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Bone Augmentation of Atrophic Alveolar Ridges Using a Synthetic Bone Substitute With Mesenchymal Stem Cells: A Randomized, Controlled Clinical Trial

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Keywords: alveolar ridge augmentation | bone regeneration | cell therapy | mesenchymal cells dental implants

ABSTRACT

Objectives: To assess the efficacy and safety of a cell-based therapy for 3D bone augmentation of severe alveolar bone defects prior to dental implant placement.

Materials and Methods: A Phase 2 randomized controlled clinical trial evaluated the safety and efficacy of a cell therapy using expanded autologous iliac crest-derived mesenchymal cells seeded on a synthetic bioabsorbable bone substitute covered with a non-resorbable membrane. The control group received an autogenous bone block graft. After 5 months, CBCT scans were compared to measure the bone volume changes achieved after the regenerative surgery. Subsequently, dental implants were placed in the regenerated areas.

Results: A total of 48 patients were included and randomized (36 patients in the test group and 12 in the control group). However, seven patients did not reach the minimum required number of expanded MSCs and were therefore unable to be treated. The tested intervention demonstrated significantly greater gains in bone volume, with a mean difference of 480.01 mm^3 ($p = 0.032$). Similarly, the mean change in bone crest volume from baseline to 5 months was notably higher in the test group (1066.91 mm^3)

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compared to the control group (586.9 mm^3). Adverse reactions and patient morbidity were minor in both groups. Implants were placed on the regenerated bone, and all were integrated successfully in both groups.
Conclusions: The cell-based therapy resulted in significant changes in bone volume compared to the control treatment, enabling dental implants in all patients. The procedure was associated with minimal adverse effects and patient morbidity.
Trial Registration: ClinicalTrials.gov: NCT03373052, NCT04297813

1 | Introduction

Reconstruction of oral and maxillofacial bone defects, mainly when segmental defects involve the inferior mandibular border, requires three-dimensional (3D) ridge augmentation, which remains a significant clinical challenge in implant dentistry (Tay et al. 2022). These regenerative interventions are technically demanding and biologically complex as they require angiogenesis, cell proliferation, and osteogenesis at a specific distance from the existing bone (Urban et al. 2023). Recent systematic reviews evaluating the effectiveness of regenerative interventions for 3D bone augmentation prior to dental implant placement indicate that these interventions can lead to successful outcomes. However, the evidence mainly comes from case series and a limited number of randomized clinical trials (Menasche et al. 2024; Robert et al. 2023; Urban et al. 2023, 2019; Zhang et al. 2022).

On-lay grafting with autologous block grafts has long been regarded as the "gold standard" for treating severe jaw atrophies, and it continues to be one of the most traditional approaches (Chiapasco et al. 2009). Nevertheless, its application is associated with significant clinical challenges, which include increased patient morbidity related to graft harvesting and a high incidence of intra- and post-operative complications, such as wound dehiscence and neurosensory disorders. Another challenge is the rapid resorptive pattern associated with autogenous bone grafts, which reduces the long-term volume gain of bone augmentation (Robert et al. 2023). These limitations have led to a preference for less invasive approaches, such as guided bone regeneration (GBR), which combines bone replacement grafts and barrier membranes of various origins (Hammerle and Jung 2003). Currently, the use of xenogeneic bone substitutes along with barrier membranes is the most employed intervention for staged bone augmentation of the jaws (Sanz-Sanchez et al. 2015). However, xenogeneic bone replacement grafts, such as deproteinized bovine bone mineral (DBBM), may impede the amount and quality of newly formed bone due to their slow resorption rates and inability to maintain the necessary space for effective 3D bone augmentation (Sanz et al. 2019).

To address these limitations, modified GBR techniques incorporating various combinations of autologous bone, bone substitutes, resorbable and non-resorbable membranes, commercially available bioactive products, and additional space-maintaining devices (such as titanium-reinforced non-resorbable membranes or resorbable membranes combined with space-maintaining devices like tenting screws or osteosynthesis plates) have been extensively studied over the last three decades. These approaches have shown considerable success in enhancing the dimensions of the alveolar ridge, thereby facilitating the placement of dental implants in ideal prosthetic positions (Urban et al. 2023). Despite their potential, these modified surgical procedures have not become standard practice except among highly experienced clinicians, as achieving favorable outcomes requires intricate flap designs to advance soft tissues while ensuring tension-free closure and optimal postoperative care for submerged healing (Urban et al. 2016).

Alternatively, tissue engineering strategies that combine cell-based approaches, utilizing mesenchymal stem cells (MSCs) seeded in bone replacement graft scaffolds and covered with barrier membranes, have emerged as a less invasive option for bone regenerative therapies (Padial-Molina et al. 2015). MSCs possess a unique ability to replicate in an undifferentiated state and differentiate along the osteogenic lineage, making them promising candidates for bone regeneration. In addition to their osteogenic potential, MSCs provide paracrine, trophic, and immunomodulatory effects that enhance regenerative therapies (Peng et al. 2024).

Recent systematic reviews examining the use of MSCs in oral and craniofacial applications have demonstrated their potential for regenerating both intraoral and extraoral bone defects, although most published studies are case reports or small case series (Ivanovski et al. 2024; Shanbhag et al. 2019). The first human case series utilizing bone marrow-derived mesenchymal stem cells (BMMSCs) for maxillary sinus (Kaigler et al. 2013) and alveolar ridge augmentation (Gjerde et al. 2018; Kaigler et al. 2015, 2013) demonstrated safe and effective bone formation after a one-year follow-up. However, the effectiveness of MSC-based therapies for orofacial bone regeneration remains uncertain, as only three phase 1 clinical trials have been published in the past 5 years (Cubuk et al. 2023; Fatale et al. 2022; Tanikawa et al. 2020). These trials reported bone formation without adverse effects, although in relatively small patient populations. Furthermore, these studies lacked full clarity regarding the source, dosage, and characterization of the MSCs used. In summary, although cell therapies combined with bone grafts and membranes have shown promise in terms of safety and bone regeneration, the evidence supporting any additional effects beyond those of the scaffold and membrane remains limited.

Therefore, this Phase 2 randomized controlled clinical trial (RCT) aimed to evaluate the safety and efficacy of a cell therapy using expanded autologous iliac crest-derived MSCs seeded on a synthetic bioabsorbable bone substitute and covered with a non-resorbable membrane for 3D bone augmentation prior to dental implant placement, in comparison to a control group receiving the established standard of care procedure that involves autogenous bone blocks.

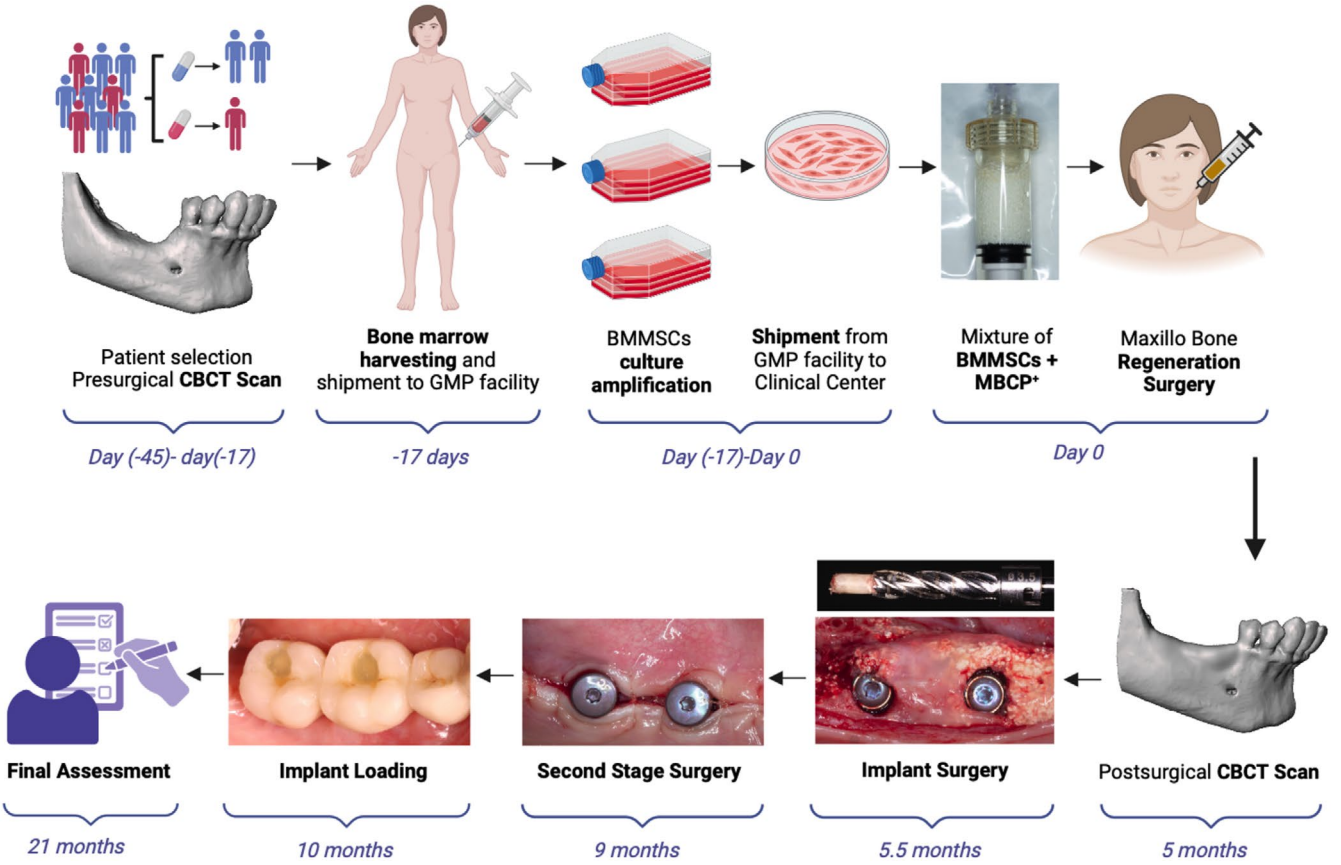

FIGURE 1 | The experimental design of the RCT in the cell-based therapy group (tested intervention), which includes CBCT analysis for patient and site selection, bone marrow harvesting, cell isolation, expansion and mixture with the bone substitute, regenerative surgery, post-surgical CBCT, implant placement, and follow-up.

