Venkataiah *et al.*
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Advancements in osteoblast sourcing, isolation, and characterization for dental tissue regeneration: a review

Venkata Suresh Venkataiah[1], Deepak Mehta[1], Mohammad Fareed[2] and Mohmed Isaqali Karobari[1,3*]

*Correspondence:
dr.isaq@gmail.com

[1] Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India
[2] Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Diriyah, P.O. Box: 71666, Riyadh 11597, Kingdom of Saudi Arabia
[3] Department of Conservative Dentistry and Endodontics, Faculty of Dentistry, University of Puthisastra, Phnom Penh 12211, Cambodia

Abstract

Background: Primary osteoblasts are essential for bone formation and regeneration, making them pivotal in dental applications, including periodontal regeneration, ridge augmentation, and implant osseointegration. Sourced from various tissues like alveolar bone, calvarial bone, mandibular and maxillary bones, long bones, and bone marrow-derived stem cells (BMSCs), each type of osteoblast presents unique advantages and limitations related to yield, accessibility, and clinical relevance. Given these variables, selecting an appropriate source is crucial for experimental consistency and translational application in dentistry.

Methods: This review synthesizes data from in vitro, animal, and clinical studies to provide a comprehensive overview of osteoblast sourcing, isolation, and characterization in dental research. Sources were reviewed based on yield, anatomical relevance, and accessibility, while isolation methods were compared to assess their impact on cell behavior and phenotype retention. The review evaluates methods such as enzymatic digestion, explant culture, and differentiation of BMSCs, alongside characterization techniques like morphological analysis, gene expression profiling, and mineralization assays.

Results: The analysis shows that alveolar bone-derived osteoblasts offer high clinical relevance due to their anatomical similarity to oral structures but are limited by low yield and invasive collection. Calvarial and long bone osteoblasts provide higher yields, making them useful for material testing, though they lack biomechanical compatibility with oral environments. BMSCs offer a renewable source with significant regenerative potential but require precise differentiation protocols. In vitro studies contribute mechanistic insights, while animal models bridge the gap to clinical application, despite challenges in standardization and interspecies variability.

Conclusion: This review highlights the importance of selecting appropriate osteoblast sources and methods for dental research to optimize outcomes in periodontal and implant-related therapies. The variability across study designs and experimental outcomes underscores the need for standardized protocols and targeted systematic reviews within specific research settings. These findings offer a framework for future osteoblast-based research and guide the effective translation of osteoblast therapies into clinical dental practice.

Keywords: Bone regeneration, Dental research, Osteoblasts, Primary osteoblast characterization, Tissue engineering

Introduction

Primary osteoblasts are essential to bone formation and regeneration, playing a pivotal role in dental applications, including periodontal regeneration, ridge augmentation, and implant osseointegration [1, 2]. These specialized cells produce the extracellular matrix and deposit minerals necessary for bone formation, making them fundamental in developing and evaluating regenerative dental therapies [3]. Osteoblast-based therapies are widely studied across in vitro, animal, and clinical settings to assess their effectiveness in promoting bone growth, integrating dental implants, and repairing bone defects [4–7].

Osteoblasts can be derived from several skeletal tissues, including alveolar bone, calvarial bone, mandibular and maxillary bones, long bones, and bone marrow-derived stem cells (BMSCs) [8–10]. Each source offers distinct advantages regarding yield, accessibility, and relevance to clinical applications [10]. Alveolar bone-derived osteoblasts may be regarded as the gold standard due to their anatomical similarity to oral structures, ensuring high translational relevance for applications like implantology and periodontal regeneration [11, 12]. However, the low cell yield (5,000–15,000 cells per gram) and invasive nature of their collection limit their use in large-scale research or routine clinical practice [13].

To address these limitations, alternative sources, such as calvarial bone and long bones, are frequently used in preclinical studies [8–10]. Calvarial bone osteoblasts provide a high yield (> 50,000 cells per gram), making them ideal for in vitro material testing. However, they differ biomechanically from alveolar bone, reducing their relevance for clinical translation to oral environments [8]. Similarly, long bones offer abundant osteoblasts but exhibit different mechanical properties, which limits their suitability for dental research beyond initial testing phases [14]. Mandibular and maxillary bones, while more closely aligned with alveolar bone in cellular behavior, are harder to obtain, requiring invasive procedures or cadaveric samples [15]. BMSCs, in contrast, provide a renewable source of osteoblast progenitors, with each milliliter of aspirate yielding 1 to 5 million stem cells. These cells offer flexibility in experimental design and potential for regenerative therapies but require precise differentiation protocols to ensure consistency in osteoblast behavior [16]. The trade-off between yield, relevance, and clinical accessibility must be carefully considered when selecting an osteoblast source for a specific study or therapeutic application.

