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Comprehensive Evaluation of Bone Marrow-Derived Mesenchymal Stem Cell Transplantation for Maxillary Sinus Floor and Alveolar Ridge Augmentation: Implications for Clinical Performance and Malpractice Prevention

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Abstract: Background: The safety and efficacy of bone marrow-derived mesenchymal stem cell (BMSC) transplantation in maxillary sinus and alveolar ridge augmentation represent a cutting-edge approach in regenerative dentistry. The findings hold significance for advancing regenerative therapies and enhancing long-term outcomes in patients requiring extensive dental interventions. Objectives: The study aimed to assess the safety, feasibility, and long-term outcomes of bone marrow-derived mesenchymal stem cell (BMSC) transplantation in maxillary sinus floor and alveolar ridge augmentation. Objectives included evaluating BMSC-induced bone regeneration, conducting safety assessments, and ensuring ethical compliance, with a 7-year follow-up to assess dental implant survival and stability. Methodology: This study outlines a meticulous methodology for bone marrow-derived mesenchymal stem cell (BMSC) transplantation in maxillary sinus floor and alveolar ridge augmentation. Incorporating safety checks, donor screening, autologous serum preparation, and a detailed surgical procedure, the study evaluated feasibility and long-term outcomes. Ethical considerations were paramount, with adherence to the Declaration of Helsinki and explicit participant consent. The comprehensive evaluation included radiographic assessments, CT scans, histomorphometric analysis, and a 7-year follow-up, emphasizing sustained success and ethical integrity. Results: Ten subjects undergoing bone marrow aspiration, two were excluded due to inadequate cell quantity and suspected bacterial contamination. The remaining eight underwent cell transplantation for sinus floor and alveolar ridge augmentation. Flow cytometry revealed BMSCs' low proportion (0.065%) compared to hematopoietic stem cells (0.89%). Transplants integrated well, evidenced by CT imaging and histology, with varying bone regeneration. Dental implant success was 93%, with no infections, over an average 56-month follow-up. The study demonstrates cell transplantation safety, effectiveness, and variability in outcomes. Conclusion: Cell transplantation, specifically using Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) in combination with Platelet-Rich Plasma (PRP) gel and β-TCP granules, is a viable and safe approach for sinus floor augmentation and alveolar ridge augmentation.

Keywords: BMSC transplantation, Maxillary sinus floor, Alveolar ridge augmentation, Safety checks, Donor screening, Autologous serum preparation, Surgical procedure

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1. Introduction

The innovative technique harnesses the regenerative potential of bone marrow-derived mesenchymal stem cell (BMSCs) to address challenging cases, such as severe atrophy or multiple tooth defects, where traditional interventions may be insufficient.[1] The method aims to optimize bone regeneration, supporting successful outcomes in dental implant procedures.[2] Research in this domain is pivotal for advancing therapeutic options and ensuring patient well-being in complex dental reconstructions.[3]

Cell-based therapies have undergone extensive exploration for bone regenerative applications, encompassing varied sources like BMSCs, PDCs, and ASCs. Despite ASCs' heightened multilineage potential, their dental utilization encounters impediments, particularly in liposuction resistance.[4] Conversely, the periosteum, housing plentiful osteoprogenitor cells, grapples with the lack of established methods for selective proliferation. Among these, BMSCs emerge prominently, heralded for their possession of both pluripotent stem cells and osteoprogenitor cells.[5] Their ease of harvesting, requiring a mere tens of milliliters of bone marrow aspirate under local anesthesia, coupled with straightforward proliferation on culture dishes, positions BMSCs as a promising cell source for bone tissue engineering.[6]

The paradigm of cell-based therapy for tissue regeneration involves three fundamental components, termed the tissue engineering triad: progenitor cells, stimulatory factors (e.g., BMPs, PDGFs, PRP), and a cell scaffold.[7] In the realm of stimulatory factors, diverse bioactive molecules, including growth and differentiation factors along with parathyroid hormone, find utilization. Notably, platelet-rich plasma (PRP) emerges as a potential candidate due to its known ability to accelerate bone regeneration coupled with the ease of preparation.[8, 9]

When considering the scaffold in bone tissue engineering, calcium phosphate materials such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) take precedence. Widely employed for their inorganic presence in bone tissue, these materials meet the scaffold requirements by constructing a 3D structure essential for shaping augmented bone. Moreover, they facilitate the attachment of cells and stimulatory factors.[10]

In the synthesis of a comprehensive approach for successful bone tissue engineering, BMSCs are selected as the stem/progenitor cells, β-TCP is chosen as the absorbable scaffold for regenerated bone, and PRP is designated as the stimulatory factor. This strategic amalgamation aligns with the tissue engineering triad, providing a nuanced and effective strategy for bone regenerative therapy.[11, 12] This study addresses a critical gap in dental implant research by exploring the safety and efficacy of bone marrow-derived mesenchymal stem cell (BMSC) transplantation in maxillary sinus and alveolar ridge augmentation.

2. Methodology

A comprehensive process of BMSC culture, transplantation, and thorough assessments, emphasizing safety, feasibility, and long-term outcomes in patients undergoing maxillary sinus floor and alveolar ridge augmentation.

2.1 Study Design

Bone marrow-derived mesenchymal stem cells (BMSCs) were cultured and expanded from iliac bone marrow aspirate for subsequent use in maxillary sinus floor and alveolar ridge augmentation. The transplantation procedure involved the combination of induced osteogenic cells with autogenous platelet-rich plasma (PRP) and β-tricalcium phosphate granules (β-TCP). The study included eight patients (two males and six females) with an average age of 54.2 years. Safety assessments were conducted through the monitoring of adverse events. Radiographic evaluation and bone biopsies were performed to assess the quality of the regenerated bone.

2.2 Participants

2.2.1 Inclusion Criteria:

Patients anticipating dental implant treatment. Patients with more than two continuous tooth defects where fixed prostheses were not applicable. Patients with severely atrophic maxilla or mandible requiring bone transplantation. Alveolar bone ridge width < 5 mm. Maxillary sinus floor-to-alveolar ridge distance < 5 mm. Mandibular ridge-to-mandibular canal distance < 5 mm. Maintenance of good oral hygiene. Age between 20 and 70 years. Ability to understand and provide written informed consent.

2.2.2 Exclusion Criteria:

Diabetes and/or autoimmune diseases. Hemorrhagic diathesis (PT < 50%, APTT < 23.5 or > 42.5 s). Uncontrollable infectious diseases. Osteoporosis. Liver dysfunction (AST < 10 or > 40 IU/L, ALT < 5 or > 45 IU/L). Pregnancy or potential pregnancy. Allergy to study medications or requiring continuous systemic medication. Other conditions deemed inappropriate by the responsible physician.

2.3 Study Scope

In accordance with the structured design of this investigation, a total of ten patients were purposefully and meticulously selected for enrollment in this phase I/II pilot study. The primary objective of this phase was to evaluate the feasibility of the procedures involved in the transplantation of induced osteogenic cells, in conjunction with autogenous platelet-rich plasma (PRP) and β-tricalcium phosphate granules (β-TCP), for maxillary sinus floor and alveolar ridge augmentation.