2 | Material and Methods

2.1 | Study Design and Ethical Considerations

This study was designed as a randomized, prospective, two-arm, multi-center clinical trial (phase 2B) involving 69 patients recruited from the following clinical centers across four European countries: University of Bergen, Norway (Department of Clinical Dentistry, Faculty of Medicine); University Complutense of Madrid, Spain (Department of Dental Clinical Specialties, Faculty of Odontology); CHU Nantes, France (Centre de Soins Dentaires, Centre Hospitalier Universitaire de Nantes); Universitat Internacional de Catalunya, Spain (University Dental Clinic); University of Southern Denmark Hospital, Esbjerg, Denmark (Oral and Maxillofacial Surgery Department, Faculty of Health); and Assistance Publique–Hôpitaux de Paris/APHP, France.

Patient selection was based on the need for bone reconstruction of mandibular or maxillary defects (width ≤4 mm) (Benic and Hammerle 2014, types 5 and 6), which require three-dimensional reconstruction of the ridge since the existing ridges were insufficient to provide adequate primary stabilization for dental implants placed in a prosthetically driven position. Recruited patients were then randomized to receive one of two interventions: Group I, the test intervention, which involved cell therapy using autologous (BMMSCs) that were isolated, cultured, and expanded ex vivo from iliac crest harvested bone marrow aspirates, combined with a synthetic bone substitute and a non-bioabsorbable membrane; or Group II, the control intervention, which involved autologous bone blocks harvested from the mental region or the posterior mandibular ramus. The use of autogenous bone blocks was chosen as the control, as it has been reported as the most used method to assess the efficacy of staged bone regenerative interventions in a recent systematic review (Sanz-Sanchez et al. 2015).

The primary objective of this clinical trial was to evaluate the efficacy of a tested cell-based guided tissue regeneration intervention, while continuing to monitor its safety when compared to a standard bone regenerative treatment (use of autologous bone block grafts) for the three-dimensional bone augmentation of severe bone defects to facilitate a prosthetically driven implant placement. The primary outcome measure was the bone volume changes, assessed radiographically using sequential cone-beam computed tomography (CBCT) images taken before the regenerative surgery and approximately 5 months later, just before implant placement. Secondary endpoints included the safety of the cell-based therapy, determined by the occurrence of adverse events (AEs), patient morbidity assessed through patient-reported outcomes (PROMs), and the evaluation of postoperative soft tissue healing. Additionally, the effectiveness of both interventions was evaluated based on the ability to place a subsequent dental implant in the regenerated site. The study design and follow-up visits are summarized in Figure 1.

The trial received approval from the National Competent Authorities (NCAs) and Ethical Committees (ECs) in each participating country through a Voluntary Harmonization Procedure (VHP) facilitated by the European Medicines Agency (EMA) network. During this process, the sponsor (University of Bergen) and the Norwegian Medical Products Agency (NOMA) coordinated the application and approval for the clinical trial, collaborating with the respective national medicine agencies in each participating country. The sponsor finalized and submitted key documents, including the clinical protocol (CP), informed consent forms (IC), clinical research forms (CRF), investigator brochure (IB), and the investigational medicinal product document (IMPD), all of which NOMA approved to conduct the study through the VHP (CLINICAL TRIAL—MAXIBONE—EUDRACT NO. 2018-001227-39/VHP1528). After VHP approval, country-specific clinical files, including patient informed consent and General Data Protection Regulation (GDPR) compliance, were submitted and approved by the respective Regional Ethical Committees in each participating country in accordance with national regulations. This multicenter clinical trial was prospectively registered on ClinicalTrials.gov and was conducted following the Declaration of Helsinki (2008), ISO 14155:2011, and the MEDDEV 2.12/2 rev 2 guidelines on Post-Market Clinical Follow-up studies issued by the European Commission's Directorate.

2.2 | Patients Sample

Adults aged 18 years and older were screened for the presence of residual maxillary or mandibular ridges that were inadequate for providing primary stabilization for dental implants, necessitating a three-dimensional bone augmentation procedure. Two experienced clinicians at each clinical center informed potential participants about the study. If patients consented to participate, they underwent a clinical examination that included clinical photographs, dental impressions, and a review of their medical history. If the patient met the inclusion criteria, CBCT scans and dental X-rays were performed. The final selection of patients was based on the absence of significant oral pathology, insufficient bone ridge width (≤ 4 mm) as measured on the CBCT, and the presence of healthy oral mucosa in the area requiring bone augmentation.

Patients were excluded if they exhibited any of these conditions:

- General contraindications for dental and surgical treatments.
- Thin mucosal biotype (thickness < 1 mm).
- Inflammatory and autoimmune diseases of the oral cavity.
- Smokers, as well as pregnant or lactating women, and patients with uncontrolled diabetes or any concurrent systemic disease that compromises adequate wound healing and bone repair.

Before final inclusion, patients received comprehensive verbal and written explanations of the interventions and conditions of the clinical trial. Upon agreeing to participate, they signed an informed consent form in compliance with Directive 95/46/EC on data protection and in accordance with current European Union legal provisions. Patients were informed that they could withdraw from the study at any time without needing to justify their decision. Additionally, the principal investigator had the right to withdraw a patient from the trial at any time if it was deemed necessary for the patient's best interest.

2.3 | Methods for Allocation and Masking

Patients were randomly allocated to either the test treatment group or the control treatment group in a 3:1 ratio, using concealed block size assignments of randomized treatment codes, which were available at each center through the digitized clinical report form (electronic CRF o eCRF).

2.4 | Preparation Procedure for the Test Intervention

In the test group, the preparation of autologous (BMMSCs) was conducted before the surgical intervention, following the protocol developed in the REBORNE project (FP7 no. 241979) (Brennan et al. 2014; Gjerde et al. 2018; Rojewski et al. 2019). The goal was to achieve a minimum concentration of 100×10^6 MSC/5 mL saline and 4%–5% human serum albumin. Bone marrow aspirates from the posterior iliac crest were collected through a sterile, minimally invasive surgical procedure performed under local anesthesia or intravenous conscious sedation (Gjerde et al. 2020). Approximately 30 mL of bone marrow was gathered in two 20 mL syringes (Omnifix 20 mL Luer Lock Solo) containing 5 mL of heparin and 45 mL of NaCl solution, which were shipped within 24 h to one of two certified cell processing centers: the Institute for Transfusion Medicine and Immunogenetics at Ulm University (Germany) or EFS Ile de France, Centre de Thérapie Cellulaire in Paris-Créteil (France). MSCs were isolated, expanded, and cultured at these centers for 15 days. If the minimum required concentration was not achieved, the sponsor withheld clearance for the regenerative surgical procedure, resulting in the patient being withdrawn from the study. Once the sponsor granted clearance, the cell therapy product was shipped back within 24 h to the respective surgical center for therapeutic use.

The synthetic bone substitute used was a biphasic micro/macro-porous granular calcium phosphate (0.5–1 mm in size) containing hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) in a 20/80 ratio (microporous biphasic calcium phosphate, MBCP Biomatlante, Vigneux de Bretagne, France). This bone substitute was supplied in specialized sterilized syringes of 5 cm^3 to facilitate controlled mixing of the cell therapy product with the biomaterial under aseptic conditions in the operating room. The cell suspension was mixed with the bone substitute materials for 60 min before the surgical procedure to ensure the adhesion of the cells on the (MBCP) granules. The mixture was maintained at room temperature in a sterile environment throughout the procedure. When the defect site was prepared, the cell-seeded material was applied directly using the syringe. A titanium-reinforced polytetrafluoroethylene (PTFE) non-bioabsorbable membrane (Cytoplast;

