ORIGINAL ARTICLE

Reduction of Osx expression in the osseointegration process of dental implants with human adipose-derived mesenchymal stem cell intervention: an experimental study

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ABSTRACT

Introduction: Peri-implantitis, an inflammatory response affecting the hard and soft tissues surrounding a dental implant, ultimately leads to a loss of osseointegration. Human adipose-derived mesenchymal stem cells (hADMSCs), sourced from adipose tissue, have high biocompatibility and regenerative capacity, with advantages such as self-renewal, plasticity, and multilineage differentiation. Alveolar bone repair using hADMSC has been demonstrated in periodontitis animal models through the STRO-1, RUNX-2, Osx (Osterix), and COL-I pathways. This study utilized hADMSC intervention to analyze Osx's impact on the osseointegration process of Y-TZP. **Methods:** This experimental study employed Wistar rats, divided into six treatment groups (three for week 1 and three for week 8) and one negative control group. The study design was a randomized post-test only control group. After Y-TZP scaffold implantation for one and eight weeks, histological analysis identified Osx expression on the mandibular bone surface of *Rattus norvegicus albinus* Wistar strain rats. Immunohistochemistry (IHC) results for Osx expression were compared using a One-Way ANOVA test for data analysis. **Results:** hADMSC cells reached optimal proliferation and maturity by passage 4, suitable for Y-TZP scaffold seeding. Characterization confirmed positive CD105, CD73, CD90, and negative CD45, CD34, CD14, CD19, HLA-DR expression. ANOVA ($p<0.05$) and post-hoc HSD showed significant Osterix differences, except between treatment groups P2, P3, and P4. **Conclusion:** Intervention with hADMSCs in Y-TZP dental implants can potentially overcome dental implant failure, as evidenced by a decrease in Osterix expression during osseointegration. This decreased Osterix expression facilitates osteoblast formation and promotes the homing of hADMSCs.

KEYWORDS

Osx expression, hADMSC, osteointegration, dental, implants

INTRODUCTION

Chewing, aesthetics, and speech are disrupted by tooth loss due to extraction. The cumulative effects of oral health issues throughout an individual's life have positioned oral problems as the tenth leading cause of "total years lived with disability" among adults over the age of 70.[1] As a vital component in dental restoration, teeth are often affected by severe trauma, foreign microorganisms, and genetic abnormalities.[2] Throughout life, untreated dental cavities and periodontitis, two of the world's most prevalent illnesses, lead to tooth loss, a decline in quality of life, and ultimately impaired masticatory function. Aging individuals and older adults may be disproportionately impacted.[3]

In addition to dysregulated host inflammation, a resulting dysbiotic microbiota promotes the development of specific microorganisms within the biofilm. These microorganisms produce compounds that exacerbate inflammation and can

occasionally lead to tissue deterioration and tooth loss.[4] Untreated dental caries and periodontitis, the most prevalent illnesses globally, contribute to tooth loss, reduced quality of life, and impaired masticatory function.[3]

A dental prosthesis can compensate for the loss of function. There are two primary varieties of dental prostheses: fixed and removable. Tooth loss is also recognized as one of the most significant causes of psychological trauma.[5] For patients who have lost teeth due to periodontal disease, rehabilitation using dental implants, fixed prostheses, and removable prostheses allows for restoring function and appearance.[6] The reliable and long-lasting performance of implant-supported prostheses has made the use of dental implants for replacing lost teeth both advantageous and increasingly common.[7] Individuals with edentulism (total tooth loss) or those with few remaining teeth that impede proper chewing often exhibit dietary changes at the end of the disease spectrum. They appear to favor softer diets containing more fat and carbohydrates and fewer fresh fruits and vegetables.[4] Full-arch implant-supported fixed dental prostheses offer advantages such as comfort and significant improvements in prosthetic function, stability, and adaptability when compared to conventional treatment options.[8]

Implants represent the optimal dexterous prosthesis. The implantation of dental implants is the most common procedure for replacing lost or missing teeth[9] Dental implants are a dependable rehabilitation option due to their excellent predictability. This, coupled with the established long-term effectiveness of implant-supported prostheses, has led to implant therapy being widely accepted by the general public.[10] Mechanical properties such as tensile and compressive strength, stress distribution at the implant-bone contact, and mechanical stability all influence an implant's long-term stability.[11] Thousands of edentulous individuals have received dental prostheses secured with titanium implants, which have improved their oral function, self-esteem, psychosocial functioning, and aesthetics.[12] Despite their high success rates, 5–11% of dental implants may fail within 10–15 years, necessitating their removal. This presents challenges for this treatment approach.[7]

