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OBM Transplantation

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Review

The Role of Bone Grafts in the Management of Complex Fractures

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Academic Editor: Maurizio Salvadori

OBM Transplantation
2026, volume 10, issue 4
doi:10.21926/obm.transplant.2604279

Received: January 09, 2026
Accepted: November 01, 2026
Published: November 06, 2026

Abstract

Complex fractures, which are characterized by substantial loss of bone, severe disintegration, or nonunion, pose significant challenges in the field of orthopedic surgery. Bone grafting is recognized as a valuable technique for enhancing bone healing, restoring structural integrity, and improving functional recovery. Bone grafts are grouped into three types: autografts, allografts, and synthetic substitutes, each with unique benefits and drawbacks. Autografts, which originate from the patient's own body, have superior osteogenic, osteoinductive, and osteoconductive characteristics, making them the gold standard. Still, donor-site morbidity and limitations on accessibility are concerns. Allografts derived from cadaveric sources offer a possibility that eliminates donor-site complications; however, they are associated with immunological rejection and disease transmission. Synthetic grafts and bioengineered scaffolds are gaining popularity due to their biocompatibility and ability to be customized to individual patient needs, which often incorporate growth factors (e.g., BMPs and VEGF) and stem cells to promote regeneration. The integration of bone grafts with modern surgical procedures, including 3D printing, tissue engineering, and growth factor therapies, has revolutionized the management of fractures. Based on the literature reviewed, 3D-printed scaffolds can offer patient-specific architectures along with controlled mechanical and biological properties, whereas newly developed bioprinting and artificial intelligence-based

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technologies may enhance scaffold design, vascularization, and regenerative performance. Despite these advances, optimizing graft selection and vascularization remains crucial to achieving optimal outcomes. This review focuses on the role of bone grafts in complex fracture care, specifically their biological and mechanical contributions to bone regeneration. Future research should focus on enhancing scaffold vascularization, biomaterial performance, personalized care, and manufacturing consistency, along with adequately powered preclinical and long-term clinical studies. An interdisciplinary approach involving regenerative medicine, biomechanics, 3D bioprinting, and artificial intelligence may be required to generate more effective and patient-specific treatments for complex bone defects.

Keywords
Bone grafts; complex fractures; fracture healing; autografts; allografts; synthetic grafts; osteogenesis; osteoinduction; osteoconduction; regenerative medicine; tissue engineering; biomaterials; orthopedic trauma; bone regeneration

1. Introduction

Osteoporotic fractures, which are also known as fractures of fragility, are often brought on by minor trauma or daily activity and occur in the proximal humerus, distal tibia, hip, distal forearm, and vertebrae of the elderly person [1, 2]. Non-union Fracture in osteoporotic bones, which has a 19.7% global occurrence rate, is a major concern in orthopedics, particularly as the global population ages and osteoporosis becomes more prevalent [3]. Because of decreased bone strength, reduced healing capacity, and difficulty achieving solid fixation, osteoporotic fractures are more likely to result in non-union. The primary reason for non-union in these patients is disrupted bone regeneration, which can be related to reduced osteoblast activity, mechanical instability, and inadequate vascularity [4, 5]. Furthermore, poor angiogenesis and chronic inflammation often hinder the natural process of bone regeneration. Recent data suggest that significant molecular pathways such as Vascular Endothelial Growth Factor (VEGF)-mediated angiogenesis and Wnt/β-catenin, which are crucial pathways for bone development and vascular integration, are dysregulated in non-unions [6, 7]. In some cases, traditional fixation approaches may not be suitable, requiring alternative methods to promote and sustain bone healing [8]. Previous clinical and animal studies have shown that bone grafting may facilitate healing in complex fractures and non-unions, respectively, by offering structural support and a biologically optimal environment for new bone growth and development [8]. Autologous bone grafts contain osteogenic cells, osteoinductive signals, and an osteoconductive matrix [9]. However, earlier studies have identified substantial constraints, including limited graft supply, donor-site morbidity, postoperative pain, and increased surgical time associated with graft harvesting. Such limitations contributed to the emergence and clinical evaluation of allografts and synthetic bone substitutes as alternative treatments [9]. Bone grafts are generally categorized into autografts, allografts, and synthetic grafts depending upon their origin and biological activity [8] (Figure 1).