The decision to limit the study to ten patients during this initial phase reflects a deliberate and cautious approach, adhering to the principles of a pilot study. Pilot studies are instrumental in gauging the viability, safety, and practicality of novel interventions before broader implementation. The sample size of ten patients was deemed sufficient for this preliminary exploration, providing an initial understanding of the procedures' effectiveness and safety.

The study's timeframe was defined by a follow-up period spanning two years after the transplantation of cells. This duration was chosen deliberately to allow for comprehensive observation, documentation, and assessment of the outcomes over an extended period. The two-year follow-up period aligns with the study's dual purpose: not only to monitor immediate responses but also to evaluate the long-term stability and success of the induced bone regeneration.

This meticulous approach to study scope not only adheres to the ethical considerations of patient safety and well-being but also allows for a phased progression of the research. The initial enrollment of ten patients and the subsequent two-year follow-up period provide a foundational understanding that can inform future phases of the study, contributing to the broader scientific knowledge in the field of bone regeneration procedures. The outcomes of this phase I/II pilot study serve as a critical foundation for potential larger-scale investigations in subsequent phases, building on the insights gained and addressing any identified limitations or opportunities for refinement.

2.4 Donor Screening

Prior to their inclusion in the study, subjects underwent a comprehensive screening process to ensure their eligibility and safety. The screening encompassed a battery of tests for infectious diseases and evaluation of biochemical markers. The meticulous nature of this screening was pivotal in establishing a cohort of participants who could undergo the subsequent procedures with a minimized risk of complications.

The screening tests included assessments for infectious diseases such as hepatitis B (HBs antigen, HBs antibody, HBc antigen), hepatitis C (HCV antibody), human immunodeficiency virus (HIV antigen and HIV antibody), syphilis (RPR, TPHA), and human T-cell lymphotropic virus type 1 (HTLV-1 antibody). The subjects' negative results in all these tests were a prerequisite for enrollment, indicating the absence of these infectious agents in their systems.

Additionally, biochemical markers such as white blood cells, red blood cells, platelet counts, hemoglobin, hematocrit, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were scrutinized. These markers provided insights into the general health and coagulation status of the potential donors.

Furthermore, as part of the screening process, autologous serum was procured from the subjects before the bone marrow aspiration procedure. This autologous serum served a dual purpose – not only did it contribute to the preparation of the culture medium for the harvested bone marrow stromal cells (BMSCs), but it also ensured that any subsequent cellular interventions were conducted using the subject's own serum, minimizing the risk of immunological reactions.

The thoroughness of the donor screening process, involving both infectious disease assessments and biochemical marker evaluations, underscores the commitment to patient safety and the ethical standards set forth in clinical research. The stringent criteria for inclusion based on these screening outcomes contribute to the overall reliability and validity of the study results, laying the groundwork for a scientifically sound and ethically responsible investigation.

2.5 Autologous Serum Preparation

To facilitate the cultivation and expansion of bone marrow stromal cells (BMSCs), a crucial step involved the preparation of autologous serum derived from the patients' own peripheral blood. This process was meticulously executed to ensure the use of personalized serum, minimizing the risk of immunological complications during subsequent cell transplantation.

The procedure commenced with the collection of 200–400 mL of peripheral blood from each participant before the bone marrow aspiration. This peripheral blood served as the source material for generating autologous serum. To maintain the integrity of the blood components, the collected samples were stored at a temperature of 4 ◦C for one hour.

Subsequently, the blood samples underwent a centrifugation process, a key step in separating the serum from other blood components. The resulting serum was then carefully transferred to fresh bags, each containing approximately 50 g, ensuring aseptic conditions throughout this cryopreservation step (Nipro Corp., Osaka, Japan). The cryopreserved serum was stored in a freezer at −20 ◦C until it was required for use in the study.

When preparing the culture medium for the BMSCs, the autologous serum was thawed and added to the medium. This addition contributed to the creation of a specialized culture medium, providing essential nutrients and growth factors necessary for the optimal growth and maintenance of the harvested BMSCs. The final concentration of autologous serum in the culture medium was set at 10%, aligning with established protocols for cell culture.

By utilizing autologous serum in the culture medium, the study aimed to create an environment that closely mimicked the physiological conditions of the subjects, fostering

the growth and expansion of BMSCs in a manner that would be well-tolerated upon transplantation. This emphasis on personalized serum not only reflected a commitment to patient-specific approaches but also aimed to enhance the overall safety and success of the subsequent cellular interventions in the study.

2.6 Harvest and Expansion of Bone Marrow Stromal Cells

The pivotal phase of this study involved the intricate process of harvesting and expanding bone marrow stromal cells (BMSCs) from the iliac bone crest. This procedure was performed under local anesthesia, ensuring patient comfort during the bone marrow aspiration.

The bone marrow, a rich source of multipotent stromal cells, was aspirated using a syringe containing 500 U of heparin, with 10 mL of marrow reserved for subsequent flow cytometric analyses. The remaining aspirated bone marrow underwent careful processing to dilute it four-fold with α-MEM (Gibco BRL, Grand Island, NY, USA). This dilution was achieved using a medium supplemented with 10% autologous serum, 2.5 µg/mL of amphotericin B, and 50 µg/mL of gentamicin sulfate.

The adherent cells, recognized as BMSCs, were selectively cultured in 150 cm2 flasks (Corning, New York, NY, USA). Non-adherent cells were systematically removed during routine medium changes, and adherent cells were maintained in the same medium under controlled conditions of 37 ◦C and a 5% CO2 atmosphere. Throughout the culture period, these cells exhibited a characteristic spindle shape, attesting to their identity as BMSCs.

Upon reaching 80% confluency, the BMSCs were sub-cultured into larger flasks, and this process was repeated until reaching the desired cell passage. At passage 2, the BMSCs underwent induction of osteogenic differentiation, a critical step for the intended applications in bone regeneration.

The osteogenic induction medium, consisting of specific factors such as 10 nM dexamethasone, 100 µM ascorbic acid, and a culture medium enriched with autologous serum, amphotericin B, and gentamicin sulfate, was applied to the cells for a period of 7 days. The induction was designed to guide the BMSCs towards adopting an osteogenic lineage, a crucial aspect for their subsequent effectiveness in bone regeneration applications.

Confirmation of osteogenic differentiation was achieved through detailed alkaline phosphatase (ALP) activity analysis. This involved incubating harvested cells with a buffer solution and Triton X-100 for protein extraction, followed by assessing ALP-specific activity through the conversion of p-nitrophenyl phosphate to p-nitrophenol. The resulting data, quantified using a spectrophotometer, provided valuable insights into the successful induction of osteogenic differentiation in the cultured BMSCs. This process ensured that the transplanted cells would contribute effectively to bone regeneration, a fundamental outcome in the context of this study.

2.7 Safety Tests

Ensuring the safety of the cell culture medium was of paramount importance in this study, and a rigorous series of tests were implemented to assess various potential sources of contamination.