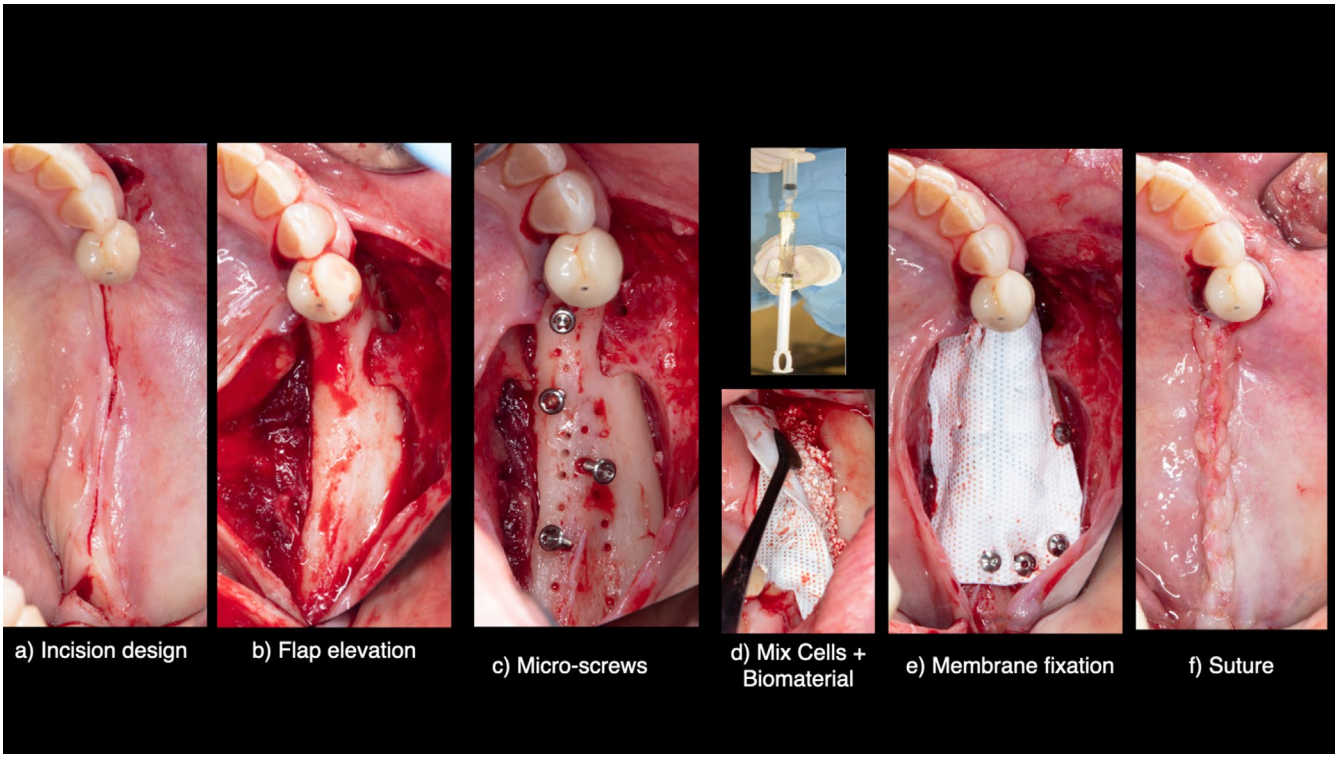

FIGURE 2 | The bone regenerative surgery in the test group. (a) Crestal incision extending to adjacent teeth; (b) full-thickness flap elevation; (c) cortical perforations and space maintainers (tenting pole micro-screws); (d) syringe for mixing and applying the cell and synthetic porous granular biomaterial mixture to reconstruct the bone defect, covered with a non-bioabsorbable membrane; (e) membrane fixation with micro-screws to ensure stable maintenance of the regenerative material; (f) flaps sutured to promote primary healing.

Osteogenics Biomedical, Lubbock, TX, USA) was utilized to cover the augmented area.

2.5 | Surgical Interventions

All surgical procedures were performed by highly trained oral surgeons, maxillofacial surgeons, or periodontal specialists. In the test group, the surgical intervention adhered to the standard GBR procedure, enhanced by cell therapy, which utilized a synthetic particulate bone replacement graft and a non-resorbable barrier membrane (Figure 2). In the control group, the surgical procedure employed an autologous bone block graft secured with micro-screws (Figure 3). For both procedures, antibiotics were administered before the surgical intervention, along with prophylactic anti-inflammatory medications following standard clinical practice at the respective surgical centers.

Briefly, under general or intravenous conscious sedation and local anesthesia, a mid-crestal incision was made, and a full-thickness flap was elevated, extending at least 10 mm mesially and distally from the augmentation site. Periosteal-releasing incisions were performed to adequately expose the bone defect and allow for tension-free primary closure over the regenerated area. The horizontal width of the alveolar crest was measured 2 mm below the crest using a bone caliper (Ivanson Measuring Calliper 0–10 mm, Stoma, Emmingen-Liptingen, Germany), with measurements recorded to the nearest 0.1 mm. To facilitate localization for implant placement surgery, the horizontal mesiodistal distance from the measuring point to the root surface of the adjacent tooth was documented. Cortical bone perforations were made to enhance blood supply to the defect site.

- In the tested intervention, two to three mini screws were placed like tenting poles in the area designated for augmentation, and a mixture of biomaterial and stem cells was injected with a syringe to reconstruct the planned bone volume. Subsequently, the augmented area was covered with a membrane and secured using micro-screws (Figure 2).
- In the control treatment, an autologous bone block was harvested, preferably from the mandibular ramus, following standard procedures. After harvesting, the block was trimmed to the desired volume and secured to the recipient site with fixation osteosynthesis screws (Figure 3).

The mucoperiosteal flaps were then advanced coronally and sutured using crossed horizontal internal mattress sutures combined with simple sutures until a tension-free primary closure was achieved.

After surgery, patients were instructed to follow the prescribed medication regimen and avoid brushing or flossing at the surgical site for the first 2 weeks. Instead, they were advised to use a standard antiseptic rinse (chlorhexidine 0.12%) and a soft bristle brush, ensuring that the grafted area remained undisturbed. Two weeks postoperatively, patients were scheduled for suture removal, healing evaluation, assessment of any

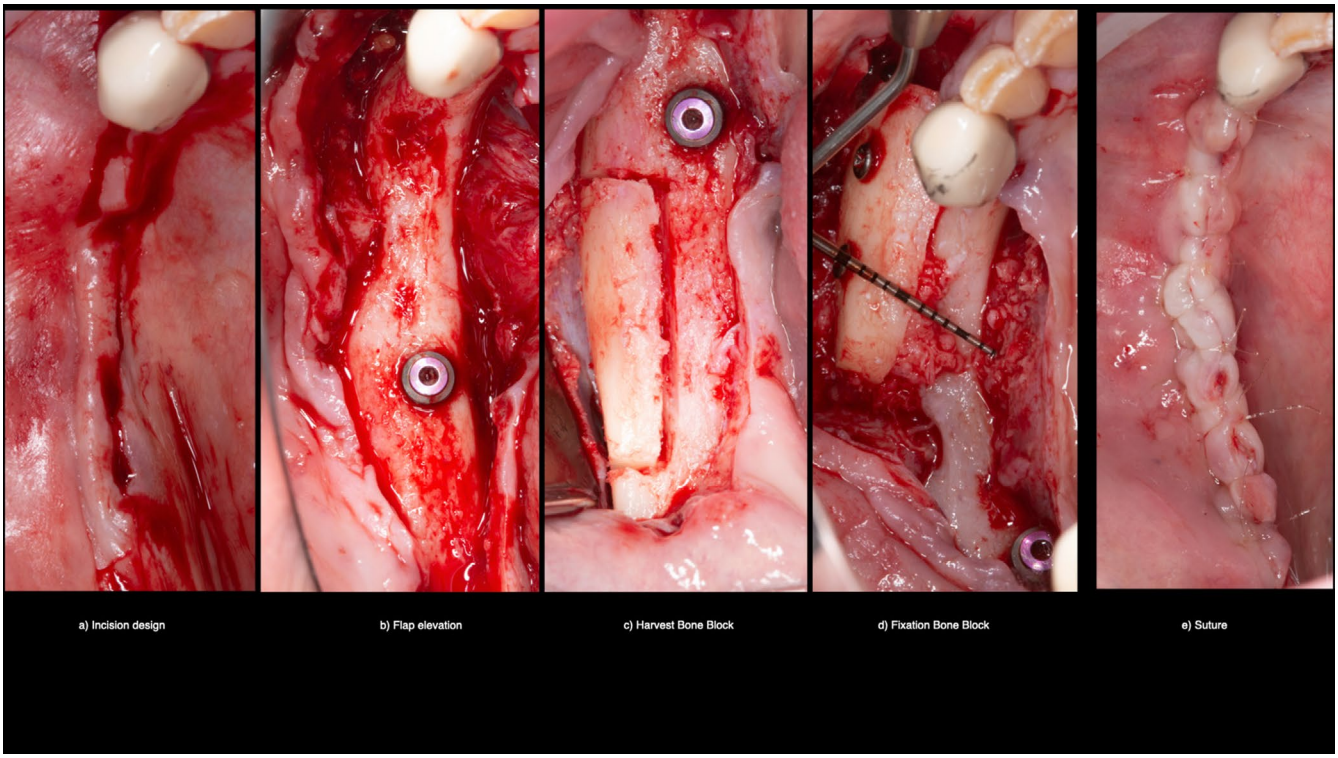

FIGURE 3 | The bone regenerative surgery in the control group. (a) Crestal incision extending to adjacent teeth; (b) full-thickness flap elevation; (c) harvesting of the bone block graft from the mandibular ramus; (d) bone block fixed on the bone defect with micro-screws; (e) flaps sutured to promote primary healing.

complications, recording of PROMs, and verification of adherence to the prescribed postoperative medications. Additional follow-up visits were arranged for 4 weeks and 4 months after surgery to monitor healing progress and assess any adverse events.

Approximately 22 weeks after the test and control bone augmentation surgeries, implant placement surgery was conducted. Two weeks before this procedure, a CBCT was performed to assess the volume of the regenerated bone area on cross-sectional views. The implant surgery was carried out under local anesthesia, using crestal and vertical incisions, followed by the elevation of full-thickness mucoperiosteal flaps to expose the underlying ridge. Whenever feasible, a surgical implant guide was prepared preoperatively to ensure prosthetically driven implant placement, aiming to maintain a minimum distance of 1.5 mm from the adjacent tooth to the implant shoulder at the bone level and 3 mm between adjacent implant shoulders.