Implants provide the best solution for chewing function and aesthetic appeal. Due to their benefits, which include flawless retention, minimal harm to neighboring teeth, and reduced foreign body sensation, dental implants have been extensively utilized in clinics to restore the appearance and functionality of lost teeth.[13] Titanium and titanium alloys are the most common materials used for dental implants, owing to their advantageous biocompatibility and excellent mechanical and physical characteristics.[14] Several factors, such as diabetes mellitus, smoking history, sulcus bleeding index, periodontal probing depth, and loss of bone mineral density,[15] influence dental implant longevity.

Technological advancements, including morphological design, surface modification, and specific coatings, continue to increase the success rate of implant restoration.[16] The most aesthetically pleasing and practical substitute for lost teeth is osseointegrated dental implants.[17] Titanium particles found in peri-implant gingival tissues can alter the polarization of macrophages and lymphocytes. This phenomenon may help explain the imbalance in osteoblast–osteoclast activity, consequently dental implant osseointegration failure.[7]

Individual differences exist in the healing process of dental implants. Microbial residues prevent re-osseointegration and wound healing.[18] Due to their lack of primary stability, dental implants might require an extended healing period following implantation into the alveolar bone to prevent repair failure.[19] A shorter healing time would be required for the new bone to attach to the implant. Moreover, angiogenesis is another factor influencing total recovery time, as tissues possess a rich vascular system.[2] Cytokines and pro-inflammatory cells can trigger local inflammation, potentially impairing bone healing and regeneration, and prevent bone integration.[20] On the implant surface, increased cell adhesion and proliferation may ultimately enhance osteogenic differentiation, improving bone regeneration and the healing process.[21]

Dental implants exhibit a high failure rate. While oral implants are a reliable and secure therapeutic option for tooth restoration, incidents of failure occur.[22] Despite their potential longevity, standard metallic alloy implants have disadvantages. Patients may experience significant bone loss around the implant due to bacterial infections in the micro-gaps caused by inadequate soft tissue attachment.[23] Typically, early failures occur before osseointegration and prosthetic loading, whereas late failures happen after loading.[9] Changes in inflammatory cytokines, implant survival rate, and periodontal indices were evaluated in individuals with chronic periodontitis and those with healthy periodontal tissue.[15]

Cytokine-induced inflammation can prevent osseointegration and cause implant loss.[17] Furthermore, some implant recipients have a history of periodontitis. Both history of periodontitis and bacterial presence are risk factors for peri-implant infections.[24]

Peri-implantitis is a major cause of implant failure. It is an inflammatory response to infection[25] and is commonly linked to implant failure. An imbalance between the host immune response and bacterial load is believed to underlie peri-implantitis (PI), which is characterized by soft tissue inflammation and bone resorption. Its impact on long-term implant success is one of the primary causes of dental implant failure.[13] The microbial biofilm that forms on implant surfaces initiates and sustains peri-implant inflammatory conditions, significantly damaging the surrounding soft and hard tissues.[26] With an incidence ranging from 9.25% to 46.83%, peri-implantitis poses a serious threat to the long-term viability of dental implant procedures.[27] It causes an inflammatory reaction in the hard and soft tissues surrounding a dental implant, ultimately leading to loss of osseointegration.[16]

Osseointegration varies from person to person. When osseointegration fails and the intended denture becomes encapsulated in connective tissue, the implant cannot secure it.[28] Osteointegration failure can occur in two phases: early, when it fails to develop, and late, when it deteriorates after a period of function. Factors such as bone quality and quantity, surgical trauma, and surgical contamination have been associated with early failures. Late failures have been attributed to peri-implantitis and occlusal overload syndrome. In addition, social and biological factors, including smoking and health issues, also contribute to implant failure.[17] In osteoporotic conditions, decreased trabecular bone thickness and increased trabecular separation lead to alveolar bone absorption and atrophy in both the upper and lower jaw.[29]

Inflammation around implants accompanies peri-implant bone loss.[18] This clarifies why dental implant osseointegration fails and why osteoblast–osteoclast activity becomes imbalanced.[7] The bone-implant contact (BIC) was calculated by dividing the length of the mineralized bone directly contacting the implant's total lateral length. BIC was calculated to evaluate the specimens' osseointegration performance, expressed as the ratio of the total contact lengths between the implant and the cortical and trabecular bones to the implant perimeter.[19]