While in vitro studies offer valuable mechanistic insights into osteoblast behavior, they often oversimplify biological processes, lacking the complex interactions present in living tissues. Animal models, such as rodent studies, provide physiological environments to test scaffolds and biomaterials but present challenges related to interspecies differences, which can limit the applicability of findings to human clinical settings. Translating preclinical successes to clinical trials remains a major challenge, with variability in patient healing responses, graft performance, and long-term outcomes complicating the development of standardized protocols.

Initially, a systematic review and meta-analysis were planned to consolidate evidence across in vitro, animal, and clinical studies on primary osteoblasts in dentistry. However,

the heterogeneity in study designs, variability in outcomes, and limited availability of clinical trials precluded a comprehensive meta-analysis. Differences in isolation techniques, characterization methods, and experimental settings further contribute to the variability in reported outcomes, making it difficult to pool data meaningfully across studies.

As such, this review provides a comprehensive synthesis of the available literature, highlighting key aspects such as sources, isolation techniques, characterization methods, applications, while offering insights into the current challenges and future perspectives (Fig. 1). It also highlights the need for focused systematic reviews on specific research settings—such as in vitro studies or clinical trials—to address variability effectively. This review may serve as a framework for future systematic review protocols, guiding researchers in developing standardized methods to better assess and translate osteoblast-based therapies into clinical practice.

Sources of primary osteoblasts for dental research

Primary osteoblasts used in dental research are derived from several skeletal tissues, including alveolar bone, calvarial bone, mandibular and maxillary bones, long bones, and bone marrow-derived stem cells (Fig. 2) [8–10, 13]. Each source presents specific advantages and limitations in terms of yield, accessibility, and relevance to clinical applications, making it essential for researchers to carefully select the most appropriate source based on their experimental goals.

Alveolar bone-derived osteoblasts are regarded as the gold standard because of their anatomical similarity to oral structures, ensuring high translational relevance for applications such as implantology and periodontal regeneration [9, 13]. However, their use is limited by the low yield (5,000 to 15,000 cells per gram) and the invasive nature of sample collection [9, 13]. Calvarial bone, often obtained from rodent models, provides

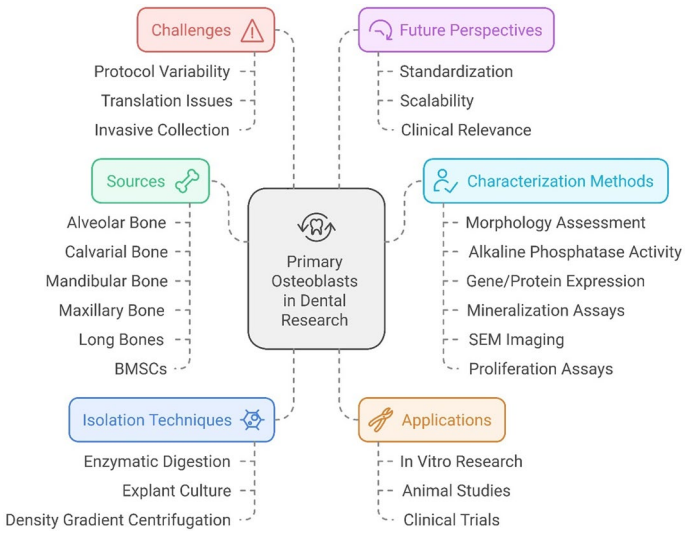

Fig. 1 The schematic summarizes key areas in primary osteoblast research for dental applications, emphasizing the need for standardization and scalability. The figure highlights how isolation and characterization techniques influence in vitro and clinical research outcomes while acknowledging challenges such as translation issues and invasive collection methods