After the micro screws were removed, the implant-bone bed was prepared using a trephine with adequate irrigation, which facilitated the harvesting of a bone core biopsy. Standard drills were then employed to achieve the correct dimensions for the bone bed. Dental implants (Straumann Bone Level Implants) with diameters of 4.1 mm and lengths ranging from 8 to 12 mm were placed according to standard procedures and the manufacturer's recommendations (Figures 4 and 5). Implant stability was assessed using an Osstell Beacon device (Osstell, Gothenburg, Sweden), with results reported as stability quotient measurements. If bone dehiscence was observed, or if the buccal or lingual bone around the implant was deemed insufficient (less than 2 mm), a secondary bone augmentation procedure was performed. This procedure employed a standard guided bone regenerative approach, utilizing synthetic bone granules and a bio-absorbable barrier membrane. Following implant placement, periapical dental X-rays were taken, and patients were prescribed standard prophylactic and therapeutic antibiotics, as well as anti-inflammatory or analgesic medications at the surgeon's discretion.

2.6 | Outcomes Variables

A central clinical coordinator (MS) was responsible for monitoring the clinical trial and ensuring the overall fulfillment of the protocol at all investigational sites. Additionally, each clinical center had an independent study monitor, separate from the surgeon and the clinical examiner, who managed the CRFs, randomization, and overall study administration. In each center, a clinical examiner, also independent from the surgeon, was blinded to the type of intervention and was responsible for collecting and entering data into a customized central electronic CRF.

The primary outcome of the trial was the changes in bone volume, measured by superimposing two sequential CBCT scans taken before and 20 weeks after the bone regenerative surgery. To summarize the process, CBCT images were captured using optimized scanning protocols developed at each center for bone volume assessment. The resulting CBCT data sets were converted to DICOM (Digital Imaging and Communications in

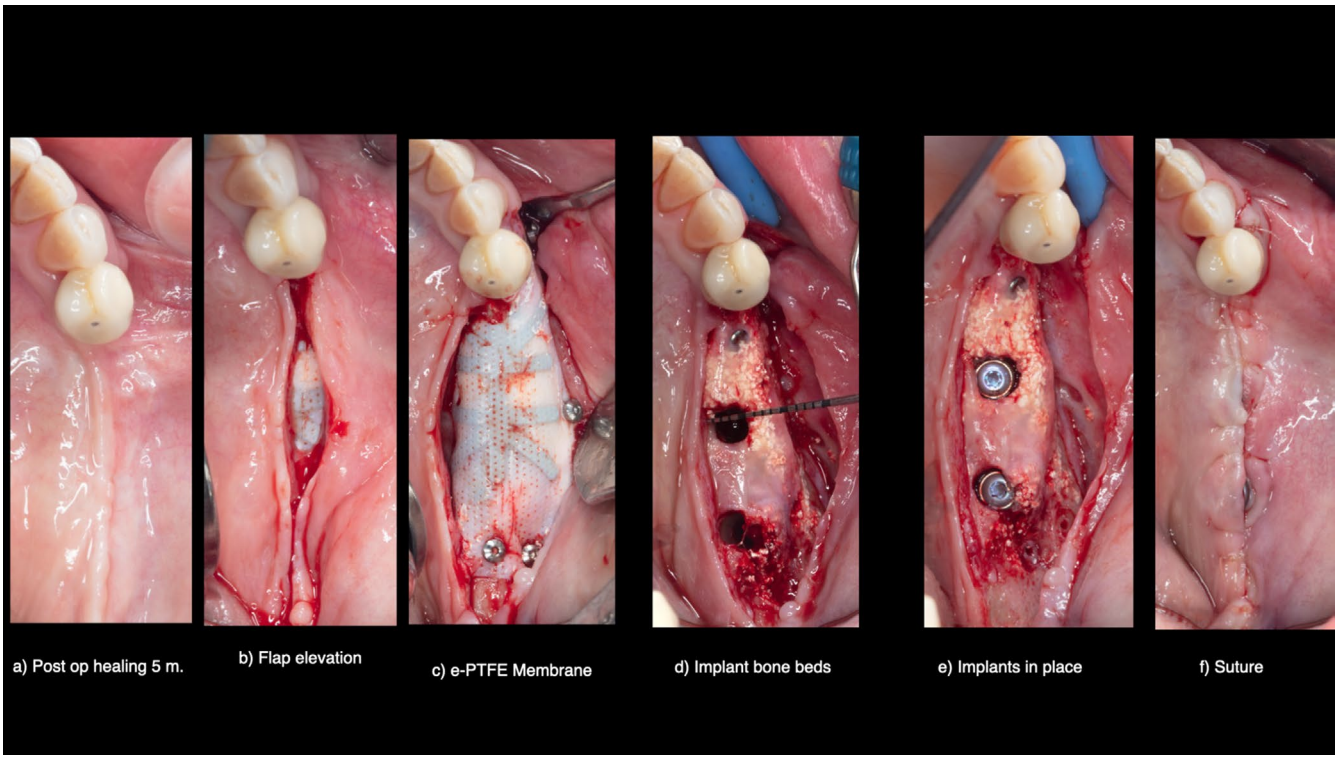

FIGURE 4 | The implant placement therapy in the test group. (a) Postoperative healing at 5 months following regenerative surgery; (b) Crestal incision to expose the regenerated bone; (c) Full-thickness flap elevation revealing the non-bioabsorbable membrane; (d) After membrane removal, bone beds for implants were prepared in the regenerated bone; (e) Implants in place with good primary stability; (f) Flaps were sutured to facilitate submerged implant osseointegration.

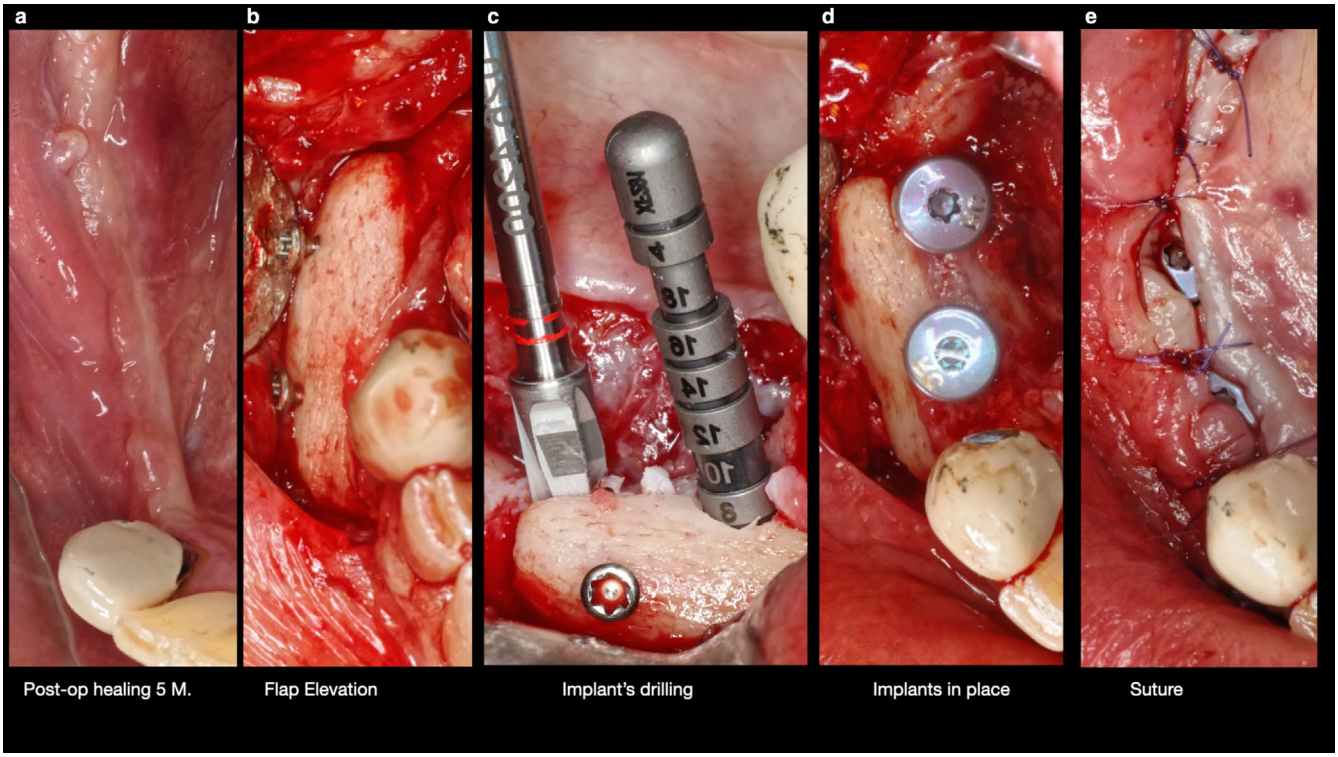

FIGURE 5 | The implant placement therapy in the control group. (a) Postoperative healing at 5 months after regenerative surgery; (b) Crestal incision to expose the regenerated bone; (c) Implant bed preparation on the regenerated bone; (d) Implants in place with good primary stability; (e) Flaps were sutured to facilitate submerged implant osseointegration.