Bacterial and Fungal Contamination: Regular safety checks for bacterial and fungal contamination were conducted on the culture medium. These checks involved aerobic and anaerobic cultures performed every two weeks and, critically, prior to any cell transplantation procedures. The goal was to promptly identify and address any microbial presence that could compromise the safety of the cell culture.

Mycoplasma Contamination: Before cell transplantation, the culture medium underwent thorough testing for mycoplasma contamination. The extraction of DNA from the prepared medium was conducted using phenol: chloroform: isoamyl alcohol (PCI), followed by a series of steps involving isopropanol and ethanol. A two-step PCR reaction was then performed to detect the presence of mycoplasma. The absence of mycoplasma contamination was essential to prevent any potential adverse effects associated with this type of microbial presence.

Endotoxin Testing: The culture medium underwent meticulous testing for endotoxins before cell transplantation. This involved diluting samples in normal saline solution and subjecting them to the Endosafe PTS system. Samples containing endotoxin levels greater than the specified threshold were deemed positive, prompting further investigation and corrective actions. This stringent testing procedure aimed to eliminate the risk of endotoxin-related complications associated with the cell transplantation process.

These comprehensive safety tests collectively ensured that the culture medium used for the expansion and induction of BMSCs was free from bacterial, fungal, mycoplasma, and endotoxin contamination. By adhering to these stringent safety protocols, the study aimed to minimize any potential risks or adverse events related to the transplantation of cultured cells, thereby safeguarding the well-being of the study participants.

2.8 Flow Cytometry

Flow cytometry played a pivotal role in characterizing the Bone Marrow Stromal Cells (BMSCs) and gaining insights into their specific surface marker expression. This method utilized specific antibodies targeting CD34, CD45, and CD73 to provide a detailed and quantitative analysis of the cell population.

Antibodies Used: Phycoerythrin-conjugated (PE-) and allophycocyanin-conjugated (APC-) antibodies were employed against CD73. A biotinylated antibody targeting CD45 was used, with detection accomplished using streptavidin Pacific Blue. CD34 expression was examined using specific antibodies.

Detection of Dead Cells: Propidium iodide was incorporated into the analysis to identify and exclude any non-viable cells. This ensured that the flow cytometric results focused on the viable and actively functioning cell population.

Controls: To enhance the specificity of the analysis, color-conjugated mouse-IgG1k was utilized as a negative control for each antibody. This control helped distinguish true antibody-specific signals from background noise, ensuring the accuracy of the flow cytometric data.

Data Analysis: Flow cytometric data were analyzed using FlowJo software. This allowed for the precise quantification and characterization of the BMSCs based on their expression of CD34, CD45, and CD73. The combination of these markers provided a unique fingerprint for the BMSCs, aiding in understanding their identity and potential therapeutic relevance. By leveraging flow cytometry, the study could discern and quantify the specific subpopulations of BMSCs based on surface marker expression. This information was crucial for ensuring the consistency and quality of the transplanted cell population and contributed to the overall safety and efficacy assessment of the cell transplantation process.

2.9 Preparation of Transplants

The day of transplantation involved a meticulous preparation process to ensure the viability and effectiveness of the therapeutic transplants. The key steps involved in the preparation of transplants are outlined below:

Autologous Platelet-Rich Plasma (PRP) Generation: On the day of transplantation, 60 mL of peripheral blood was collected from the patient. This blood was then processed to extract autologous PRP, a rich source of growth factors and platelets essential for tissue regeneration. The PRP preparation utilized a specialized kit (Smart Prep, Harvest Technologies Corp., Plymouth, MA, USA) following the manufacturer's protocol.

Harvesting and Counting BMSCs: The Bone Marrow Stromal Cells (BMSCs), previously cultured and expanded from the iliac bone crest, were detached using TrypLETM Select. A precise cell count was performed using a hemocytometer to determine the cell concentration for subsequent steps.

Alkaline Phosphatase (ALP) Assay: Prior to transplantation, a portion of the harvested BMSCs underwent an ALP assay, confirming their osteogenic differentiation and activity. This assay served as an additional quality control step to ensure the therapeutic potential of the transplanted cells.

Combination with Autologous PRP: The harvested BMSCs were then combined with the autologous PRP. This combination aimed to enhance the regenerative capabilities of the BMSCs, leveraging the growth factors present in the PRP to promote tissue healing and augmentation.

Addition of β-Tricalcium Phosphate (β-TCP) Granules: To provide structural support and a scaffold for tissue regeneration, the BMSC-PRP mixture was further combined with β-Tricalcium Phosphate (β-TCP) granules. This composite formulation ensured a well-supported environment for cell attachment, proliferation, and subsequent tissue regeneration.

Gel Formation: The PRP/cell mixture, along with β-TCP granules, was combined with autologous thrombin and 10% $CaCl_2$ to induce gel formation. This gel served as a carrier for the transplanted cells and provided a three-dimensional matrix for their integration into the target tissues.

Transplantation: The final gel-β-TCP-BMSC-PRP composite was then transplanted into the target sites. The specific transplantation sites included the maxillary sinus floor and alveolar ridge, addressing the predetermined defects and augmenting the bone structure. This comprehensive preparation protocol ensured the synergy of autologous PRP, BMSCs, and β-TCP granules, optimizing their regenerative potential and enhancing the success of the transplantation process.

2.10 Surgical Procedure

The surgical procedures were conducted with precision and adherence to established protocols, encompassing sinus floor elevation and alveolar ridge augmentation. The detailed steps of the surgical procedure are elucidated below:

Anesthesia and Sedation: The transplantation procedures were executed under the administration of local anesthesia to ensure the comfort of the patient. Additionally, intravenous sedation with propofol was employed, contributing to a controlled and relaxed surgical environment.

Sinus Floor Elevation: For patients undergoing sinus floor elevation, the lateral sinus wall was carefully opened, and the sinus floor mucous membrane was methodically separated and elevated inward with the accompanying bone fragment. This technique

created a space between the sinus floor and the elevated membrane, facilitating the subsequent transplantation process.

Alveolar Ridge Augmentation: In cases necessitating alveolar ridge augmentation, the mucoperiosteal flap was elevated, and the transplant was meticulously placed on the atrophic alveolar ridge. This targeted the specific areas where dental implant installation was planned. The transplant was then covered with a Goretex membrane (Goretex® TR Membrane, Japan Goretex Co. Ltd., Tokyo, Japan), securing it in place and optimizing the environment for tissue regeneration.

Membrane Removal and Implant Installation: For alveolar ridge augmentation cases, the Goretex membrane was removed before dental implant installation. The subsequent step involved the careful installation of dental implants six months after the initial transplantation. This timeline allowed for sufficient tissue healing and integration of the transplants into the existing bone structure.

Muco-periosteal Flap Repositioning and Suturing: Following the completion of the transplantation and implant installation, the muco-periosteal flap, whether used in sinus floor elevation or alveolar ridge augmentation, was repositioned meticulously. Suturing was performed to secure the flap in its new position, ensuring optimal coverage and protection of the transplanted material. This surgical protocol, meticulously executed under local anesthesia and intravenous sedation, aimed at achieving successful sinus floor elevation and alveolar ridge augmentation. The subsequent installation of dental implants after a specified duration allowed for the integration of transplanted materials, contributing to long-term stability and functionality. The procedure followed established guidelines for optimal outcomes and patient safety.