Inflammatory factors and biomarkers can affect osseointegration. In both innate and adaptive immunity, glycolysis is the primary metabolic pathway for almost all pro-inflammatory immune cells.[20] First-generation biomaterials were selected for dental implants primarily for their mechanical and corrosion resistance. However, the potential immune responses and hypersensitivity reactions that may arise several years post-implantation, attributed to ion release and protein interactions with the implant surfaces, were not adequately addressed in the selection process.[30]

Inadequate surface characteristics that result in poor soft tissue repair around implants induce inflammation, infection, and dysregulated keratinocyte and macrophage activity.[25] This process can hasten tissue breakdown surrounding implants through the production of cytokines, proteases, and prostaglandins.[13] T and B lymphocytes, macrophages, endothelial cells, polymorphonuclear neutrophils, osteoclasts, osteoblasts, fibroblasts, and keratinocytes are among the

cell types that may trigger an abnormal immune-inflammatory response that destroys peri-implant and periodontal tissues.[17]

In addition to their capacity for superior-quality tissue repair and biocompatibility, human adipose-derived mesenchymal stem cells (hADMSCs), which are obtained from adipose tissue, offer advantages such as self-renewal, plasticity, and multilineage differentiation. Alveolar bone repair using hADMSC occurs in periodontitis animal models via the STRO-1, RUNX-2, OSX, and COL-I pathways.[30] Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is currently under extensive scientific and clinical investigation due to its exceptional mechanical, aesthetic, and biocompatible properties.[32]

Dental implants made of Y-TZP ceramic biomaterial show improved osteointegration with hADMSC intervention. The study's novelty lies in elucidating the mechanism accelerating mandibular bone regeneration following Y-TZP scaffold-hADMSC implantation. This study aims to analyze the reduction of Osx expression in the osseointegration process of dental implants with human adipose-derived mesenchymal stem cell intervention.

METHODS

This study is a true experimental investigation using Wistar rats as test animals, divided into six treatment groups: three groups at the first week (positive control/without treatment/K1, Y-TZP defects/P1, and defects with Y-TZP seeded with hADMSC/P2), and three groups at the eighth week (positive control/K2, Y-TZP defects/P3, and defects with Y-TZP seeded with hADMSC/P4), plus one negative control group (KN). Each group followed a randomized post-test-only control group design, with four samples per group, resulting in a total of 28 required samples. Adipose tissue was collected from a donor undergoing elective procedures without health risks at Airlangga University Hospital, Surabaya, after providing a thorough explanation and obtaining signed informed consent.

This method of extracting fat does not affect the area's function or appearance, as it collects fat from the upper surface while leaving the inner surface, along with other muscles and tissues, intact—within an area of approximately 5 cm^3. Isolation and expansion culture of hADMSC were performed at Universitas Airlangga's Center for Stem Cell Research and Development Laboratory, Surabaya.

Two immunostaining techniques—namely immunocytochemical staining and flow cytometry—were used to characterize the phenotype of mesenchymal stem cells cultured from hADMSC following the fourth passage. The hADMSC exhibited negative expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR, and positive expression of CD73, CD90, and CD105. CD90, CD105, and CD73 were confirmed by positive staining using the Accuri C6 Flow Cytometer (BD Biosciences, San Jose, CA, USA), while CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR were negatively stained. Commercially available kits (Cyagen Biosciences Inc., Sunnyvale, CA, USA) were then used to identify these cells in adipogenic, osteogenic, or chondrogenic conditions.

The Y-TZP implant used was a Type 2p, with dimensions (Ø = 2.9 mm, P = 3 mm) and included rod grooves and holes for attachment. For the hADMSC seeding process, the Y-TZP scaffold was immersed in DMEM/F12 medium for 24 hours. The medium was then replaced with fresh medium, and the scaffold was placed into a 24-well culture plate (M24). A 200 µL suspension containing 2 x 10^6 cells was added to each well. The mixture was incubated for one hour at 37^0 C in 5% CO^2. Afterward, an additional 1.3 mL of medium was added to each well, and the cells were further incubated at 37^0 C in 5% CO^2. To ensure even distribution of cells across the suspension and scaffold, the wells were shaken periodically over the next three days.