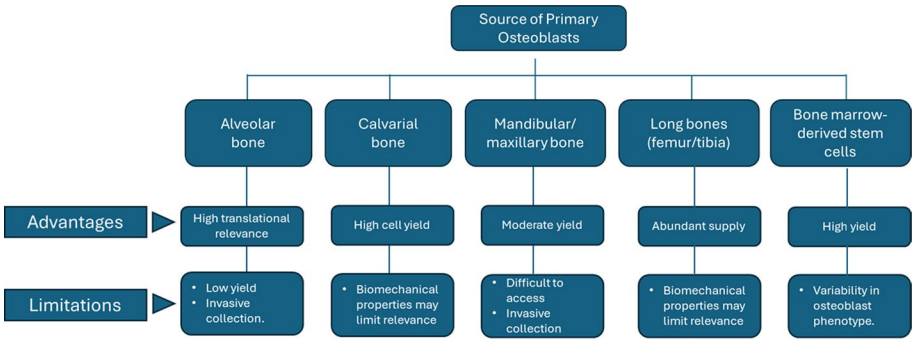

Fig. 2 A schematic representation of different sources of primary osteoblasts, highlighting their advantages and limitations

Table 1 Comparison of osteoblast sources for dental research applications [8–10, 13, 14, 17]

Source	Yield (cells/g or mL)	Relevance to dentistry	Advantages	Limitations
Alveolar bone	5,000—15,000	Very high	High relevance to oral applications	Invasive collection, low yield
Calvarial bone	> 50,000	Low	High yield, easy to obtain from rodent models	Limited biomechanical relevance
Mandibular/maxillary bone	10,000—20,000	High	Similar to alveolar bone in behavior	Invasive collection, limited accessibility
Long bones (femur/tibia)	30,000—50,000	Moderate	High yield, practical for animal models	Different biomechanical properties
BMSCs	1—5 million MSCs per mL aspirate	High	Renewable source, regenerative potential	Variability in differentiation

higher yields exceeding 50,000 cells per gram, which makes it suitable for preliminary material testing [8, 10]. However, the biomechanical properties of calvarial bone differ from those of alveolar bone, reducing its relevance for dental applications [8, 14].

Mandibular and maxillary bones offer osteoblasts with behavior closely aligned to alveolar bone, providing better translational relevance. However, these bones are more difficult to access and require invasive procedures, resulting in moderate cell yields of 10,000 to 20,000 cells per gram [9, 13]. Long bones such as the femur and tibia, commonly used in animal models, provide abundant osteoblasts with yields between 30,000 and 50,000 cells per gram [10, 14]. Despite their accessibility, the biomechanical differences between long bones and alveolar bone limit their direct application to dental research beyond early-stage testing [8, 14].

BMSCs represent a renewable source for regenerative studies and yield between 1 and 5 million stem cells per milliliter of aspirate. These cells offer flexibility in research design, but their differentiation into osteoblasts introduces variability, requiring precise control to maintain phenotype consistency [12, 17]. A summary of these sources, their yields, and their advantages and limitations is presented in Table 1.

Choosing the right source for osteoblast research involves balancing yield, accessibility, and relevance. While alveolar bone osteoblasts remain the gold standard for

dental research due to their high clinical relevance, their low yield and invasive collection limit their widespread use [9, 13]. Calvarial and long bone-derived osteoblasts are more accessible and offer higher yields, making them ideal for early-stage material testing, though they lack the biomechanical properties necessary for dental applications [8, 10, 14]. Mandibular and maxillary bone-derived osteoblasts strike a better balance between relevance and behavior but are challenging to obtain [9, 13]. BMSCs offer a renewable source with regenerative potential, though the differentiation process adds complexity to their use in research [12, 17].

Techniques for isolating primary osteoblasts in dental research

The method used to isolate primary osteoblasts is critical, as it directly influences the yield, viability, and behavior of the cells. Different skeletal tissues require distinct isolation techniques, such as enzymatic digestion, explant culture, and bone marrow flushing, each with specific benefits and limitations. These methods not only affect the number of cells obtained, but also influence their phenotype, proliferation, and differentiation potential, which are essential considerations for dental research and clinical applications [9, 18, 19]. Table 2 provides a comparative summary of isolation methods and their outcomes for different osteoblast sources.

Alveolar bone osteoblasts are typically isolated using enzymatic digestion with collagenase and dispase to break down the extracellular matrix and release cells [9, 20]. This method ensures that the cells retain their phenotype but is limited by the relatively low yield obtained per gram of tissue. Calvarial bone, often used in animal models, undergoes sequential enzymatic digestion using collagenase II and trypsin, which results in higher yields suitable for preliminary testing of biomaterials and scaffolds [21, 22].