2.11 Evaluation

The comprehensive evaluation process encompassed multiple modalities and time points to assess the effectiveness and progress of the transplantation procedures. The evaluation components are outlined below:

Radiographic Evaluation: Panoramic X-rays were employed as a diagnostic tool before the operation and at specific intervals, including 6, 12, and 24 weeks, and 1 and 2 years post-operation. These X-rays provided a detailed visual representation of the treated areas, facilitating the monitoring of changes in bone structure and density.

Computed Tomography (CT) Scans: CT scans were conducted at critical junctures, including before the operation, at 6 months, and 1 and 2 years post-operation. The utilization of CT scans allowed for a three-dimensional assessment of the regenerated bone. SimPlant software was utilized for precise quantification of the amount of regenerated bone, providing valuable insights into the spatial distribution and density of the new bone tissue.

Bone Biopsy: At the crucial 24-week mark post-cell transplantation, bone biopsies were performed concurrently with dental implant installation. A trephine bur (2 mm inner diameter and 3 mm outer diameter) facilitated the extraction of biopsy samples, ensuring a representative specimen for subsequent analysis.

Histomorphometric Analysis: The collected biopsy samples underwent histomorphometric analysis, a meticulous examination of tissue structure and composition. This analysis included the assessment of new bone area, the area of remaining scaffold, the area of fibrous tissue, and the area of bone marrow-like tissue. ImageJ software was employed for precise measurements, and the results were expressed as a percentage of the total area of the section. This multifaceted evaluation approach, integrating radiographic assessments, CT scans, and histomorphometric analysis, provided a comprehensive understanding of the bone regeneration process. The

combination of qualitative and quantitative data allowed for a nuanced interpretation of the outcomes, contributing to a robust assessment of the success and efficacy of the transplantation procedures.

2.12 Long-Term Follow-Up

A subset of the study participants, specifically five out of the initial eight subjects, underwent an extensive long-term follow-up to gauge the enduring success and stability of the implemented interventions. The key components of this long-term follow-up are elucidated below:

Subjects Selected for Long-Term Follow-Up: Out of the original cohort of eight subjects, five were chosen for the long-term follow-up based on their availability and willingness to participate. These individuals, having previously undergone the transplantation procedures, became the focal point for an extended observation period.

Duration of Follow-Up: The long-term follow-up spanned a notable duration of 7 years post-transplantation. This extended timeframe facilitated a thorough examination of the sustained outcomes and allowed for the identification of any potential changes or developments over an extended period.

Assessment of Dental Implant Survival: A pivotal aspect of the long-term follow-up was the evaluation of dental implant survival. The integrity and longevity of the dental implants, which had been installed into the regenerated alveolar bone during the earlier phases of the study, were systematically assessed.

Imaging Analysis: To complement the clinical evaluations, imaging analysis was conducted during the long-term follow-up. Panoramic X-rays and potentially other imaging modalities were employed to scrutinize the condition of the dental implants and the regenerated bone. This comprehensive imaging analysis offered a visual perspective on the enduring stability and structural integrity of the treated areas. The long-term follow-up, extending to 7 years, served as a critical juncture to appraise the sustained success of the transplantation procedures. By focusing on dental implant survival and employing advanced imaging techniques, this phase of the study provided valuable insights into the long-term efficacy and durability of the implemented regenerative strategies.

2.13 Ethical Considerations

The study unequivocally adhered to the principles set forth in the Declaration of Helsinki, a cornerstone document guiding ethical conduct in medical research involving human subjects. This commitment underscored the researchers' dedication to upholding the rights, well-being, and confidentiality of the study participants. Prior to participation, all subjects involved in the study provided written informed consent. This crucial ethical requirement ensured that participants were adequately informed about the study's objectives, procedures, potential risks, and benefits. The voluntary and informed nature of participant consent underscored the respect for individual autonomy and decision-making.

3. Results

Study population

Ten individuals who had their bone marrow aspirated. There were two men and six females among the research subjects. Two cases, however, had issues: in one, the total quantity of cells did not match the threshold for the research procedure, and in another, there was a suspicion of bacterial contamination of the serum, which resulted in the cells

being discarded. Sadly, it was necessary to remove these two individuals from the trial because they refused to have a second bone marrow aspiration. Table 1 shows that of the eight patients that were left for cell transplantation, two were male and six were female, with an average age of 54.2 years. Alveolar ridge augmentation was done at four locations and sinus floor elevation was done at nine sites in seven individuals. Furthermore, in two instances were both operations carried out.

Table 1. Summary of patient information.

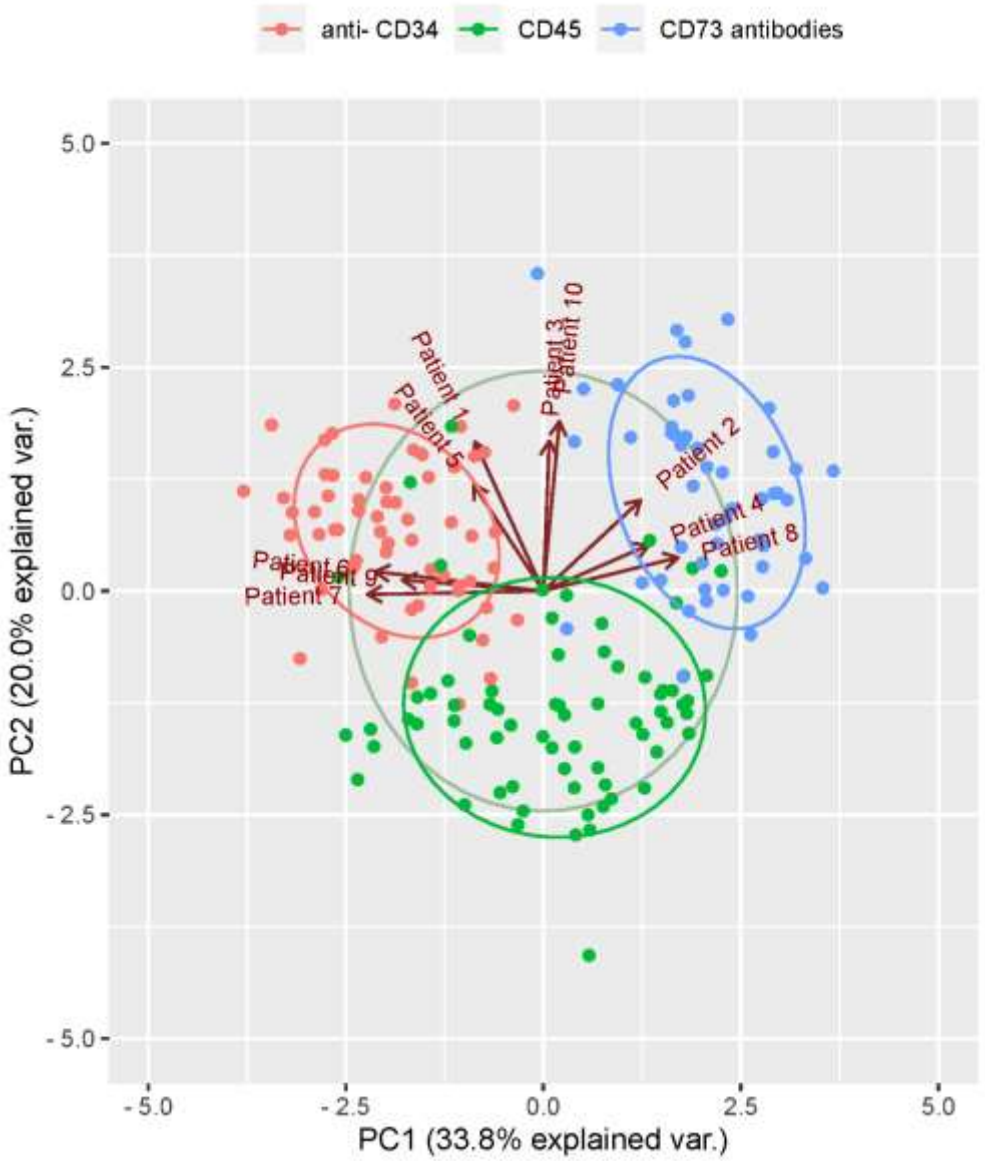
No.	Age	Sex	Target Region and Implants	Cell No. (10^4/uL)	PRP (mL)	Plt. Count	β-TCP (g)	No. of Procedure	($\times 10^6$)
1	40	F	rt. SFA	13.3	2.8	ND	2.0	2	
2	52	M	rt. SFA	8.2	3.0	ND	1.5	2	
3	53	F	lt. SFA	6.0	3.5	73	2.0	3	
4	52	F	lt. SFA	26.8	2.7	49.3	1.4	2	
5	51	F	-	-	-	-	-	-	
6	38	F	-	-	-	-	-	-	
7	63	M	rt. SFA	39.6	4.0	57.3	1.0	4	
8	57	F	bil. SFA 22~23 ARA	4.5	8.0	ND	3.0	6	
9	60	F	15~12, 23~24 ARA.	5.2	4.0	ND	1.0	5	
10	57	F	bil. SFA 24~25 ARA.	21.1	10.0	80.2	3.0	5	