Osx expression was detected through histological analysis of the mandibular bone surface of *Rattus norvegicus* albinus (Wistar strain) following Y-TZP scaffold

implantation at one and eight weeks. Each tissue sample was sectioned into 4-µm-thick slices, and Osx expression was analyzed using immunohistochemistry. The isolation, expansion, and culture of stem cells were conducted at the Laboratory of the Stem Cell Research and Development Center of Airlangga University, while hADMSC sample collection took place at the Airlangga University Hospital, Surabaya. The experimental animal procedures were conducted at the Faculty of Veterinary Medicine, Airlangga University.

Tissue preparation in the treatment groups involved single staining against Osx at weeks one and eight. Samples were soaked in primary antibodies (mouse anti-human Osx monoclonal antibody, Novus Biological, USA) and secondary antibodies (human anti-rat Osx polyclonal antibody, Bioss, USA) for 30 minutes each, rinsed twice with PBS, and soaked for five minutes in chromogenic substrate.

After three PBS washes and aquadist rinsing, the tissue sections were stained with Mayer's hematoxylin for six minutes, rinsed under running water, mounted, and covered with glass cover slips for microscopic analysis. For each sample, the average IRS value was determined from 20 fields of view at 1000x magnification, with each field containing approximately 1500 cells, to ensure data representation and minimize inaccuracy in the results.

The Remmele Scale Index (Immuno Reactive Score, or IRS) was calculated by multiplying the percentage score of immunoreactive cells by the staining intensity score. A semi-quantitative evaluation of Osx expression in each sample was performed using the modified Remmele method. All investigations were conducted using a Nikon H600L light microscope (Nikon Corporation, Tokyo, Japan), equipped with a 300-megapixel DS-Fi2 digital camera (Nikon Corporation, Tokyo, Japan) and Nikon Image System image processing software (Nikon Corporation, Tokyo, Japan). Results for each field of view were recorded on a worksheet, and average scores were calculated. Data analysis of Osx immunohistochemistry (IHC) expression results was conducted using a one-way ANOVA test.

RESULTS

The hADMSC cells exhibited sufficient proliferation and maturity by passage 4, making them ready for seeding onto the Y-TZP scaffold. The cell density at passage four is illustrated in Figure 1. In the characterization analysis of hADMSC (Table 1), CD (Cluster of Differentiation) markers CD105, CD73, and CD90 were considered positive, as their values were above the NLT (Not Lower Than) threshold of 95%. Conversely, CD45, CD34, CD14, CD19, and HLA-DR (Human Leucocyte Antigen-DR) were considered negative, with values below the NLT threshold of 2%. Based on the expressions of these biomarkers, the adipose-derived cells were identified as hADMSC.

Normality and homogeneity tests confirmed a standard and homogeneous distribution of Osterix (Osx) expression differences across groups. The one-way ANOVA test yielded a statistically significant result with $p<0.05$. The post-hoc HSD multiple comparisons test showed that treatment groups P1, P2, P3, and P4 differed significantly from groups KN (normal control), K1 (positive control 1), and K2. However, P2 and P3 showed no significant difference from P4 (Figure 1). The expression of osterix in each treatment group appeared as a brownish reaction to the anti-polyclonal anti-Osterix antibody, as shown in Figure 3.

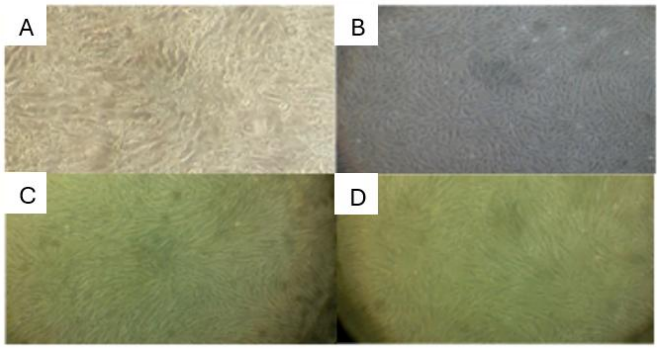

Figure 1. Isolation and expansion of hADMSC cultures across passages: (A) Passage 1, (B) Passage 2, (C) Passage 3, (D) Passage 4