Mandibular and maxillary bone samples can be processed through explant culture or enzymatic digestion. Explant culture allows cells to migrate out of tissue fragments over time, preserving their native phenotype, though it is time-consuming [13, 23]. Enzymatic digestion is faster but requires invasive collection procedures, limiting its practicality.

Table 2 Comparative analysis of isolation methods for primary osteoblasts in dental research [9, 13, 19–28]

Source	Isolation method	Yield (cells/g or mL)	Advantages	Limitations
Alveolar bone	Enzymatic digestion (collagenase, dispase)	5,000—15,000	High relevance to dental research	Low yield, invasive collection
Calvarial bone	Sequential enzymatic digestion (collagenase II, trypsin)	> 50,000	High yield, fast isolation	Limited dental relevance
Mandibular/maxillary bone	Explant culture or enzymatic digestion	10,000—20,000	High relevance, better mimicry of alveolar bone	Time-consuming explant method, invasive collection
Long bones (femur/tibia)	Bone marrow flushing and enzymatic digestion	30,000—50,000	High yield, abundant cells for animal models	Different biomechanical properties
BMSCs	Density gradient centrifugation, osteogenic differentiation	1—5 million MSCs per mL aspirate	Renewable source, regenerative potential	Requires controlled differentiation, variable results

Long bones, such as the femur and tibia, are processed through bone marrow flushing and digestion, yielding large numbers of osteoblasts [19, 24]. However, these cells are less relevant for dental applications due to differences in mechanical properties compared to alveolar bone [2, 25].

Bone marrow-derived stem cells (BMSCs) are isolated using density gradient centrifugation and subsequently differentiated into osteoblasts in vitro using osteogenic media containing dexamethasone, ascorbic acid, and β-glycerophosphate [26, 27]. While BMSCs offer a renewable source, the differentiation process introduces variability, necessitating precise control to maintain osteoblastic function [24, 28].

Characterization techniques for primary osteoblasts in dental research applications

Accurate characterization of primary osteoblasts is essential to confirm their phenotype and functionality, ensuring the reliability of research findings in dental applications. The characterization process verifies that isolated cells exhibit the expected behavior of osteoblasts, including proliferation, differentiation, and mineralization [9, 13]. In dental research, several techniques are used to validate cell identity and assess their ability to interact with scaffolds, biomaterials, and implants. These methods involve morphological assessment, gene expression analysis, biochemical assays, and mineralization tests to confirm that the cells maintain their osteoblastic properties throughout experimentation [29, 30]. Table 3 summarizes these techniques, their purpose, and limitations.

Morphological assessment through light or phase-contrast microscopy is the first step in characterizing osteoblasts. Osteoblasts display a cuboidal or polygonal shape with prominent nuclei and abundant cytoplasm, reflecting their active state [20, 31]. While this provides a quick overview of the cell's phenotype, morphology alone is insufficient to confirm osteoblastic identity. Alkaline phosphatase (ALP) is a key enzyme involved

Table 3 Characterization methods for primary osteoblasts in dental research [9, 13, 20, 29–38]

Technique	Purpose	Markers/assays used	Advantages	Limitations
Morphological analysis	Confirms characteristic osteoblastic shape	Phase-contrast microscopy, light microscopy	Simple, fast, non-invasive	Insufficient alone to confirm identity
ALP assay	Evaluates early differentiation and activity	Spectrophotometric ALP enzyme activity	Indicates functional osteoblast activity	Limited to early differentiation
Gene expression analysis	Confirms osteoblast phenotype at molecular level	qPCR, RT-PCR (Runx2, ALP, COL1A1, OCN)	Precise molecular insights	Requires RNA extraction and careful handling
Immunocytochemistry/Western blotting	Validates protein expression of osteoblast markers	Osteocalcin, osteopontin, COL1	Confirms phenotype at protein level	Labor-intensive, requires specific antibodies
Mineralization assays	Assesses ability to form mineralized matrix	Alizarin Red, von Kossa staining	Visual confirmation of calcium and phosphate deposition	Semi-quantitative, may require complementary assays
SEM	Examines surface morphology and ECM organization	High-resolution SEM imaging	Evaluates cell–material interactions	Requires expensive equipment and preparation
Proliferation assays	Measures metabolic activity and growth	MTT, BrdU, XTT assays	Quantifies cell viability and biocompatibility	Cannot differentiate osteoblasts from other cell types