Ten patients were entered; however, patients no. 5 and 6 were dropped because of the risk of contamination in the serum and insufficient cell numbers, respectively. Sinus floor augmentation (SFA) and/or alveolar ridge augmentation (ARA) were performed in 7 and 3 cases, respectively. rt: Right side, lt: left side, bil: bilateral side, PRP: platelet-rich plasma, Plt.: platelet, β-TCP: tricalcium phosphate, ND: not detected.

Cell fraction analysis of BMA

The study utilized flow cytometry with anti-CD34, CD45, and CD73 antibodies to investigate the fraction of Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) within the whole bone marrow aspirate. Figure 1 The findings revealed that the major population of BMSCs fell within the CD34-, CD45dim, and CD73+ fraction, constituting only 0.065% of the total bone marrow cells. Critically, this proportion was notably lower than the putative fraction of hematopoietic stem cells (CD34- and CD45-), which accounted for 0.89%. This significant difference in percentages suggests that BMSCs constitute a relatively minor subset within the bone marrow aspirate compared to hematopoietic stem cells. While the study sheds light on the phenotypic characterization of BMSCs, acknowledging the limitations is essential. The low percentage of BMSCs within the bone marrow may pose challenges in terms of isolation and utilization. The study could benefit from discussing potential implications and practical considerations associated with the low abundance of BMSCs. Additionally, the study mentions that the characteristics of the cultured BMSCs were not defined with the samples used in this particular investigation. This lack of characterization may limit the comprehensive understanding of the isolated BMSCs in this specific study. It would be beneficial to provide more context on the relevance of these findings, especially in comparison to the results from the previous study that used the same protocol. Understanding the consistency or variations between studies could enhance the reliability and generalizability of the findings. Furthermore, the results from the previous study, which indicated that the dominant cell type was positive for CD10, CD29, CD73, CD90, CD146, and HLA-ABC, and negative for CD3, CD14, CD19, CD34, and CD45, could be discussed in more detail. Elaborating on how these characteristics align with the observed phenotypic markers in the current study would provide a more comprehensive understanding of the BMSCs and their potential applications.

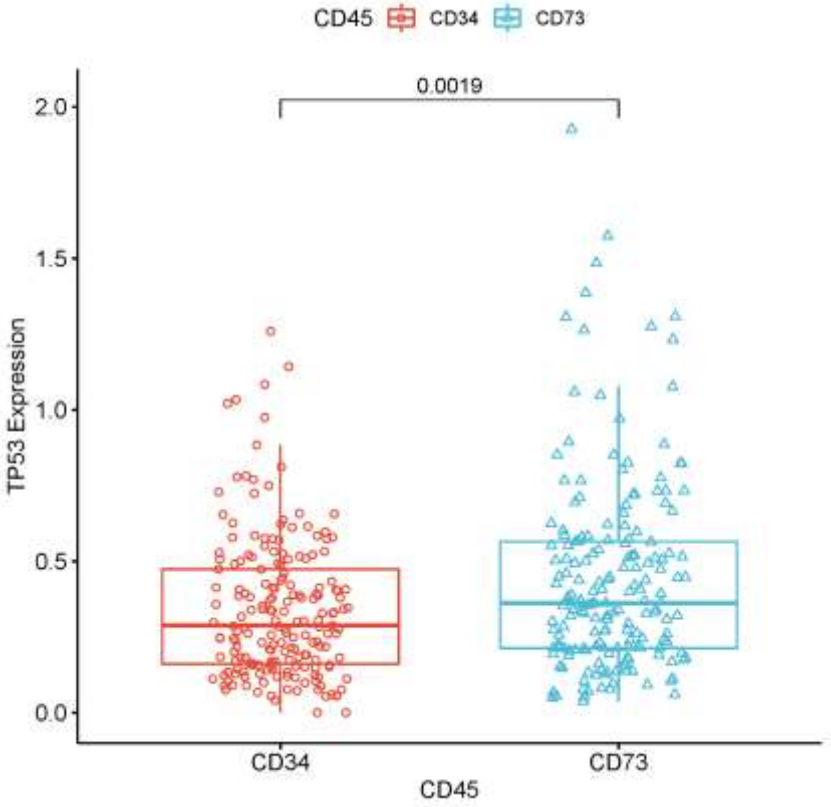
Figure 1: flow cytometry with anti-CD34, CD45, and CD73 antibodies to investigate the fraction of Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) within the whole bone marrow aspirate.