Table 1. hADMSC characterization results

Test Method	Specifications	Result
		ADSC-HMN-P4-220518
Viability	FIO	94.87%
Biomarker CD105[2]	NLT 95%	97.41%
Biomarker CD73[2]	NLT 95%	99.56%
Biomarker CD90[2]	NLT 95%	97.61%
Biomarker CD45[2]	NMT 2%	1.42%
Biomarker CD34[2]	NMT 2%	1.45%
Biomarker CD14[2]	NMT 2%	0.74%
Biomarker CD19[2]	NMT 2%	0.83%
Biomarker HLA-DR	NMT 2%	0.84%

Cell count : 647,500 Cells
Remark: FIO : For Information Only
NLT : Not Lower Than
NMT : Not More Than

Table 1 demonstrates that CD105, CD73, and CD90 are identified as positive markers, with values exceeding the NLT threshold of 95%. In contrast, CD45, CD34, CD 14, CD 19, and HLA-DR are identified as negative markers, with values below the NLT threshold of 2%. Based on the expression of these four biomarkers, the adipose-derived cells are confirmed as hADMSC.

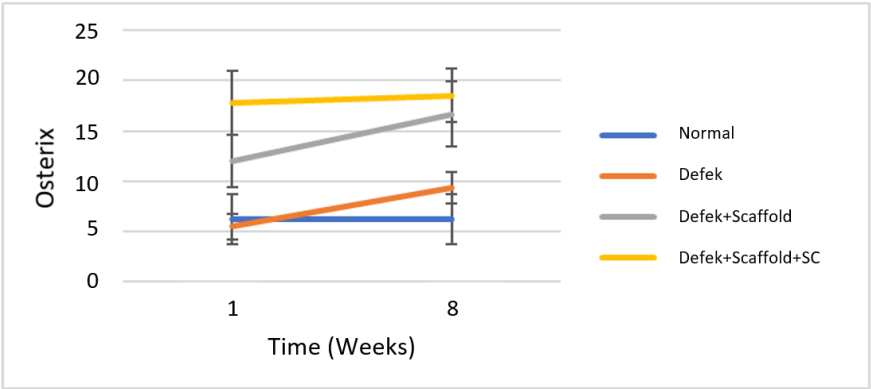

Figure 2. Intergroup Osterix diagram.

A significance level of $p<0.05$ is observed according to the one-way ANOVA test in Figure 2. The HSD multiple comparisons test reveals significant differences between groups the KN, K1, and K2 groups and the treatment groups P1, P2, P3, and P4. No significant differences are observed between P4 and either the P2 or P3 treatment groups.

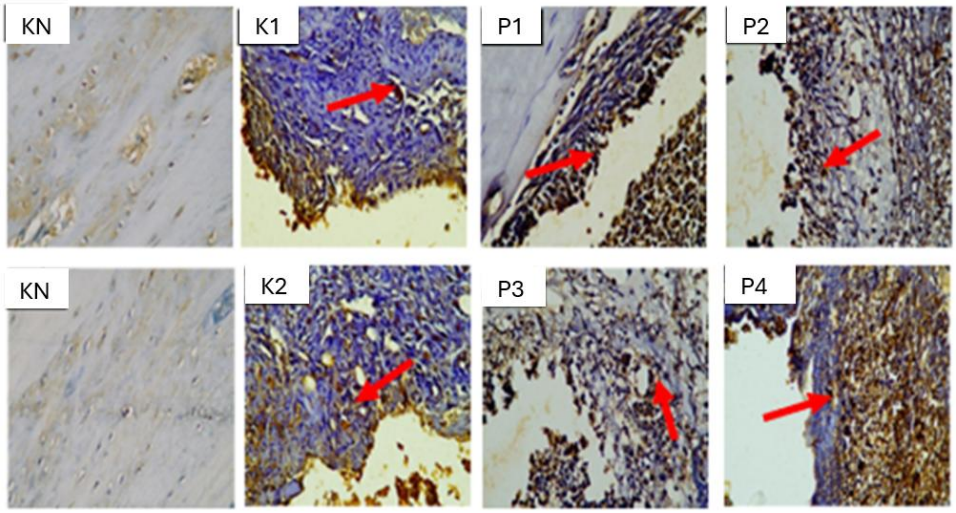

Figure 3. Osterix expression at week 1 (KN,K1,P1 andP2) and week 8 (KN,K2,P3 and P4) of treatment.

Osterix expression was indicated by a brownish reaction to the anti-Osterix polyclonal antibody, as marked by red arrows in Figure 3. Images were captured using a Nikon Eclipse Ci microscope equipped with a 16.25-megapixel DS-Ri camera at 400x magnification.