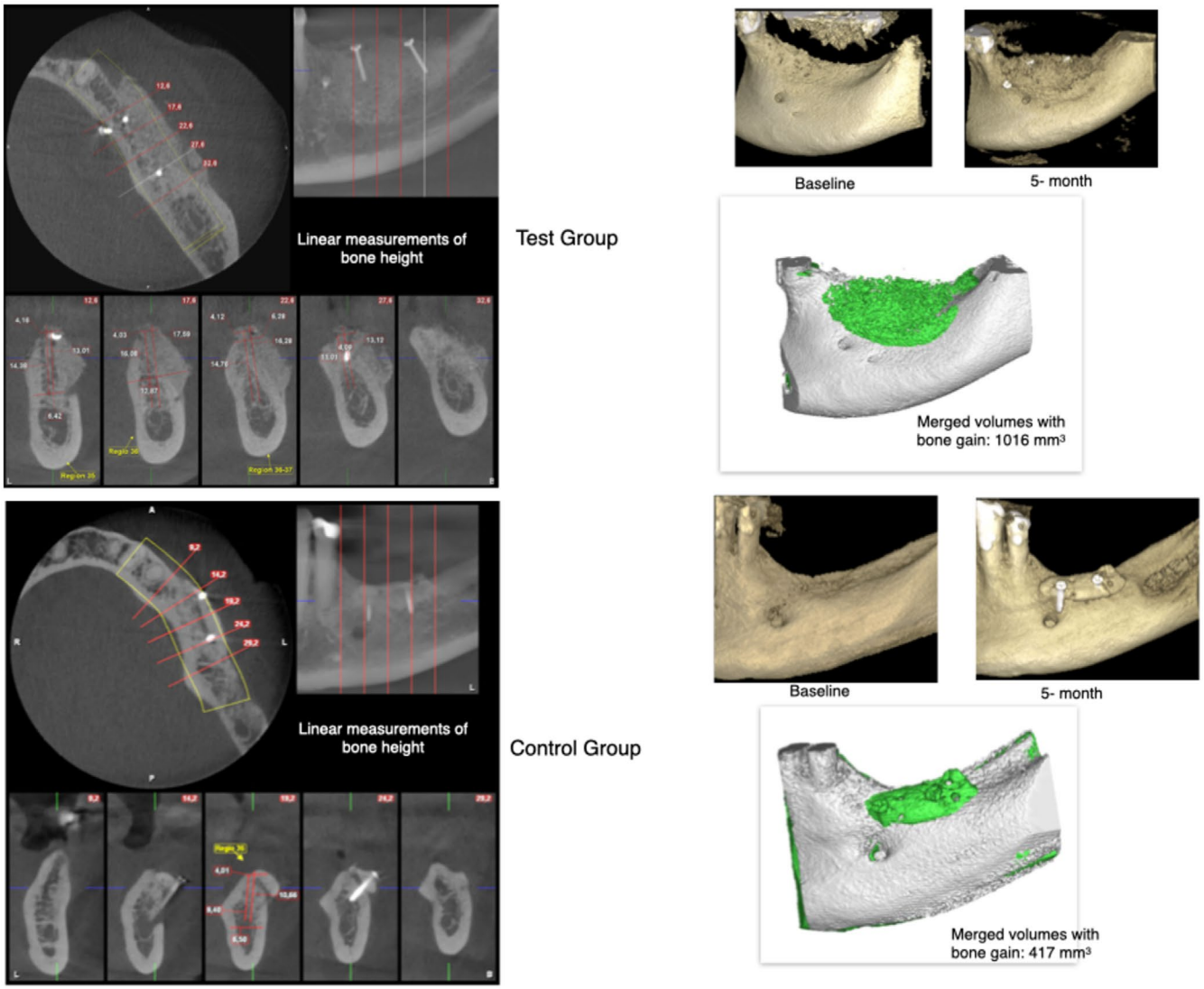

FIGURE 6 | The methodology for assessing bone volumetric changes by superimposing the two CBCTs, one taken prior to the regenerative surgery and the other before the implant therapy, in both the test and control groups. 3-D reconstructions enable the visualization of bone volume gain in both groups.

Medicine) format and imported into the software Mimics 17.0 (Mimics Innovation Suite, Materialize NV, Belgium). Using this software, the DICOM data from baseline and 5-month CBCT scans were superimposed in the region of interest (ROI) through operations such as thresholding, automated segmentation, and rendering. A 3D measurement tool calculated the changes' volume in cubic millimeters. To standardize the localization of the ROI, the distance from the distal root surface of the adjacent tooth was used. The volume of the 3D models was automatically computed and visually represented using color-coded 3D models. The Standard Tessellation Language (STL) registration method was employed to align images taken before and after treatment. Once optimal superimposition was accomplished, 3D models were reconstructed from the corresponding regions in the pre- and post-treatment images. The difference in bone volume was then calculated by subtracting the pre-3D volume from the post-3D volume (Figure 6).

The secondary outcomes of the trial were:

- The safety of the interventions was assessed by recording adverse events (AEs) at 2 and 4 weeks, as well as at 5, 6, 9, and 10 months. Any AE occurring during the clinical study, from the enrollment of the first subject/patient until the last subject/patient visit, was documented using the Adverse Event form, and any suspected unexpected serious AE was reported to the sponsor and trial coordinator.
- The soft tissue healing (uneventful healing, wound dehiscence, membrane exposure, redness, signs of infection, swelling) was assessed at 2, 4 weeks, and 5 months.
- The morbidity associated with both procedures was evaluated by tracking the usage of postoperative anti-inflammatory medications and the level of reported pain, measured using a VAS scale.
- Patient satisfaction was assessed using completed questionnaires from patients that employed a Likert scale.

Overall quality of life was measured through structured self-administered questionnaires, which included Health-related Quality of Life (HRQoL) and Oral Health-Related Quality of Life (OHRQoL).

- The effectiveness of the interventions was evaluated by assessing the ability to place implants in the bone-regenerated area. Additionally, implant stability was recorded at the time of placement, along with the need for secondary bone augmentation in cases of dehiscence or the presence of thin bone (less than 2 mm) on the buccal surface of the implants positioned correctly according to prosthetic guidelines.

This Phase 2 randomized clinical trial adheres to the CONSORT 2010 checklist, which outlines the information to include when reporting a randomized trial.

2.7 | Sample Size Calculation

The sample size calculation for this study was constrained by the limited availability of prior randomized controlled trials (RCTs) that compared the same procedures. Nevertheless, the calculation was derived from two prospective case series studies, which utilized both the test and control interventions and assessed similar primary outcomes (Gjerde et al. 2018; Ortiz-Vigon et al. 2018). The estimation was based on a mean treatment effect difference between groups of 0.5 cm^3, with a standard deviation of 1 cm^3. The resulting sample size included 85 patients in the test group and 28 patients in the control group, corresponding to a 3:1 ratio between the groups. This calculation was performed with a type I error probability of 0.05 (5%) and a statistical power of 0.8 (80%).

2.8 | Statistical Analysis

The statistical unit of analysis was the individual patient; thus, outcomes were calculated per patient and subsequently by group. For continuous variables, the difference between baseline and 5-month visits for both groups was assessed. An intention-to-treat (ITT) analysis was conducted. The analysis of specific variables was performed using data from participants for whom those data were available. Data were presented as means with 95% confidence intervals (CIs), standard deviations, medians, interquartile ranges, and extreme values. Shapiro–Wilk tests were utilized to assess normality in the analyses. Prevalences and 95% confidence intervals were reported for categorical outcomes.

"The primary outcome (volumetric bone gain) was compared using a student's *t*-test when the data followed a normal distribution. If this assumption was not met, the Mann–Whitney *U*-test was applied. For intragroup comparisons, the paired student's *t*-test or the Wilcoxon signed-rank test was used, depending on the normality of the data. Levene's test was used to assess the homogeneity of variances before performing the student's *t*-test."

For intragroup comparisons, the paired Student's *t*-test or the Wilcoxon signed-rank test were used, depending on the normality of the data. For secondary quantitative outcomes, intergroup differences at baseline and each postoperative visit were analyzed using a two-group *t*-test (if the data were normally distributed) or the Mann–Whitney *U*-test (for non-normally distributed data). For secondary categorical outcomes, intergroup comparisons were made using Chi-squared tests or Fisher's exact test. The statistical software used for the analysis included SPSS, Stata, and R.

3 | Results

A total of 69 patients were screened for eligibility to participate in this clinical study across the respective clinical centers from June 2020 to September 2023. Of these, 48 patients met all the inclusion criteria and were randomized into two groups: 36 patients in the test group and 12 in the control group. Among the 36 patients in the test group who underwent bone marrow harvesting, 29 achieved the required minimum expansion of 100×10^6 MSCs, which allowed the sponsor to release the cell therapy for the bone augmentation procedure. Of these 29 patients, 26 successfully underwent implant surgery. All 12 patients in the control group also received the implant surgery. Certain centers did not provide specific patient data for some variables; therefore, only patients with available data were included in the analysis of the corresponding variables. Figure 7 illustrates the flowchart of the clinical trial, detailing the reasons for the patient dropouts at various stages.

Table 1 presents the demographic and bone defect characteristics of the patients who underwent bone augmentation surgery, including both the test and control groups. The study population was predominantly adult women, with average ages of 53 years in the test group and 62 years in the control group (82% and 75%, respectively). Although plaque control was significantly worse at baseline in the test group compared to the control group (30% vs. 18.67% in FMPS), these differences were not significant throughout the study period. Additionally, the bone defect characteristics, including apico-coronal height and bucco-lingual width, were similar between the test and control groups. Radiographic measurements from the CBCT scans showed comparable crestal width volumes in mm^3 (3732; SD = 1932 vs. 2739; SD = 1165; $p > 0.05$).