Cell growth and differentiation

The average cell number at the time of transplantation, specified as 1.6 × 10^7 cells, provides a quantitative measure of the cells administered during the transplantation process. This numerical value is crucial for understanding the scale of the procedure. The statement goes further by acknowledging the variability in cell proliferating capability among individuals, emphasizing that not all patients exhibited the same rate of cell proliferation. This individualized response may be attributed to inherent biological differences, health status, or other factors influencing cell behavior. Moreover, the statement delves into cell differentiation, explaining that despite implementing the exact same induction protocol, there were variations in the final harvested cell number and ALP activity levels. Cell differentiation is a critical aspect of stem cell therapy, as it determines the types of cells that the transplanted cells can mature into. The mention of ALP activity specifically suggests an interest in the osteogenic potential of the cells, as ALP is often associated with bone-forming activity. Figure 2

Figure 2: Total cell number (CD34) and alkaline phosphatase (ALP) activity (CD73) of the cultured cells at harvest before transplantation. ALP activity was expressed as µmol p-nitrophenol/min/µg protein.



Sinus Floor Augmentation (SFA) Procedure

In the case of Sinus Floor Augmentation (SFA), the procedure involved creating a space (figure 3) that was subsequently filled with a transplant comprising Platelet-Rich Plasma (PRP) gel combined with Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) and β-TCP (beta-tricalcium phosphate) granules, as previously described. This composite material was used to facilitate the regeneration of bone in the sinus floor region. (figure 4-6)

Figure 3: Sinus Floor Augmentation (SFA), the procedure involved creating a space that was subsequently filled with a transplant

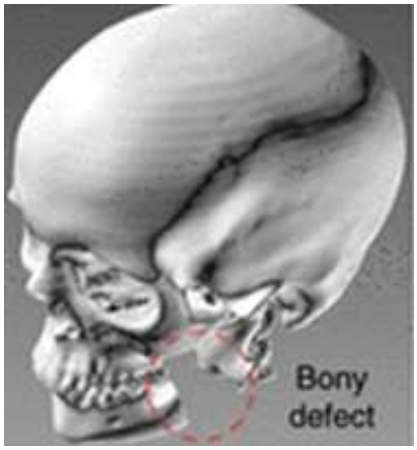


Figure 4: 3D print module of the composite material was used to facilitate the regeneration of bone in the sinus floor region

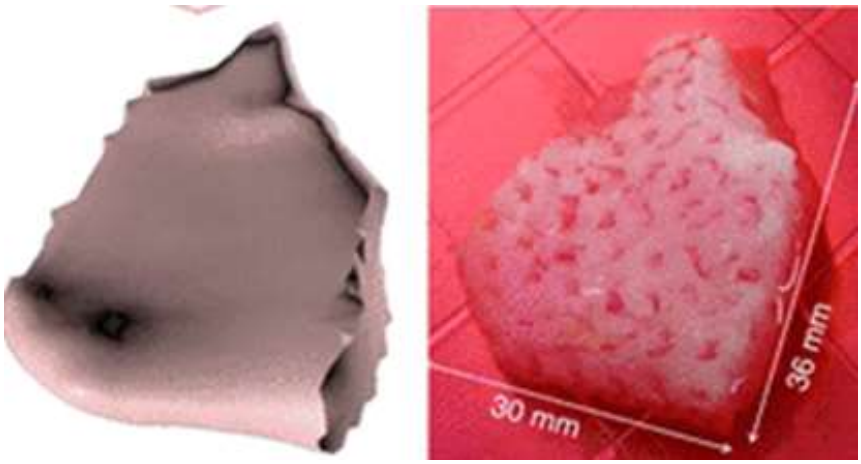


Figure 5: Thermal analysis of Platelet-Rich Plasma (PRP) gel combined with Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) and β-TCP (beta-tricalcium phosphate) granules

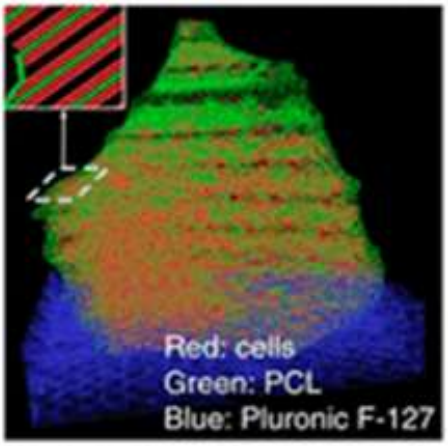
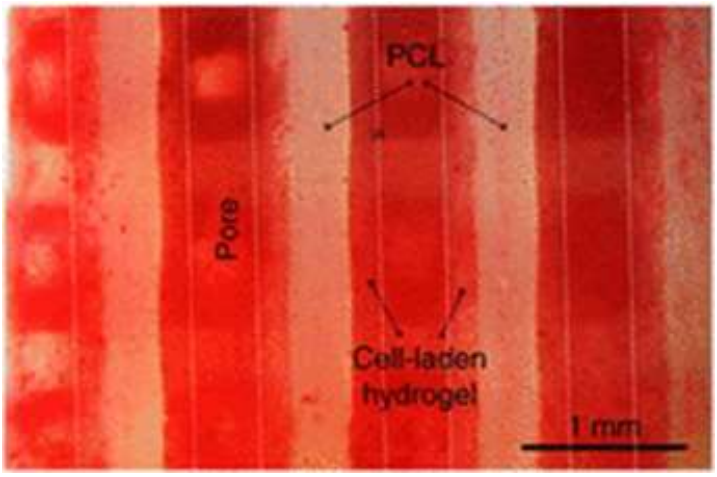


Figure 6: Microscopic magnification of transplant comprising Platelet-Rich Plasma (PRP) gel combined with Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) and β-TCP (beta-tricalcium phosphate) granules



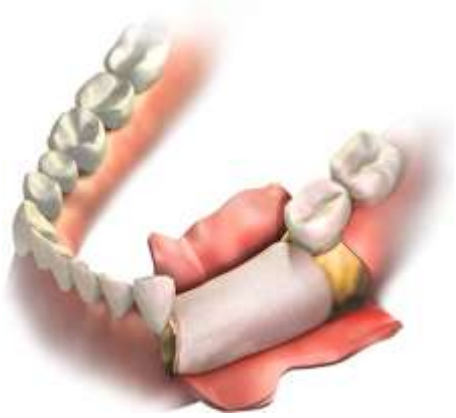
Post-Transplantation Evaluation

Six months after the cell transplantation, a mucoperiosteal flap was re-elevated. At this point, the regenerated bone was observed, indicating successful outcomes of the transplantation procedure. Subsequent to the observation, drilling was performed in the maxilla, and dental fixtures were installed. However, it is noteworthy that the statement mentions variations in the texture and hardness of the regenerated bone among cases.

Alveolar Ridge Augmentation (ARA) Procedure

For Alveolar Ridge Augmentation (ARA), subjects with severely atrophic alveolar ridges were recruited. The procedure involved guided bone regeneration, where the transplant material was applied to foster bone regrowth. Six months post cell transplantation, the mucoperiosteal flap was re-elevated, revealing a surface covered with relatively hard bone. Subsequently, drilling was performed, and dental implants were installed. Similar to the SFA procedure, the statement indicates variability in the hardness of the regenerated bone following ARA. Figure 7

Figure 7: Alveolar Ridge Augmentation (ARA), subjects with severely atrophic alveolar ridges were recruited. The procedure involved guided bone regeneration, where the transplant material was applied to foster bone regrowth. Six months post cell transplantation, the mucoperiosteal flap was re-elevated, revealing a surface covered with relatively hard bone.