in early bone formation and is used as a biochemical marker for osteoblast differentiation. Elevated ALP activity, assessed through colorimetric or spectrophotometric assays, indicates that the cells are functionally active and preparing for matrix mineralization [9, 32]. Gene expression analysis through quantitative real-time PCR (qPCR) or RT-PCR quantifies the levels of osteoblast-specific markers such as Runx2, ALP, COL1A1, and osteocalcin (OCN). These markers reflect the different stages of osteoblast differentiation, from the early pre-osteoblast phase to mature osteoblasts capable of mineralizing bone. This technique provides precise molecular-level insights, but requires careful RNA handling [20, 33]. Protein-level analysis is performed to validate the expression of key osteoblast proteins, such as osteocalcin, osteopontin, and collagen type I (COL1A1). Techniques such as immunocytochemistry and Western blotting confirm the presence of these proteins, providing further evidence of the osteoblastic phenotype [33, 34]. However, these methods are labor-intensive and require specific antibodies. Mineralization assays, such as Alizarin Red and von Kossa staining, evaluate the ability of osteoblasts to deposit calcium and phosphate minerals, respectively. These techniques provide visual confirmation of matrix mineralization, which is essential for assessing the cells' ability to form bone-like structures on biomaterials [18, 35]. SEM offers high-resolution imaging to assess the surface morphology of osteoblasts and their interaction with biomaterials. This technique is particularly useful for evaluating the organization of the extracellular matrix and cellular responses to different surface modifications [36]. Cell proliferation assays, such as MTT, BrdU, or XTT assays, assess osteoblast viability and metabolic activity under different conditions. These assays help evaluate the biocompatibility of dental materials and scaffolds by quantifying the rate of cell growth [34, 37].

The selection of appropriate characterization techniques is crucial for ensuring that primary osteoblasts maintain their phenotype and functionality throughout experimentation. Morphological analysis offers a quick but preliminary assessment, while ALP assays and gene expression profiling provide deeper insights into differentiation stages. Protein-level analysis, using immunocytochemistry and Western blotting, ensures that key osteoblastic proteins are expressed, while mineralization assays confirm the functional ability of cells to deposit bone-like matrix on dental biomaterials. Finally, SEM and proliferation assays provide additional information on cell–material interactions and viability, essential for evaluating the biocompatibility of implants and scaffolds in dentistry [33, 38]. Together, these techniques provide a comprehensive toolkit to assess osteoblast behavior, ensuring the reliability of results and supporting the development of regenerative dental therapies.

Summary of primary osteoblasts in various research contexts

Primary osteoblasts have been investigated in in vitro, animal, and clinical settings to understand their behavior, potential applications, and limitations in dental research. Each setting offers unique insights into osteoblast function, interaction with biomaterials, and regenerative potential, but also presents distinct challenges in translating results to clinical practice. This section synthesizes the available evidence and highlights both the successes and limitations of primary osteoblasts in different experimental contexts.

In vitro studies provide a controlled environment to investigate the behavior, adhesion, proliferation, and differentiation of primary osteoblasts on various biomaterials

and dental implants. Alveolar and mandibular osteoblasts cultured on titanium surfaces exhibit enhanced adhesion and osteogenic differentiation, suggesting that surface modifications can improve implant integration [27, 35]. These studies also allow for the screening of biomaterials and scaffolds for biocompatibility and osteoconductive potential [39, 40].

Mineralization assays, such as Alizarin Red staining, confirm the capacity of osteoblasts to deposit calcium-rich matrices, demonstrating their ability to form bone-like structures [33, 41]. However, despite these promising results, in vitro experiments lack the complex biological interactions found in living systems. The simplified environment may overestimate the osteogenic potential of biomaterials, necessitating further validation in animal models [23, 42].