DISCUSSION

Accelerated healing in dental implants is achieved by controlling inflammatory factors. Proper bone repair depends on effective contact between the implant surface and surrounding bone tissue.[21] Advances in surface modifications and coatings for dental implants have aimed to improve osseointegration and reduce inflammation.[2] A major barrier to the repair of extensive bone lesions is the rapid proliferation of connective tissue, which hinders new bone formation.[33] Barrier membranes must maintain their biological function during the four-month alveolar bone repair period.[34] To ensure successful osseointegration, it is essential to promote new bone tissue formation and support the healing process around the implant.[35]

Implant failure may result from biological causes (peri-implantitis and mucositis), technical factors (e.g., immediate implant instances), age (elderly patients), hormonal changes (e.g., menopause), immunocompromised states (e.g., diabetes), and the use of non-biocompatible biomaterials. Peri-implantitis can be challenging to treat. While peri-implant mucositis can often be managed effectively, non-surgical treatment of peri-implantitis frequently leads to recurrence.[18] Reducing inflammation surrounding the implants and enhancing osseointegration are two essential elements for achieving the clinical success of dental implantation.

Moreover, the challenges posed by inflammation and periodontal regeneration introduce cellular mechanisms that recapitulate key elements of bone regeneration.[2] Cold atmospheric plasma (CAP), which contains reactive oxygen species (ROS), exhibits antibacterial properties and increases implant surface hydrophilicity, thereby promoting cell adhesion and early healing in fluid-inaccessible areas.[18] Drug delivery systems incorporating several biomolecules have also enhanced implant integration surrounding bone. Chemically modified mRNA (cmRNA) is a new therapeutic strategy to trigger bone healing.[36] Additionally, mesenchymal stromal cells (MSCs) may influence immune responses through their secretion of paracrine factors, making them a promising therapeutic option.[37]

Bioceramics such as alumina and zirconia form biological and chemical bonds and are characterized by low toxicity, favorable tissue responses, strong

mechanical properties, and high compressive strength. Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is a dental material that is now the subject of scientific and clinical research because of its exceptional mechanical, cosmetic, and biocompatible properties.[32] In dentistry, Y-TZP is increasingly used as an alternative to metal frameworks due to its comparable survival rate.[38] Lithium and zirconia disilicates may also offer higher stress absorption.[39]

Human Adipose Mesenchymal Stem Cells (hADMSC) are incorporated with Y-TZP to prevent implant failure. Mesenchymal stem/stromal cells (MSCs) and their extracellular vesicles (MSC-EVs) contribute to tissue regeneration by promoting angiogenesis, regulating extracellular matrix remodeling, reducing inflammation, and repairing tissue damage.[40] Adequate osseointegration is established by osteoinductivity, which is the osteoblast growth of mesenchymal stem cells (MSC), and osteoconductivity, which is the deposition of new bone matrix on the material surface.[21] During bone remodeling, MSCs utilize a range of metabolic pathways to obtain the energy required for differentiation and function, with mitochondrial metabolism showing a notable increase. [41] Osteogenic and adipogenic differentiation, surface marker expression, and shape were assessed to verify that the separated cells possessed MSC phenotypic traits. [42] Assays were employed to measure ROS generation in hADMSCs and its impact on the gene and protein levels. [43]

The development of osteoblasts and the formation of bone are regulated by transcription factors, including Osterix (Osx). Elevated osteoclast activity, observed via the Wnt/β-catenin pathway, leads to increased RANKL release from osteoblasts.[44] Osteix and Runt-related transcription factor 2 (Runx2) serve as alternative signaling molecules crucial for regulating osteoblast growth.[45] Our previous research in rat osteoblast cultures demonstrated that while collagen-coated plates did not significantly affect osteogenic gene expression, cultivating cells in three-dimensional gels considerably enhanced it.[46] Osteonectin protein, found in both bone and dentin, plays a role in osteogenesis.[47] In animal models of bone defects, hADMSC osteogenic differentiation and post-implantation regeneration have shown effectiveness.[48]