CT Imaging Analysis

CT images from representative cases were included, showcasing the timeline of imaging before the operation, at 6 months, and at 12 months after cell transplantation. In the cases of Sinus Floor Augmentation (SFA) and Alveolar Ridge Augmentation (ARA), the images revealed that the borderline between the transplants and the original bone was still visible at 6 months but became almost continuous after 12 months, indicating a gradual integration of the transplanted material with the existing bone.

Volume Analysis

The study employed CT data and software to analyze the volume of regenerated bone at 6, 12, and 24 months after cell transplantation. The analysis revealed a general decrease in volume over time, with individual variations in the time course. On average, the volume of regenerated bone was 75.2% at 1 year and 62% at 2 years after transplantation compared to that at 6 months. Figure 8

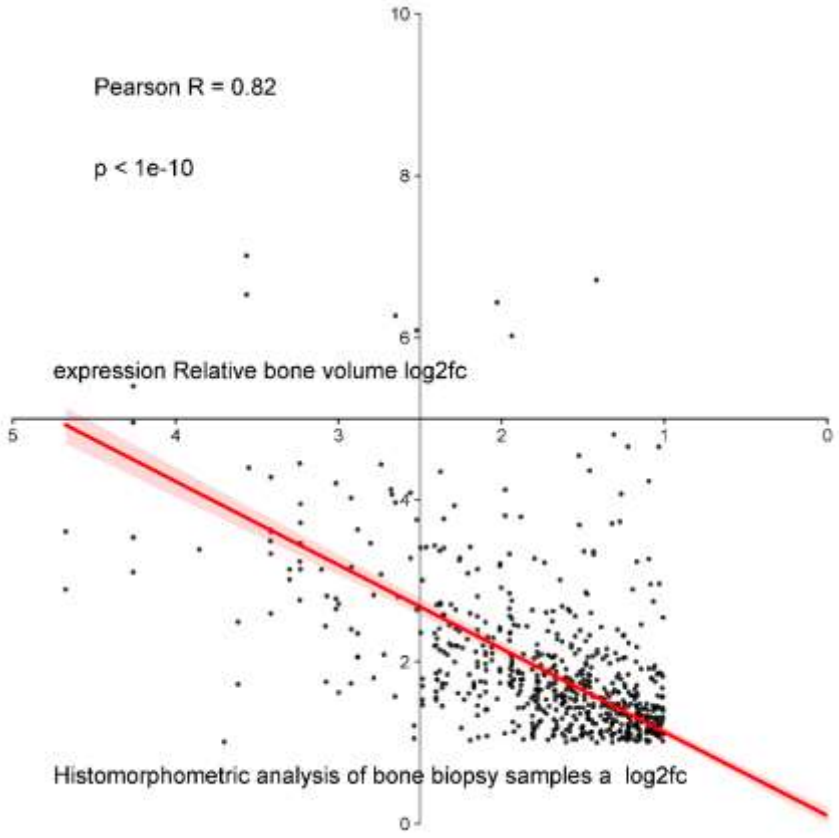


Figure 8: CT data and software to analyze the volume of regenerated bone at 6, 12, and 24 months after cell transplantation. The analysis revealed a general decrease in volume over time

Bone Biopsy and Histological Analysis

Bone biopsies were performed at 6 months at the site of implant installation using a trephine bur. Histological analysis, involved non-decalcified sections stained with H&E, Villanueva bone staining, and Villanueva–Goldner staining. While bone formation was observed in all cases, the area of new bone formation and remaining scaffold varied among patients. Bone formation was predominantly observed adjacent to the scaffold. The average new bone area was 41.9 ± 27.8% at 6 months, with variability among individuals.

Implant Installation and Integration

A total of 29 dental implants were installed in the regenerated bone, with 27 showing integration (93%). Two implants were removed during the abutment connection process, and there were no signs of infection or pathological changes in cases of unintegrated implants. The follow-up period, spanning 45–66 months with an average of 56 months after cell transplantation, reported no trouble for the integrated implants.

Long-Term Follow-Up and Health Outcomes

During the treatment and follow-up period, which extended up to approximately 66 months after cell transplantation, there were no noted side effects or health concerns. This indicates the stability and safety of the treatment over an extended period, highlighting the long-term success and biocompatibility of the cell transplantation approach for bone regeneration.

4. Discussion

In participants with severely atrophic maxilla in need of bone transplantation, the study shows remarkable success in achieving both dental implant placement and bone regeneration. Participants in the research had significantly atrophic maxilla, a difficult condition that frequently necessitates bone grafting in order to facilitate dental implants.[13] Under local anesthetic, the crucial stage in the bone regeneration process—the bone marrow aspiration—was completed quickly—in only thirty minutes. Crucially, there were no unfavorable outcomes seen during or following the bone marrow aspiration process, proving its viability and safety. This helps the bone regeneration strategy work better overall. The study emphasizes the benefits of employing BMSCs for bone tissue engineering, pointing out that the more conventional method of collecting autologous bone is accompanied with difficulties. [14]

On the other hand, using BMSCs provides a less intrusive option, examined were the safety and clinical effectiveness of alveolar bone tissue engineering with BMSCs; during the treatment and follow-up periods, no transplant-related problems were noted.[15] A portion of the patients underwent follow-up for more than eight years following transplantation, which gave important information on the procedure's long-term results. The long-term follow-up showed that the alveolar bone that had grown back was stable. This is a crucial evidence of the procedure's effectiveness and capacity to provide long-lasting effects.[16]

Patients who underwent long-term observation also had stable dental implants, indicating that the integration and placement of the implants were made possible by the bone regeneration. The work is consistent with other studies on BMSC-based bone tissue synthesis, where there were no known issues. [17] This uniformity across research indicates a strong safety profile for the method and enhances the validity of the findings. Recognize that the study only included a small number of instances and that the conclusions are based on a subgroup of eight people. Because of the small sample size, a careful interpretation is necessary despite the encouraging results. While acknowledging the need for more research with bigger cohorts, this acknowledgment encourages scholars to view the work as a worthwhile addition.[18]

This study evaluated the effectiveness of the bone regeneration process in detail, offering important new information on the properties and outcome of the tissue-engineered bone. Bone regeneration was shown in each of the eight patients that were part of the study, showing a 100% success rate in reaching the principal aim of the transplant protocols.[19]

At six months after transplantation, histomorphometric studies showed an average bone area of 41.9%, giving a numerical indication of the degree of bone regeneration attained during the first evaluation period. The paper states that the reported new bone regions are nearly equivalent to those found in trials utilizing autologous bone grafts for sinus floor elevation, notwithstanding the possibility that direct comparisons with other studies may be difficult due to differences in methodology and patient demographics.[20]

The average new bone area in trials involving autologous bone transplantation to the sinus floor or alveolar ridge varied from 31.2% to 37.7%. The new bone area following autologous bone transplantation with calcium phosphate was found to be 44.24% ± 13.79%.