The primary outcome measure assessed the changes in bone volume at the selected crestal sites between pre-surgery and 5 months post-bone augmentation (Table 2). The test group exhibited significantly greater changes in bone volume compared to the control group, with a mean difference of 480.01 mm^3 ($p = 0.032$). At 5 months post-surgery, the mean difference in bone volume between the test and control groups was 1473.23 mm^3 ($p = 0.091$). Although both regenerative treatments significantly increased crestal bone volume, the effects in the test group (cell therapy) were more pronounced than those in the control group (block graft). Specifically, the test group demonstrated a mean bone volume of 4799.13 mm^3 (SD = 2448.41), compared to 3325.90 mm^3 (SD = 1559.62) in the control group. The mean change in bone crest volume from baseline to 5 months was 1066.91 mm^3 (SD = 677.89) in the test group, while the control group showed a change of approximately half that value (586.9 mm^3; SD = 453.20). The

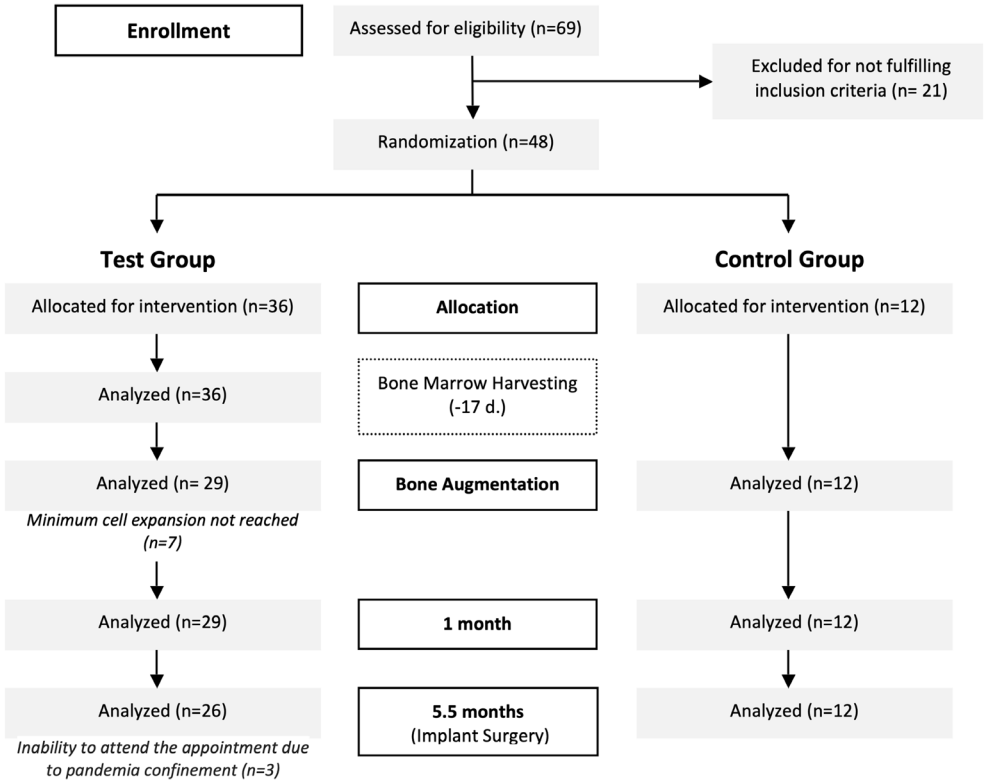

FIGURE 7 | The flow diagram of the RCT, detailing the patients screened, included, randomized, and finally analyzed, along with the reasons for patient dropouts at various stages.

mean bone crest height at 5 months post-surgery, in the area corresponding to the minimum baseline crest width (≤4 mm), was 11.96 mm (SD = 4.22) for the test group and 11.31 mm (SD = 4.81) for the control group. However, these differences were not statistically significant.

Adverse events related to the augmentation procedure were initially recorded exclusively in the test group, associated with bone marrow harvesting, and subsequently noted in both groups concerning the bone regenerative intervention (Table 3). Two minor adverse events occurred during bone marrow aspiration. One patient developed a minor hematoma, resulting in a 2-day sick leave, while another patient experienced pain and nausea, leading to a 4-day sick leave. Both events resolved without any lasting effects.

Adverse events related to the surgical intervention were reported in two patients from the test group and one patient from the control group; these incidents were minor and included malaise, inflammation, and pain. Additionally, six patients—four from the test group and two from the control group—experienced adverse events in the late postoperative period (i.e., more than 2 weeks post-surgery). These events primarily involved sensory disturbances, such as hypoesthesia, paresthesia, or both. The distribution of these events is summarized in Table 3. No statistically significant differences between the groups were observed. During the first 2 weeks after surgery (Table 4), seven patients in the test group experienced soft tissue dehiscence, with no cases reported in the control group. However, these differences were not statistically significant. Inflammation and infection events were infrequent in both groups, with eight cases in the test group and two in the control group. In the test group, minor membrane exposure occurred in seven patients (24.1%), but this did not require early membrane removal. Regarding late healing (one-month post-surgery), soft tissue dehiscence was observed in six patients from the test group, while none were noted in the control group; however, these differences were not statistically significant. Similarly, inflammation persisted in five patients from the test group and in one from the control group, although all cases resolved without complications. Healing after implant placement was generally uneventful in both groups. A minor infection occurred in one patient from the test group, and slight soft tissue dehiscence was noted in three patients from the test group and one from the control group. Again, these differences were not statistically significant.

Table 5 presents the results of the PROMs. In the test group, patients reported mild pain during the bone marrow aspiration procedure, with a mean VAS score of 2.96. For the first 2 weeks following bone regenerative surgery, pain was similarly minimal and comparable between the test and control groups, with mean VAS scores of 4.0 and 3.7, respectively. Likewise, 30 days after surgery, pain levels remained low concerning overall pain during the regenerative procedure, with mean VAS scores of 4.6 for the test group and 5.2 for the control group. The duration of pain was also reported as mild, with mean VAS scores of 3.6 in the test group and 4.0 in the control group; overall comfort during the procedure received mean VAS scores of 3.6 and 4.5, respectively. None of these differences were statistically significant. Overall satisfaction with the intervention was high or very high in both groups, with 84% in the test group and 92% in the control group.

TABLE 1 | Baseline patient and bone defect characteristics.

Variable	*n* (T/C)	Test group	Control group	*p*
Age, mean (SD)	29/12	53.41 (13.31)	62.23 (7.19)	0.037[a]
Gender (% females)	29/12	82.8	75.0	0.671[b]
%FMPS, mean (SD)	29/12	30.03 (15.9)	18.67 (13.32)	0.036[a]
KT in mm, mean (SD)	29/12	3.03 (0.98)	3.18 (0.75)	0.591[c]
Intra-surgical measurements at implant site 1				
Apico-coronal bone length in mm, mean (SD)	29/12	9.72 (3.08)	8.5 (1.38)	0.357[c]
Basal width in mm, mean (SD)	29/12	6.55 (1.99)	7.25 (3.25)	0.405[a]
Crestal width in mm, mean (SD)	29/12	3.14 (0.74)	3.08 (0.9)	0.899[c]
Intra-surgical measurements at implant site 2				
Apico-coronal bone dimension in mm, mean (SD)	29/12	10.93 (2.66)	9.25 (1.42)	0.068[c]
Basal width in mm, mean (SD)	29/12	7.76 (2.65)	8.42 (3.2)	0.501[a]
Crestal width in mm, mean (SD)	29/12	4.17 (2.04)	4.08 (2.02)	0.989[c]
Intra-surgical measurements at target defect				
Apico-coronal bone length in mm, mean (SD)	29/12	9.48 (3.32)	8.5 (1.38)	0.767[c]
Crestal width in mm, mean (SD)	29/12	3.38 (1.47)	3.08 (0.9)	0.524[c]
Radiographic measurements in CBCT scan				
Crestal width in mm^3, mean (SD)[d]	23/10	3732.21 (1932.85)	2739.00 (1165.44)	0.143[a]

Note: Statistically significant differences for $p \leq 0.05$.
Abbreviations: FMPS, full-mouth plaque score; KT, keratinized tissue; *n*, number of subjects; SD, standard deviation.
[a]Student's *t* test.
[b]Fisher's exact test.
[c]Mann Whitney *U* test.
[d]Data for 6 patients from the test group and 2 patients from the control group were not available for the analysis of the variable "crestal width (mm)."

TABLE 2 | CBCT radiographic ridge bone measurements.

Variable	Group	*n*	Mean	SD	Mean Diff. (95% CI)	*p*
A. Inter-group comparisons						
Ridge bone volume changes baseline-5 M. (mm^3)[c]	Control	10	586.90	453.20	480.01 (0.19; 959.83)	0.032[a]
	Test	23	1066.91	677.89		
Ridge bone volume at 5 M. (mm^3)[c]	Control	10	3325.90	1559.62	1473.23 (3194.00; −247.37)	0.091[b]
	Test	23	4799.13	2448.41		
Ridge bone height at 5 M.* (mm)[c]	Control	10	11.31	4.81	0.65 (−2.74; 4.05)	0.70[b]
	Test	23	11.96	4.22		
B. Intra-group comparisons						
Control group; ridge bone volume (mm^3)[c]	Baseline	10	2739.00	1165.44	−586.9 (−911.00; 262.69)	0.003[b]
	5 M.		3325.90	1559.62		
Test group; ridge bone volume (mm^3)[c]	Baseline	23	3732.21	1932.85	−1066.91 (−1360.00; −773.77)	0.000[b]
	5 M.		4799.13	2448.41		

Note: Statistically significant differences for $p \leq 0.05$.
Abbreviations: CBCT, cone-beam computerized tomography; CI, confidence interval; M., month; mean diff., mean difference; *n*, number of subjects; SD, standard deviation.
[a]Mann Whitney *U* test.
[b]Student's *t* test.
[c]Data for 6 patients from the test group and 2 patients from the control group were not available for the analysis of the variables ridge bone volume changes baseline-5 M, ridge bone volume at 5 M., ridge bone height at 5 m., control ridge bone volume and test ridge bone volume.

TABLE 3 | Adverse events.