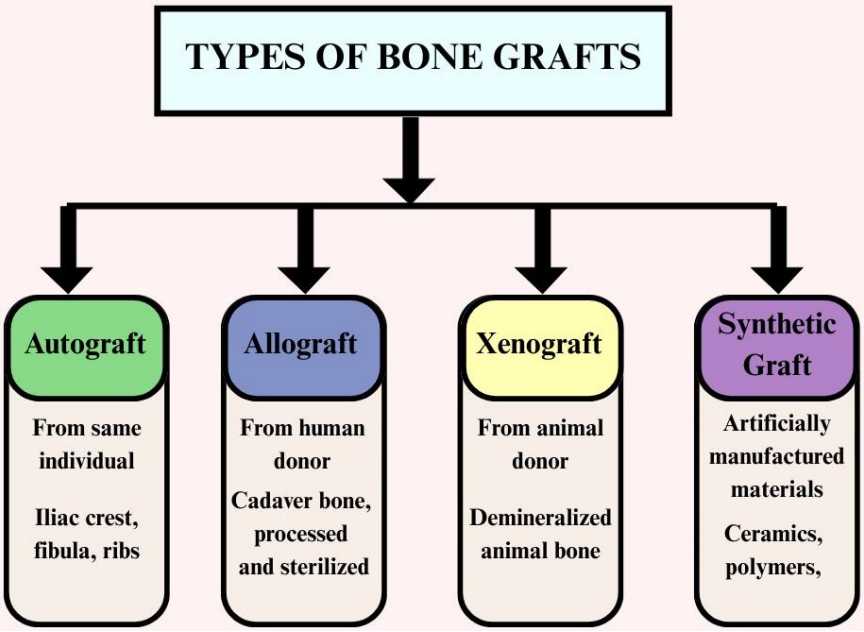


Figure 1 Classification of Bone Grafts Based on Origin and Biological Activity.

Autografts obtained from the patient's own body, usually from the iliac crest, have remained the gold standard for their broad spectrum of regeneration properties [9]. These grafts support osteogenesis by providing living bone-forming cells, osteoinduction by activating progenitor cells, and osteoconduction by functioning as a framework for the growth of new bone. Donor-site morbidity and limited availability may limit their application despite these benefits [9]. Human donor-derived allografts undergo processing and sterilization to minimize immunogenic and infectious risks. They are more readily available and provide structural integrity; however, they are less osteoinductive than autografts and lack cellular vitality [10].

Several preclinical and clinical investigations have evaluated the potential of synthetic and bioengineered grafts. Calcium phosphate-based materials, such as Hydroxyapatite (HA) and β-Tricalcium Phosphate (β-TCP), exhibit good biocompatibility and osteoconductive properties. To enhance biological activity and mechanical performance, researchers have investigated bioactive glasses and composite scaffolds [11, 12].

Simultaneously, tissue-engineering approaches incorporating growth factors, Mesenchymal Stem/stromal Cells (MSCs), nanomaterials, and Three-Dimensional (3D)-printed scaffolds have been studied to overcome shortcomings associated with conventional grafts and to enable more controlled and patient-specific regenerative strategies [8]. However, the biological conditions of the fracture, mechanical loading, vascularity, graft composition, defect size and location, and patient characteristics all influence the outcomes. Despite the growing number of studies comparing conventional grafts to emerging biomaterial-based techniques, the available data remain dispersed among clinical, preclinical, and materials science journals [11, 12]. Direct comparisons between graft types are particularly difficult because biological activity, mechanical properties, fabrication procedures, characterization approaches, and translational development vary. Thus, a comprehensive analysis of the available data is necessary to better understand the current clinical roles of autografts, allografts, and synthetic substitutes, and to assess whether emerging approaches, such as nanomedicine, stem-cell-based tissue engineering, and 3D printing, may overcome the challenges associated with conventional grafting. Accordingly, this review examines

the biological principles, types, clinical applications, and recent developments in bone grafting for managing complex fractures and non-unions, with particular emphasis on emerging technologies and their potential for future clinical translation.

2. Types of Bone Grafts

2.1 Autografts

Autologous bone has been described as the "gold standard" bone grafting material since it comprises all of the qualities required for a bone graft: osteoinduction [Bone Morphogenetic Proteins (BMPs) and other growth factors], osteogenesis (osteoprogenitor cells), and osteoconduction (scaffold) [3]. It is often used in a variety of orthopedic and oral and maxillofacial operations for augmentation and acceleration of bone regeneration (fusion, non-union, fracture and osteotomy healing) or restoration of bony abnormalities (traumatic, congenital, after tumour or infection) [3]. Autologous bone grafts may consist of cortical, cancellous, or corticocancellous bone, depending on the anatomical donor site and the recipient defect requirements (Figure 2).