Mineralized collagen scaffolds have previously proven to be a promising biomaterial implant for accelerating bone repair. In Fluffy-PLG composites, enhanced cell survival, induction of the RUNX2, Osx, and COL1A2 genes, and increased mineral production were observed. Remarkably, composites supplemented with hyperelastic bone mesh led to a markedly higher release of osteoprotegerin, an endogenous inhibitor of osteoclast activity and a soluble glycoprotein.[49] Conversely, reduced expression of marker genes (Runx-2, Tnalp, and Osx) dramatically decreases osteogenic differentiation. Gene expression analysis revealed high beta-2 adrenoreceptor expression in hADMSC, suggesting this as a potential mechanism to promote their action. In bone formation tests, all three cognitive enhancers significantly reduced the calcified matrix and early cell viability without inducing necrosis or apoptosis. Their diverse effects on the production of osteoprotegerin (OPG), Osx, and receptor activator of NF-κB ligand (RANKL) in the later stages of osteoblast development indicate distinct modes of action. Additionally, significant impairment of hADMSC migratory capacity was observed, potentially compromising cellular homing.[51]

During the current study, hADMSC underwent isolation and culture expansion until passage 4 (Figure 1). Passage 1 was initiated on day 3 and continued through passages 2, 3, and 4 by day 12. Once adequate cell density was achieved by passage 4, the cells were subjected to phenotypic characterization and evaluated for adipogenic and osteogenic differentiation. In comparison to the control cells, the results of the oil red-O staining examination of the adipogenic differentiation of hADMSC revealed a large number of vacuoles. These findings demonstrate the ability of hADMSC to develop into adipogenic cells.[52]

Investigations of hADMSC osteogenic differentiation, conducted using Alizarin Red S staining, confirmed the cells' capacity to differentiate into

osteogenic cells. This differentiation was identified by the presence of brownish vacuoles characteristic of osteoblast morphologies. The differentiation and characterization of adipose phenotypes further revealed that adipose tissue is composed of mesenchymal stem cells (MSCs), specifically human adipose-derived mesenchymal stem cells (hADMSC). Upon differentiation, hADMSC exhibited both adipogenic and osteogenic potential. Adipogenic differentiation was verified using Oil Red O staining, while osteogenic differentiation was assessed by Alizarin Red staining. To be classified as MSCs, the cells must demonstrate malleability, express specific surface antigens (Ag), and possess multipotent capacity differentiation.

The cells exhibited positive expressions of CD73, CD90, and CD105, with levels exceeding the minimum threshold of 95%, while CD45, CD34, CD14, CD19, and HLA-DR showed negative expression, with values below the maximum threshold of 2% (Table 1). MSCs typically express HLA-DR surface molecules when activated by IFN-γ, but not under quiescent conditions. However, provided other requirements are satisfied, cells can still be referred to as MSC even if HLA-DR expression is detected for a specific application. In such cases, they must be considered eligible for MSC stimulation or given an alternative designation that indicates they are not in the initial state. Based on these findings, the isolated and cultured adipose tissue, following differentiation and characterization, was indeed confirmed to be MSCs, or hADMSCs. In subsequent *in vivo* investigations, it was anticipated that hADMSCs seeded with Y-TZP would undergo differentiation (both adipogenic and osteogenic) and be classified as MSC.[52]

In the first week, the K1 group's Osx reduction did not significantly affect bone density. While not examined in this study (Figure 2), other indicators such as Col1a1, Col1a2, Col5a1, Col5a3, Dkk1, MMP13, Sost, and VEGF may represent additional pathways contributing to the significant bone density deficiencies observed in the K1 group during week 1. Bone density issues stemming from errors in the eighth week did not affect Osx expression. By week eight, it can be concluded that the K2 treatment had no discernible effect on bone density. The absence of bone regeneration processes during the eighth week can account for the observed bone density problems. In the first week, the P1 group exhibited accelerated woven bone development, approximately 10 μm/day, as evidenced by uneven collagen fibers, large osteocytes within the lacunae, and woven bone encircling the blood vessels.

Because pericytes are a crucial component of blood vessels that contain osteoblasts, bone growth is fundamentally dependent on the presence of blood vessels. The gap between bone and the Y-TZP scaffold or other osteoconductive biomaterials is filled by woven bone. In the P2 group during week 1, a decrease in Osx was observed in preosteoblasts, followed by a subsequent drop in Runx2 and an increase in Osx in osteoblasts (Figure 3). If the relationship between Runx2 and Osx persists without a rise in Osx, there may be a risk of bone tissue cancers due to excessive proliferation occurring without cellular maturation.