	Test group		Control group		
Adverse effects	***n***	**%**	***n***	**%**	***p***
Bone marrow aspiration[a]	2.0	7.1	—	—	—
Bone aug. surgery-intraoperative[b]	2.0	6.9	1.0	8.3	1.000[e]
Bone aug. surgery-postoperative[c]	4.0	66.7	2.0	33.3	1.000[e]
Implant placement-postoperative[d]	5.0	20.0	0.0	0.0	0.152[e]
Sensory disturbances (2 w. postoperative)					
Hypoesthesia recipient site	4.0	13.8	1.0	7.0	0.384[f]
Paresthesia recipient site	2.0	6.9	0.0	0.0	
Hypoesthesia and paresthesia recipient site	2.0	6.9	1.0	7.7	
Paresthesia donor site	0.0	0.0	1.0	7.7	
Total	8.0	27.6	3.0	25.0	1.000[e]

Note: Statistically significant differences for $p \leq 0.05$.
[a]Only in test group: 1st case—hematoma, 2 days sick leave; 2nd case—pain, nausea, 4 days sick leave.
[b]Test: 1st case—moderate malaise; 2nd case—pain; control: 1st case-Oro-antral communication.
[c]Test: in 2 subjects—sensory disturbances; in 1 subject—tachycardia; in 1 subject—sublingual swelling; Control: in 2 subjects-sensory disturbances. Recorded 14 ± 2 days after bone augmentation surgery.
[d]Test: In 2 subjects—sensory disturbances; in 2 subjects—postoperative infection; in 1 subject—vomits. Recorded 14 ± 15 days after implant placement surgery.
[e]Fisher's exact text.
[f]Likelihood ratio.

TABLE 4 | Mucosa post-operative healing.

Variable	Test group		Control group		*p*
2 weeks after bone augm. surgery	*n*	%	*n*	%	
Membrane exposure	7.0	24.1	—	—	—
First intention healing	22.0	78.6	12.0	100.0	0.085
Second intention healing	7.0	24.1	0.0	0.0	
4 weeks after bone augm. surgery	*n*	%	*n*	%	
Membrane exposure	6.0	22.0	—	—	—
First intention healing	21.0	77.8	12.0	100.0	0.151
Second intention healing	6.0	22.2	0.0	0.0	
Inflammation	5.0	17.9	1.0	8.3	0.648
Infection	1.0	3.7	0.0	0.0	1.000
2 weeks after implant surgery	*n*	%	*n*	%	
First intention healing	22.0	88.0	11.0	91.7	1000
Second intention healing	3.0	12.0	1	8.3	
Inflammation	0.0	0.0	0.0	0.0	—
Infection	1.0	4.0	0.0	0.0	1.000

Note: Statistically significant differences for $p \leq 0.05$. Fisher's exact test used for all comparisons.

Table 6 highlights the outcomes associated with the implant placement procedure in both groups. Implants were successfully placed in the augmented sites of all treated patients (26 in the test group and 12 in the control group). The implants exhibited high mean ISQs (approximately 70 for both groups) in the mesiodistal and buccolingual directions. No statistically significant differences were found between the groups for any of these parameters.

4 | Discussion

The results of this Phase 2 randomized clinical trial demonstrate the efficacy, safety, and effectiveness of the proposed cell-based guided tissue regeneration intervention. This intervention involves using BMMSCs isolated and cultured from iliac crest bone marrow aspirates, which were seeded in a synthetic biphasic micro/macro-porous granular calcium phosphate scaffolding biomaterial composed of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) in a ratio of 20/80. The mixture of biomaterial (scaffold) and stem cells was applied to the defect sites to reconstruct bone volume, which was subsequently covered with a titanium-reinforced PTFE non-bioabsorbable membrane. Compared to standard treatment using autologous bone block grafts, the primary outcome, measured by three-dimensional bone volume changes at selected sites with minimal alveolar ridge dimensions (≤ 4 mm), showed significantly greater gains in the test group, with a mean difference of 480.01 mm^3 ($p = 0.032$). The use of autogenous bone blocks was chosen as the control, as it has been reported as the most used method to assess the efficacy of staged bone regenerative interventions in a recent systematic review

TABLE 5 | Patient-reported outcome measures: morbidity and patient satisfaction.

Morbidity	Test group	Control group	*p*
Pain—bone marrow aspiration (VAS scale (mean; SD))	2.96 (2.87)	—	—
Pain—bone aug. surgery-postoperative (2 w.) (VAS scale (mean; SD))	4.00 (3.28)	3.67 (3.17)	0.921[a]
Pian in recipient site—bone aug. surgery-postoperative (4 w.) VAS scale (mean; SD)	0.74 (1.67)	0.92 (1.56)	0.708[a]
Pain in donor site—bone aug. surgery-postoperative (4 w.) VAS scale (mean; SD)	—	0.58 (1.24)	
Overall pain, VAS scale, mean (SD)	4.62 (2.80)	5.25 (2.41)	0.466[a]
Pain duration postsurgically, days, mean (SD)	3.62 (1.29)	4.00 (1.41)	0.312[a]
Comfortability of the procedure, VAS scale, mean (SD)	3.65 (2.97)	4.50 (3.47)	0.743[a]
Pain—implant surgery-postoperative (4 w.) VAS scale (mean; SD)	0.64 (1.68)	1.00 (2.37)	0.911[a]
Patient satisfaction[c]	**Test group**	**Control group**	***p***
Very satisfied, *n* (%)	14 (53.80)	5 (41.70)	0.504[b]
Satisfied, *n* (%)	8 (30.80)	1 (50.00)	
Neither satisfied nor dissatisfied, *n* (%)	4 (15.40)	1 (8.30)	
Dissatisfied, *n* (%)	0	0	
Very dissatisfied, *n* (%)	0	0	

Note: Statistically significant differences for $p \leq 0.05$.
Abbreviation: SD, standard deviation.
[a]Mann Whitney *U* test.
[b]Likelihood ratio.
[c]Data for 3 patients from the test group and 5 patients from the control group were not available for the analysis of the variable patient satisfaction.

TABLE 6 | Measurements at implant placement surgery.

Variable	*n* (T/C)	Test group	Control group	*p*
Implant location (% in mandible)	19/8	82.60	80.00	0.605[a]
Possibility to place an implant in the regenerated area				
Implant site # 1	26/19	100.00	100.00	
Implant site # 2	26/19	100.00	100.00	
Bleeding at the bone bed for implant placement				
Implant site # 1	25/12	96.20	91.70	0.538[a]
Implant site # 2	24/12	96.00	91.70	1.00[a]
Implant stability RFA (mesio-distal)[d]				
Implant site # 1 ISQ, mean (SD)	23/12	70.87 (7.68)	70.09 (10.62)	0.809[b]
Implant site # 2 ISQ, mean (SD)	23/12	69.74 (7.40)	68.27 (14.49)	0.586[c]
Implant stability RFA (bucco-lingual)[d]				
Implant site # 1 ISQ, mean (SD)	23/11	67.91 (9.83)	70.36 (10.01)	0.504[c]
Implant site # 2 ISQ, mean (SD)	23/11	69.43 (8.59)	71.27 (10.87)	0.596[c]
Changes in planned implant position (%)	5/3	19.20	25	0.689[a]
Additional bone regeneration needed (%)	0/1	0.00	11.10	0.281[a]

Note: Statistically significant differences for $p \leq 0.05$.
Abbreviations: ISQ, implant stability quotient; RFA, resonance frequency analysis; SD, standard deviation.
[a]Fisher's exact test.
[b]Student's *T* test.
[c]Mann Whitney *U* test.
[d]Data for 6 patients from the test group were not available for the analysis of the variables implant stability RFA-mesio distal. Data from 6 and 1 patients from test and control group, respectively, were not available for the analysis of the variables implant stability RFA-bucco-lingual.

(Sanz-Sanchez et al. 2015). This significant change in bone volume between the test and control interventions may not fully reflect the regenerative effect of the MSCs, which could be attributed to differences in the graft volumes at the time of surgery. To discard this effect, we would have needed a CBCT scan immediately after the surgery to provide this information. However, these radiation-based evaluations are not without risks, and we did not justify this additional CBCT for the patient's safety considerations.

While both bone regenerative treatments resulted in a significant 3D increase in crestal bone volume, the mean change in bone crest volume from baseline to 5 months was notably higher in the test group (1066.91 mm^3) than in the control group (586.9 mm^3). These findings are difficult to compare directly with other studies due to limited clinical investigations using cell therapies in the orocraniofacial region. A recent systematic review highlighted the potential of MSC-based therapies for bone regeneration. However, these therapies have primarily been tested in experimental in vivo studies, as clinical evidence is still lacking and mainly consists of case series and Phase 1 trials.

In fact, only three Phase 1 clinical studies have been published evaluating the effect of MSCs on orofacial bone regeneration (Cubuk et al. 2023; Fatale et al. 2022; Tanikawa et al. 2020). Cubuk et al. (2023) compared autologous MSCs with leukocyte- and Platelet-Rich Fibrin (L-PRF) carriers for treating mandibular third molar extraction sockets and found no significant differences between the two interventions regarding vertical bone gains or relative bone density. Similarly, MSCs derived from exfoliated deciduous teeth (SHED) were evaluated versus iliac crest bone grafts in patients with cleft lip and palate, reporting comparable bone fill in both groups (Tanikawa et al. 2020) and autologous maxillary periosteal MSCs with a β-TCP/HA carrier showed no significant differences in lateral or vertical bone augmentation in maxillary sinus lift procedures despite noting improved "mature" bone formation in the MSCs group (Fatale et al. 2022). However, these studies involved small patient populations and lacked clear reporting on the source, dosage, and characterization of the MSCs used.

The BMMSCs protocol used in this RCT study was based on the findings of a previous European Research Project, REBORNE (FP7 project no. 241979), which utilized a minimal concentration of 100×10^6 MSC/5 mL from iliac crest aspirates (Rojewski et al. 2019). This protocol, previously employed in a prospective case series study, demonstrated its safety and efficacy in three-dimensional bone augmentation, primarily in posterior mandibular sites (Gjerde et al. 2018).