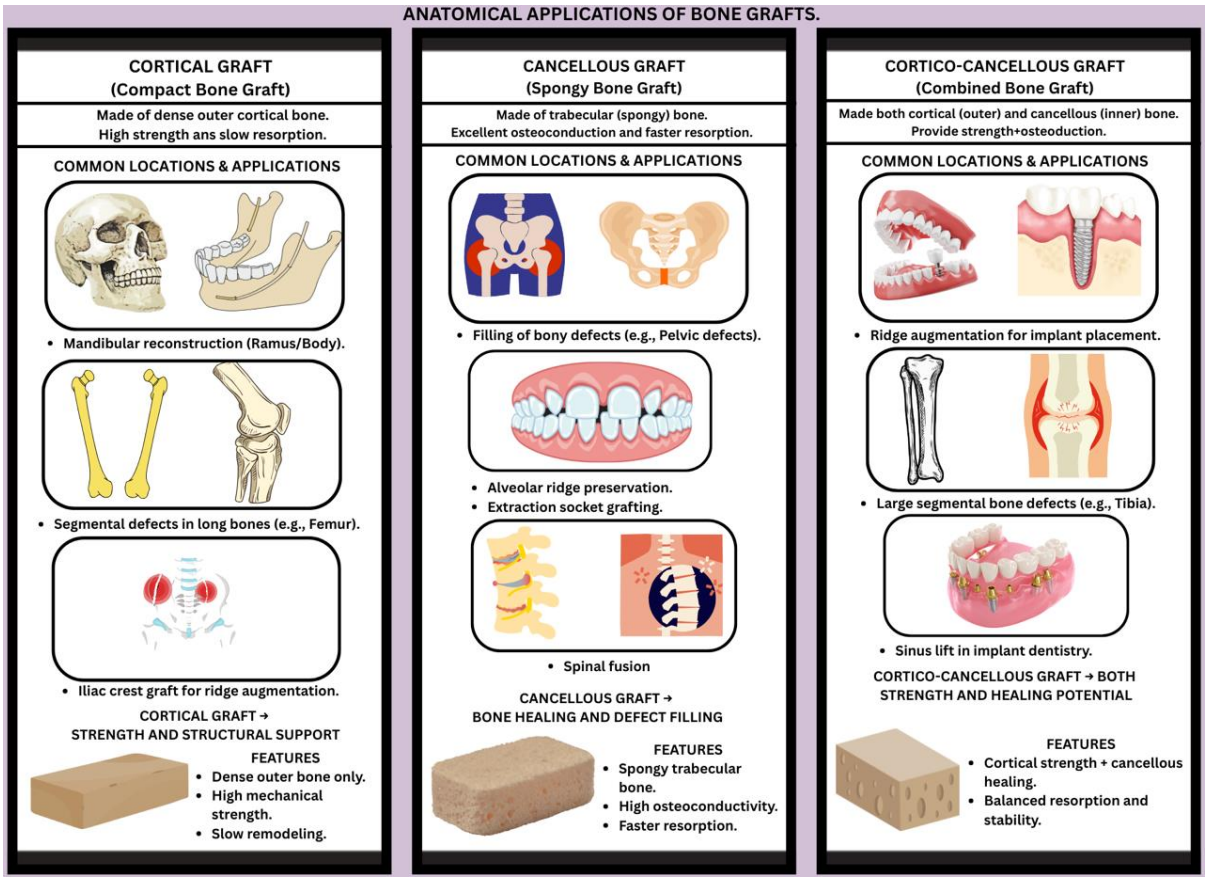


Figure 2 Anatomical Sites of Autologous Bone Grafts. The image was created manually using Canva.

Osteogenesis is facilitated by live osteoblasts and osteoprogenitor cells, which can directly contribute to new bone creation. Osteoinduction occurs when naturally occurring growth factors such as BMPs recruit and stimulate mesenchymal stem cells at the recipient site. In contrast, the graft's trabecular structure provides osteoconduction by acting as a scaffold for vascular and cellular

ingrowth [3]. Age may be considered as one of several patient-related factors when selecting an autograft, although graft selection should not be based on chronological age alone. Autologous bone grafting has been widely used across pediatric and adult populations, particularly when osteogenic, osteoinductive, and osteoconductive properties are required, and sufficient donor bone is available [13]. Pediatric studies have also demonstrated the feasibility of iliac crest autograft harvesting, although donor-site morbidity should be considered [13]. However, autografts include limitations alongside their curative properties. The limited amount that may be extracted is one of the biggest disadvantages, and it becomes especially important when extensive defect repair is required. Furthermore, donor site morbidity, which may involve persistent discomfort, infections, hematoma, neurovascular damage, or abnormal gait, is associated with autograft harvest [14]. Although it is the most typical procedure, iliac crest harvest has a 10-39% complication risk, depending on patient characteristics and the method of surgery [15]. To overcome these restrictions, alternative grafting methods such as allografts and synthetic substitutes are frequently investigated, particularly in patients who are elderly or those with comorbidities that increase the likelihood of surgery. However, because of their exceptional regenerative potential, autografts remain the initial treatment in many orthopedic and trauma situations. Current developments aim to improve autograft performance while limiting morbidity. For example, minimally invasive harvest procedures, Bone Marrow Aspirate Concentrates (BMAC), and the incorporation of autografts with biological enhancers such as Platelet-Rich Plasma (PRP) and stem cells are all being studied to maximize efficacy while minimizing risk [16].