The research notes that in situations of autologous bone transplantation, absorption is a major problem. Limited data are available on the stability of tissue-engineered bone compared to autologous bone transplants. In one research, graft height reductions of 67.8–80.7% were seen after one year and 55.8–72.2% after five years,

utilizing autologous bone grafts to the sinus. Although direct comparison is problematic, the study finds that absorption happens in the tissue-engineered bone comparable to autologous bone transplants. The characteristics of the scaffold material used during the transplantation procedure may have an impact on the tissue-engineered bone's rate of absorption.[21]

A study that was cited showed that engineered bone made from human cells seeded into a resorbable scaffold made of polyglycolic and polylactic acid underwent 90% resorption. Within two years of the transplant, host bone should take the place of the β-tricalcium phosphate (β-TCP) utilized in the procedure. Following regeneration, the regenerated bone could remodel with native bone, signifying a dynamic process of integration and change over time. Analysis of Decreasing Rates: The findings show that the enhanced bone decreased at a faster pace during the first two years than it did during the next six. This discovery implies that the early phases' usage of β-TCP may be replaced relatively rapidly, with a more stable remodeling phase occurring in the years that follow.[22, 23]

The study's conclusions highlight a number of fascinating details about the features and temporal alterations of the bone that regenerates after cell transplantation, particularly with regard to β-tricalcium phosphate (β-TCP) granules and their effect on bone regeneration as well as the integration of dental implants that follows. After cell transplantation, significant changes were seen in CT scans six months and a year later. In most cases, at six months, it was still easy to distinguish the transplants from the surrounding bone. After a year, though, the boundary was hardly noticeable. It is proposed that this alteration reflects the β-TCP granules' disintegration process, pointing to a dynamic development of the transplanting site.[24, 25]

Six months following cell transplantation, histological examinations showed bone regrowth. The β-TCP breakdown process persisted for a full year, during which time the regenerated bone progressively matured. According to CT studies, the bone that developed after a year resembled normal bone tissue. This discovery suggests that the regenerated bone has successfully integrated and matured, taking on traits similar to those of natural bone. The percentages of newly formed bone area seems to be correlated with the regenerated bone's mechanical strength. This association could have had an impact on dental implants' early stability.[26, 27]

Fascinatingly, effective dental implant integration happened at the moment of abutment connection, which happened six months following implant installation, even in situations where the regenerated bone exhibited reduced new bone area and minimum implant stability at that time. After six months, the bone texture in early bone regeneration instances was observed to be similar to native bone. It is proposed that osteoconduction, rather than osteogenesis or osteoinduction, occurred in patients with delayed bone regeneration. This suggests that bone was formed throughout the regeneration process under the direction of pre-existing bone structures. Two implants in the trial failed to integrate. The study indicates that variations in bone quality or the implant placement technique may have contributed to implant failure, even if the dental implants' initial stability was adequate and infection was not visible.[28]

According to the study, BMSCs made up more than 0.065% of the total bone marrow, which is a rather tiny percentage. Owing to the restricted portion, it was determined that in vitro growth was required in order to generate an adequate quantity of cells for transplantation. After adhering to the study's methodology, the increased BMSCs were classified as multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs). According to earlier publications, this characterisation was based on the expression of cell surface markers. There were differences in the in vivo

bone quality and in vitro cell characteristics among the patients despite the overall identification. The gender, age, or implantation place of the patient did not appear to be associated with these variances. According to the study, a number of variables, including the anatomical setting, the surgical technique, the amount of the transplant, and individual variations in the properties of the acquired cells, may have an impact on clinical effectiveness.[29, 30]

Limitations and Considerations:

The lack of a control group is one of the study's stated limitations. This suggests that, in addition to the transplanted cells, the scaffold biomaterial, β-TCP implantation alone, or platelet-rich plasma (PRP) may have aided in bone repair. The report acknowledges that PRP or only β-TCP may have contributed to bone repair. It implies that the vitality of transplanted cells may have an impact on bone regeneration and that living cells in transplants may not be the only factor impacting bone creation. The report highlights different approaches by referencing previous studies. For example, Rickert et al. showed the efficacy of bone marrow aspirates without cell culture, whereas Kaigler et al. reported on the utilization of CD90+ stem cells, highlighting the possibility for consistent outcomes. These contrasts highlight how different approaches and results may be found in the same subject. Although stem cell treatment is known to be safe, the study highlights the need for more research. To make this procedure a regular treatment for massive alveolar bone regeneration, more parameters need to be investigated, such as the type of scaffold to use and the methods used for cell preparation.

Implications:

The study implies that bone marrow-derived mesenchymal stem cells (BMSCs) in combination with a scaffold material (β-TCP) show promise in bone tissue engineering. This has implications for addressing atrophic maxilla conditions, providing an alternative to autologous bone grafts, and potentially reducing complications associated with traditional methods. The study suggests that bone tissue engineering with BMSCs is advantageous as a less invasive treatment compared to harvesting autologous bone, which is associated with complications. This has implications for patient comfort, reduced surgical risks, and potentially faster recovery. The observation of stable regenerated alveolar bone and dental implants over eight years in some cases suggests the long-term stability of the treatment. This has implications for the durability and effectiveness of the bone regeneration process using BMSCs. The study compares the new bone area after transplantation with outcomes reported in other studies using different techniques. This implies a need for comparative efficacy studies to understand how BMSC-based treatments measure up against other approaches in terms of bone regeneration.

Recommendations:

Given the observed individual variations in in vitro cell character and in vivo bone quality among patients, further research is recommended to understand the factors contributing to these differences. This could involve investigating the influence of anatomical factors, surgical procedures, and transplant volume. To address variations in cell characteristics and ensure consistent clinical efficacy, the study recommends the establishment of parameters for in vitro expansion. Standardizing protocols for obtaining and expanding BMSCs may contribute to more predictable outcomes. The study acknowledges the limitation of not having a control group. Future research is recommended to include control groups to better understand the specific contributions of BMSCs, scaffold biomaterial, and other factors in the observed bone regeneration. The study mentions the use of β-TCP as a scaffold material. Further investigation into the use of alternative scaffold materials and their impact on bone regeneration is recommended

to optimize the effectiveness of BMSC-based therapies. The study raises the possibility that PRP might have contributed to bone regeneration. Research focused on understanding the specific roles of various components, including PRP, in the bone regeneration process is recommended. To establish BMSC-based treatments as a standard therapy for large alveolar bone regeneration, the study recommends further clinical trials. These trials should involve larger sample sizes, control groups, and longer follow-up periods to validate the safety and efficacy of the approach.

5. Conclusion

Cell transplantation, specifically using Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) in combination with Platelet-Rich Plasma (PRP) gel and β-TCP granules, is a viable and safe approach for sinus floor augmentation and alveolar ridge augmentation. Despite variations in cell proliferation, differentiation, and bone regeneration outcomes among individuals, the procedure showed promising integration and stability, as evidenced by CT imaging, histological analysis, and successful dental implant integration. The long-term follow-up of approximately 66 months revealed no side effects or health concerns, supporting the overall efficacy and biocompatibility of the cell transplantation method for bone regeneration in dental applications.

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