Comparing the results of the present study with those from clinical trials utilizing other regenerative technologies for three-dimensional augmentation of heavily resorbed alveolar ridges is challenging. This difficulty arises mainly because most reported studies have relied on linear measurements (vertical bone gains) as their primary outcome rather than volumetric bone changes. A systematic review of thirty-six publications on the use of GBR, using autologous or non-autologous grafting material, bone blocks, and distraction osteogenesis, has shown their efficacy for vertical bone augmentation based on significant vertical bone gains as the primary outcome measure, reporting a significant weighted mean effect of 4.16 mm (95% CI 3.72–4.61; $p < 0.001$) across all treatment approaches (Urban et al. 2019). More recent reviews (Shi et al. 2023; Urban et al. 2023) have corroborated these findings, showing vertical bone gains from GBR interventions ranging from 4 to 5 mm, with the most favorable outcomes observed when non-resorbable titanium-reinforced membranes were employed. When using onlay bone blocks, vertical gains ranged from a mean of 4.12 mm for autogenous bone blocks to 2.03 mm for allogenic bone blocks, with the best results reported when autogenous blocks were combined with modified techniques (tunnel and shell techniques, respectively) (De Stavola and Tunkel 2013; Khoury and Hanser 2019). Only one recent clinical trial comparing GBR interventions for vertical ridge augmentation with customized reinforced PTFE mesh and titanium meshes reported both linear and volumetric data. The changes in bone volume were reported as 1.46 ± 0.48 cc for PTFE and 1.26 ± 0.55 cc for titanium mesh (Cucchi et al. 2024). These volumetric data are comparable to the 1.067 mm^3 observed in the cell-therapy group in the present study. However, they are clearly superior to the 586.9 mm^3 observed in the control group, which only used block grafts.

In this study, we selected a macro-porous granular synthetic biomaterial consisting of HA and β-TCP in a 20/80 ratio (MBCP) as the scaffold material. This bone substitute was combined with a cell therapy product prior to surgery, ensuring complete seeding and impregnation of the MBCP granules. The density of cells and cell-to-biomaterial ratio used in this investigation was adapted from a previous preclinical study, in which 20×10^6 MSCs were mixed with 1 cm^3 BCP (Brennan et al. 2014). Compared to xenogeneic bone substitutes, highly porous calcium phosphate-based synthetic biomaterials have demonstrated comparable bone regenerative outcomes in both preclinical and clinical studies (Cha et al. 2024), without the potential risks of cross-infection associated with xenogeneic or allogenic biomaterials. As a barrier membrane, we selected a titanium-reinforced PTFE non-bioabsorbable membrane. This choice was based on the capacity of non-resorbable titanium-reinforced barrier membranes, when combined with granular bone substitutes, to provide and maintain a secluded space that prevents the collapse of soft tissues within the defect (Zhang et al. 2022). Indeed, comparative studies have demonstrated that titanium-reinforced PTFE non-bioabsorbable membranes yield superior outcomes in terms of vertical bone gains and increased bone volume when compared to resorbable membranes or titanium meshes (Merli et al. 2013; Ronda et al. 2014).

Consistent with earlier case series and clinical trials investigating the outcomes of cell-based therapies for orocranial applications (Ivanovski et al. 2024), the cell-based therapy used in this study was determined to be safe. This conclusion was drawn from the absence of significant adverse events during the bone marrow procedure and in the intraoperative and postoperative periods. When complications such as hematoma, malaise, pain, or sensory disturbances were noted, all resolved favorably in the early postoperative phase. Although the morbidity associated with the control intervention (bone blocks) was consistently higher than that of the cell-based therapy, especially regarding overall pain (mean VAS score of 4.62 versus 5.25 for the test and control groups, respectively), procedure duration, and discomfort, these

differences were not statistically significant. In contrast, the pain from bone marrow aspiration in the test group measured 2.96, while the VAS score for pain at the graft donor site was only 0.58. We interpret this difference as both block harvesting and bone augmentation surgeries being performed simultaneously, and since most treatment sites were mandibular, the surgical site was the same, causing patients to have difficulty distinguishing between the two procedures. Conversely, the bone marrow aspiration was done as a separate procedure. Although the reported pain was low (2.96 on a 1–10 VAS scale), it was significantly higher than what patients in the control group reported associated with the graft donor site. These morbidity results, however, are difficult to compare with those from other studies evaluating similar three-dimensional bone augmentation procedures, even with the use of similar tools for assessing postoperative morbidity outcomes. In a systematic review by Shi, only 2.2% of the selected studies assessed the effectiveness of bone regenerative interventions using PROMs as the primary outcome (Shi et al. 2023). This is despite the recent recommendations from the ID-COSM initiative urging the mandatory use of PROMs in all clinical studies related to implant dentistry, including those involving bone augmentation procedures conducted either simultaneously or staged with implant placement, with special emphasis on patient satisfaction, surgical morbidity, and the advent of complications (Tonetti et al. 2023).

In relation to postoperative healing, flap dehiscence and membrane exposure occurred in 24% of cases 2 weeks after surgery in the cell-based therapy group. In contrast, no flap dehiscence was reported in the control group, indicating that this complication was more likely associated with the use of the barrier membrane. These findings align with other studies on bone augmentation procedures. A systematic review of 29 clinical studies (Urban et al. 2019) reported an overall weighted mean incidence of complications at 16.9%. Higher complication rates were noted in studies involving staged procedures (22.3%), with the highest complication rates linked to distraction osteogenesis (47.3%), followed by onlay bone blocks (23.9%) and GBR (12.1%).

Although the results of this clinical trial demonstrated a significantly higher efficacy of the tested cell-based guided bone regenerative intervention in achieving higher bone volume gains when compared with the standard therapy control, autogenous bone blocks, both interventions were similarly effective, since they achieved implant placement on the regenerated bone in a prosthetically driven position in 100% of the cases. Similar outcomes have been noted in studies assessing implant placement in augmented sites (Urban et al. 2019). In this systematic review, 27 out of 29 studies reported successful implant placement, with an overall mean implant survival rate of 98.95% (ranging from 90.5% to 100%).

The strength of this randomized clinical trial lies in its strict control measures for standardizing stem cell therapy. It clearly reports the source, dosage, and characterization of the MSCs used while employing bone volume changes as the primary outcome to demonstrate effective 3D bone augmentation. In fact, this study represents the first Phase 2 clinical trial of a cell therapy application in the orofacial region to demonstrate a significant effect on bone augmentation, which enabled the successful placement of dental implants.

This clinical study, however, has important limitations, primarily due to the inability to achieve the planned sample size calculated prior to the study's commencement. The reasons for this are varied, primarily related to the difficulties inherent in cell therapy studies. The strict inclusion criteria regarding the minimal dosage of expanded cells prevented seven of the randomized patients from the test group (20%) from undergoing regenerative surgery after donating iliac crest bone marrow. Furthermore, the strict regulatory conditions associated with these therapies in some European countries prevented one of the surgical centers from enrolling patients for 3 years, until they obtained final permission 6 months before the study's required closure, as mandated by the EU Horizon 2020 project regulations. Finally, this clinical study, which began in 2018 and concluded in 2022, was significantly impacted by the COVID-19 pandemic, resulting in a complete inability to perform surgeries for over a year and substantially limiting our recruitment capacity. Furthermore, 20% of the randomized patients (7 patients) were unable to be treated since they did not reach the minimum threshold of expanded MSCs, as required by the protocol, which may be due to a reduced number of stem cells from the iliac crest bone marrow harvesting. Another limitation was the inability to blind the surgeons and participants due to the inherent characteristics of the tested interventions, which required secondary interventions for either bone marrow aspiration or bone block harvesting. This may have introduced potential performance bias.

In conclusion, this Phase 2 RCT demonstrated that the proposed cell-based guided tissue regeneration intervention for three-dimensional bone volume augmentation of alveolar ridges prior to dental implant placement was efficacious, safe, and effective. It led to significant gains in bone volume compared to the control standard treatment, autogenous bone blocks, enabling the placement of dental implants in all patients. The procedure was associated with minimal adverse effects and a comparable incidence of postsurgical healing complications and patient morbidity when compared to the standard therapy used for this clinical indication.

However, these results must be interpreted with caution since a valid comparison of the increase in volume between test and control groups could not be given, as the possibility cannot be ruled out that the baseline augmentation volume was significantly higher in the test group. Moreover, this Phase 2 RCT was underpowered for the reasons reported earlier, which makes the clinical translation of this cell-based bone regenerative therapy challenging. Moreover, the use of autologous mesenchymal stem cells has significant limitations, particularly regarding its costs, regulatory constraints, and inefficiency, as 20% of patients did not produce a minimum volume of expanded MSCs. The encouraging results from this clinical trial, however, should stimulate further research and development on the application of novel technologies and processes to address these current limitations.

Author Contributions

Mariano Sanz: conceptualization, investigation, writing – original draft, supervision, writing – review and editing, methodology. **Cecilie Gjerde:** conceptualization, investigation, funding acquisition, writing – review and editing, methodology. **Bjørn Tore Gjertsen:** investigation, methodology, supervision, writing – review and editing. **Alberto**

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Ethics Statement

The trial received approval from the National Competent Authorities (NCAs) and Ethical Committees (ECs) in each participating country through a Voluntary Harmonization Procedure (VHP) facilitated by the European Medicines Agency (EMA) network VHP (CLINICAL TRIAL—MAXIBONE—EUDRACT NO. 2018-001227-39/VHP1528). After obtaining VHP approval, country-specific clinical files, including patient-informed consent and compliance with the General Data Protection Regulation (GDPR), were submitted and approved by the respective Regional Ethical Committees in each participating country, in accordance with national regulations (see appended documentation).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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