Animal models play a crucial role in bridging the gap between in vitro findings and human clinical trials. Studies using rodent and canine models demonstrate that osteoblast-seeded scaffolds enhance bone regeneration and osseointegration in critical-size defects [8, 29]. For example, calvarial bone-derived osteoblasts have been shown to promote matrix deposition and vascularization when combined with β-tricalcium phosphate (β-TCP) scaffolds [23, 30]. Similarly, studies using long bones in animal models confirm the ability of osteoblasts to support the repair of large bone defects [43, 44]. While animal models provide valuable insights into the physiological responses to osteoblast-based therapies, interspecies differences remain a challenge. The behavior of osteoblasts in animal models may not fully replicate their function in human bone, which can complicate the translation of preclinical findings into effective clinical therapies [10, 16].

Clinical trials involving primary osteoblasts have primarily focused on implant integration, ridge augmentation, and periodontal regeneration. Alveolar bone-derived osteoblasts seeded on custom-made scaffolds have shown promising results in enhancing bone volume and implant stability [8, 34]. In one study, scaffolds seeded with alveolar osteoblasts improved implant outcomes in terms of bone-to-implant contact and healing times [9, 20].

BMSCs differentiated into osteoblasts have also demonstrated potential for regenerative therapies in dentistry, particularly in cases of severe bone loss [45, 46]. However, clinical variability in patient healing responses, combined with the invasive nature of harvesting alveolar bone samples, presents challenges in standardizing these approaches. Long-term studies are still needed to establish the efficacy and safety of these therapies under diverse clinical conditions [11, 47].

The evidence from in vitro, animal, and human clinical studies underscores the potential of primary osteoblasts to support bone regeneration and improve implant outcomes [48]. However, each setting presents unique limitations. In vitro studies provide valuable mechanistic insights but lack biological complexity. Animal models offer physiological relevance but are limited by interspecies differences. Clinical studies confirm the feasibility of osteoblast-based therapies, but face challenges related to variability in outcomes and the invasiveness of sample collection.

Emerging technologies such as 3D bioprinting offer the possibility of creating highly structured and functional bone grafts with precise cellular organization, which could significantly improve regenerative outcomes [17, 49]. Additionally, gene-editing techniques like CRISPR–Cas9 present opportunities for refining osteoblast differentiation

and enhancing their regenerative capabilities, particularly in patients with genetic disorders affecting bone metabolism [50, 51]. Furthermore, advancements in differentiation protocol such as the optimization of biomimetic environments, growth factor modulation, and the use of small-molecule inducers are progressively improving the scalability and clinical applicability of osteoblast-based therapies [52]. These innovations hold promise for overcoming current translational hurdles, making osteoblast research more applicable to clinical practice.

While the advantages of osteoblast-based regenerative approaches have been well explored, it is equally important to acknowledge the challenges and limitations that persist in this field. Scalability remains a significant concern, as translating laboratory-scale osteoblast production into clinically relevant applications requires extensive optimization of culture conditions, bioreactors, and automated expansion systems [53]. Additionally, ethical considerations surrounding the use of stem cell-derived osteoblasts, particularly from embryonic sources or genetically modified cells, pose regulatory challenges that must be addressed before widespread clinical implementation [54, 55]. Another critical issue is the cost associated with isolation and differentiation methods, which often require expensive reagents, prolonged culture times, and highly specialized facilities. These limitations highlight the need for continued research and innovation to refine osteoblast-based regenerative strategies and ensure their feasibility for widespread clinical use.

Despite these challenges, the collective findings from these settings contribute to the development of osteoblast-based regenerative therapies for dental applications. Future research should focus on addressing the variability between experimental conditions and exploring non-invasive methods for obtaining primary osteoblasts or their progenitors, such as BMSCs [8].

Conclusion

Primary osteoblasts are essential for advancing dental research, particularly in periodontal regeneration, ridge augmentation, and implant osseointegration. Each source—whether from alveolar bone, calvarial bone, long bones, or BMSCs—offers distinct trade-offs in terms of yield, accessibility, and clinical relevance. While alveolar bone osteoblasts are ideal for dental applications, their low yield and invasive collection limit routine use, prompting the need for alternative sources like BMSCs and animal models. In vitro studies provide insights into osteoblast behavior but lack biological complexity, while animal models help bridge the gap to clinical trials but face interspecies challenges. Clinical studies demonstrate potential for regenerative therapies but encounter variability in patient outcomes and practical challenges with cell harvesting.

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Author contributions
The authors (V.V.S, M.I.K) were responsible for the design of the study, the review, and the first interpretations. The authors (D.M., MF and M.I.K) provided feedback, comments, and discussion leading to the final manuscript.

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