were observed in relation to sex, age or location of extracted teeth, so that the percentages of new vital bone in the different groups were similar. This concurs with other studies using different bone graft materials, whereby age does not seem to be a determinant of bone healing outcomes (Papageorgiou et al., 2016; Čandrlić et al., 2022).

ATG has a chemical structure similar to human bone (Ling et al., 2024; Khurshid et al., 2024), in both its inorganic and organic components, and its water content. Dentin is composed of approximately 70 % minerals, 20 % organic matrix, and 10 % water, while bone consists of 65 % minerals, 25 % organic matrix, and 10 % water. The inorganic component includes four types of calcium phosphates: hydroxyapatite, tricalcium phosphate, octacalcium phosphate, and amorphous calcium phosphate, which give ATG its osteoconductive properties by functioning as a scaffold (Kabir et al., 2017). The organic content is mainly composed of type I collagen, with smaller proportions of types III, V, and XII, providing elasticity and fracture resistance (Sieverts et al., 2022; Grawish et al., 2022). The rest includes non-collagenous proteins such as osteopontin, dentin sialoprotein and bone sialoprotein, osteocalcin, dentin matrix protein-1, bone morphogenetic protein type 2 (BMP-2), insulin-like growth factor (IGF), and transforming growth factor β (TGF-β), which are crucial to osteoinduction.

Likewise, ATG is an autogenous material, with all the advantages this entails. On the one hand it presents a cost-effective alternative to biomaterials, and on the other hand it enjoys greater acceptance by some patients, who for various ethnic and cultural reasons might reject certain biomaterials of animal origin (Bucchi et al., 2019). Finally, unlike autologous bone, a donor site is not required, so reducing morbidity,

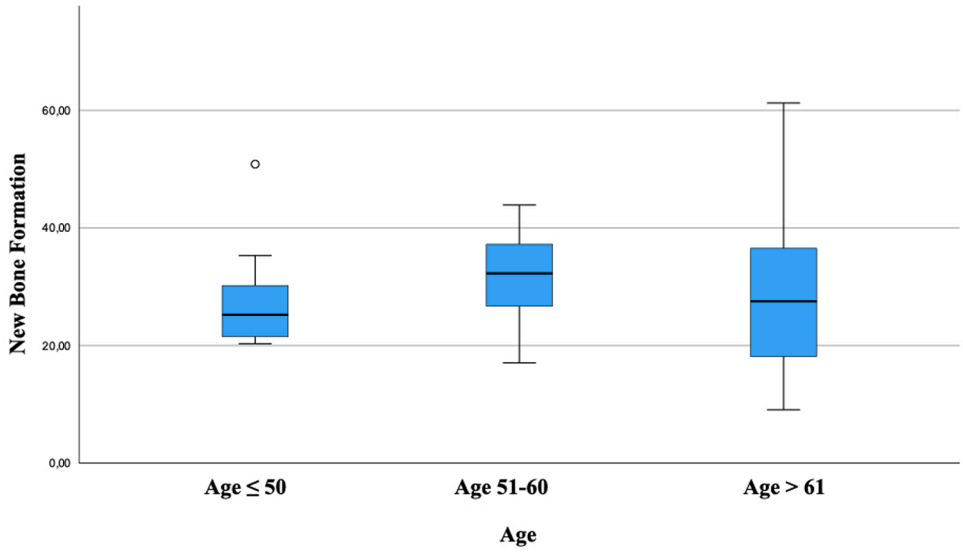

Fig. 7. Boxplot representing the differences in new bone formation between different age groups.

pain and other associated risks (Migliorini et al., 2021).

ATG does present drawbacks such as its limited availability, although it would appear to behave well if it becomes necessary to combine it with other bone substitutes (Umebayashi et al., 2020). Moreover, a dental extraction must be carried out to obtain the ATG (Kim et al., 2010).

No complications were associated with the ATGs performed in the present study, although the scientific evidence have just reported as complications graft resorption and occasional inflammatory reactions (Solyom et al., 2023). In contrast, when ARP procedures with other biomaterials is performed, some complications can occur, including delayed healing with partial exposure of the graft material, post-operative pain, swelling, and implant failure (Avila-Ortiz et al., 2014).

In addition, a high implant survival rate is obtained in this prospective clinical study (97.22 %), which is similar than the data obtained by Minetti et al. (2021), with 98.2 % after one-year follow-up.

The present study suffers from some limitations, derived mainly from the absence of a control group to compare the results, with other commonly used graft materials or spontaneous healing. This could lead to possible biases in the interpretation of efficacy. In addition, the relatively small sample size and lack of greater consistency in the locations of tooth extractions, although sufficient to provide an initial assessment of the potential of ATG in ARP procedures, limits extrapolation of the results. Furthermore, histomorphometric study was limited to basic metrics and more advanced approaches, such as immunohistochemical or molecular analyses, could provide a deeper understanding of the biological activity and integration of the ATG. Also, the lack of long-term follow-up data on placed implants limits the ability to assess marginal bone loss over time. Further prospective studies with larger sample sizes, control groups, and with long-term follow-up are needed to better interpretate the results obtained in this clinical study.

Among its strengths are the fact that all ATGs were prepared with the same device and that each biopsy was performed at the same time in all cases (5 months).

5. Conclusions

Within the limitations of the present study, according to the histomorphometric data obtained, it may be affirmed that ATG appears a good bone substitute for ARP procedures, achieving percentages of new vital bone at 5 months comparable to those obtained in other similar studies with other biomaterials. The procedure allowed implant placement with adequate insertion torque and no major complications. Further comparative studies with longer follow-up periods are needed to understand the clinical behavior of ATG in different clinical scenarios.

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Ethical statement

The study protocol was assessed and approved by the Research Ethics Committee at the San Carlos Hospital of Madrid, Spain in July 2022 (Registration Code Nº 22/464-EC_X).

CRediT authorship contribution statement

Luis Sánchez-Labrador: Writing – original draft, Visualization, Investigation, Data curation. **Leticia Alejandra Blanco-antona:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jorge Cortés-Bretón Brinkmann:** Writing – review & editing, Formal analysis. **Tomás Beca-Campoy:** Writing – original draft, Visualization, Investigation, Data curation. **José María Martínez-González:** Writing – review & editing, Supervision, Resources, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Informed consent

Each of the patients signed an informed consent form for this study.

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