In week 8, the P3 group, with reduced Osx, might have experienced an increase in osteoblasts, which would consequently raise ALP. Mandibular bone density increased following Y-TZP-hADMSC implantation due to an increase in osteocytes. After Y-TZP-hADMSC implantation in rats, increased mandibular bone density accelerated the regeneration of the mandibular bones. During week 8, growth factors, niches (including microvesicles, small compounds, biomimetic materials that facilitate recruitment, proliferation, and differentiation), and the immunological response of bones all impacted the P4 group. A niche provides the biophysical and pharmacological cues necessary to promote stem cell activity and self-renewal. The niche regulates the quantity of stem cells and also promotes cell differentiation as the cell moves away from the signal specific to self-renewal. To enhance bone density, the Y-TZP-hADMSC progenitor's maturation during osteogenesis in week eight decreased compared to week one.

The study's findings demonstrate the superiority of the Y-TZP-hADMSC implantation mechanism for accelerating mandibular bone regeneration, as this

process occurs during the first week of treatment. This observation provides an opportunity for further research in experimental animal models, with the ultimate goal of clinically applying these findings to humans as dental implant materials, specifically Y-TZP-hADMSC.

Result of previous research stated that administering a combination of ADMSCs-DDM scaffolding derived from bovine teeth, which naturally contains growth factors including type I collagen and Bone Morphogenetic Proteins (BMP), can speed up the regeneration of alveolar bones by the 28th day. The periodontitis model demonstrates a process of alveolar bone regeneration via the STRO-1, RUNX-2, Osx, and COL-I pathways.[53] Consistent with this, the research cited above suggests that bone regeneration can be enhanced through Y-TZP seeded with hADMSC. When hADMSC is added, Y-TZP, a bioceramic dental implant, increases osseointegration by reducing Osx expression, which accelerates bone regeneration process. The excellent biocompatibility of Y-TZP biomaterials as implants and other orthopedic biomaterials makes their usage in tissue engineering applicable to various medical specialties.

Increased bone density following Y-TZP-hADMSC implantation in the mandibular bone was influenced by decreased Osx expression at week 1. Interestingly, increased Osx expression at week 8 had no discernible influence on this increased bone density. The limitations of this study include the need for further investigation into several areas. Specifically, the impact of the socket tensile test and Western blot analysis, a longer study period (beyond eight weeks), the identification of additional biomarkers to explore other pathways in the mechanism of accelerated mandibular bone regeneration, and Bone-to-Implant Contact (BIC) following implantation of Y-TZP–hADMSC in the mandibular bone all warrant more comprehensive study.

Further investigation is necessary to fully understand the impact of gene transcription methods (qPCR) as a complement to immunohistochemical methods. Additionally, the exact mechanism by which stem cells work to speed up mandibular bone healing requires more comprehensive research. This study is in accordance with the existing hypothesis that a decrease in Osx can increase the osseointegration of dental implants when Y-TZP is seeded with hADMSC. Our findings serve as a valuable reference for future research in dental implants, suggesting that Y-TZP material seeded with hADMSC holds significant potential for applications in medical science, particularly in implants and orthopedics.

This study faced several limitations. A longer research period would have allowed for a more comprehensive evaluation of optimal implant osseointegration outcomes. We also encountered limited technological and research infrastructure for preparing Y-TZP cutting tools and processing samples from rat mandibular bones. This constraint, specifically the inability to perform vertical cuts to observe the entire tissue curve into the Y-TZP, hindered our assessment of the Y-TZP tensile test from the socket and Bone-to-Implant Contact (BIC). Furthermore, future studies should investigate additional biomarkers to identify further pathways involved in the mechanism of accelerated mandibular bone regeneration and incorporate gene transcription techniques (qPCR) to complement immunohistochemical methods, which would provide a more complete understanding of osseointegration between Y-TZP and the mandibular bone.

CONCLUSION

Osseointegration in dental implants can overcome dental implant failure with hADMSC intervention in Y-TZP, which has been shown to decrease Osterix expression. This reduction in Osterix expression may facilitate osteoblast development, and hADMSC homing could further prevent dental failure. These discoveries are expected to be applied clinically in humans as dental implant materials (Y-TZP–hADMSC). Implication of this study leads to new opportunities using non-human primate experimental animals. Given their excellent

biocompatibility as implants and other orthopedic biomaterials, Y-TZP biomaterials have broad applicability in other medical fields.

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