2.1.1 Recent Evidence and Clinical Outcomes of Autografts

A systematic review and meta-analysis of autologous bone grafting for post-traumatic fractures comprised numerous studies including human participants and evaluated results following autograft procedure. The results indicated a high bone union rate of 91% across the entire data set, which improved to 98% when implantation or biological augmentation were performed [17].

Furthermore, the results revealed that when using autologous graft strategies, the size of the bone defect had no discernible impact on union percentages, showing that autografts can successfully treat both minor and substantial lesions. Additionally, because of their enhanced circulatory supply and better biological integration, vascularized autografts were connected to fewer instances of postoperative infection than non-vascularized grafts [17].

In accordance with these findings, Malik et al. (2024) conducted a randomized clinical study in patients with mandibular (jaw) fractures to assess the beneficial effects of autograft alone vs autograft in combination with Concentrated Growth Factor (CGF) and xenograft + CGF [18]. The study enrolled 60 patients divided into three groups: Group A received only autograft, Group B received autograft + CGF, and Group C received xenograft + CGF. Over a 6-month follow-up period, bone density was measured via cone-beam computed tomography at 4 and 6 months. All groups showed a statistically significant increase in bone bulk ($p < 0.001$) [18]. Importantly, while CGF-augmented grafts (both autograft + CGF and xenograft + CGF) achieved somewhat higher bone density compared to autograft alone, the mean difference in bone density between the autograft-only group and the autograft + CGF group was minimal (-229.2 vs -227.7, respectively). However, the authors concluded that CGF with xenograft might be preferable clinically because it avoids donor- site morbidity associated with harvesting autograft [18].

In a 2024 review, Sun et al. (2024) describe dentin-derived autogenous grafts prepared from a patient's own extracted teeth as a promising alternative to traditional autologous bone grafts for maxillofacial guided bone regeneration. Across 46 human clinical studies (including 17 comparative trials), the grafts showed enhanced new-bone formation, improved implant stability, and effective ridge preservation. Histologic biopsies demonstrated higher bone-to-graft contact (≈69% at 6 months) and greater new-bone percentage compared with xenografts (e.g., 60.75% vs 42.81%; p = 0.0084) [19]. The review further emphasises that while autogenous dentin shares the favourable biological profile of autograft bone (osteoconductivity, some osteoinductivity, tissue integration), it avoids many donor-site morbidities because the material is harvested from extracted teeth rather than major surgical donor site. Limitations flagged by the authors include the restricted volume available for grafting, variability in processing protocols, and a lack of large-scale long-term follow-up studies. Overall, Sun et al. conclude that autogenous dentin matrix grafts show compelling clinical performance in dental and maxillofacial applications and may serve as an effective adjunct or replacement for autologous bone grafting in selected patient settings [19].

Overall, given its significant bone regeneration and mineralisation results, these clinical data support the ongoing use of autologous bone grafting as a physiologically superior choice for managing mandibular fractures.

2.1.2 Translational Evidence from Preclinical Autograft Models

A durable rat model of Autologous Cancellous Bone (ACB) grafting was created by harvesting iliac cancellous bone and transplanting it into a femoral segmental bone defect for the purpose of analyzing the biological mechanisms of autologous bone grafting. After creating surgical defects, autologous cancellous bone from the rats' own iliac crests was transplanted into the model to correct them [20]. Complete bone integration was observed eight weeks after transplantation, and radiographic and histological tests revealed marked neo-osteogenesis along with gradual development of new bone volume. Histological findings revealed that the transplanted cancellous bone functioned as a scaffold for osteoblast infiltration, vascular ingrowth, and bone formation, resulting in structural regeneration at the fractured location. The cancellous autografts' extraordinary ability to promote bone union was shown by their strong osteogenicity and rapid incorporation into the host bone [20].

The impact of autologous cancellous bone grafts on the healing of bones was studied in an experimental rabbit model of tibial bone lesions to assess their regeneration ability [21]. The transplant group's lesions were filled with cancellous bone harvested from the same animal, but the control group's lesions remained unfilled. At 35 and 70 days aafter surgery, radiological, histological, and clinical examinations revealed that autograft-treated defects had significantly higher bone healing scores than untreated ones [21]. Furthermore, autologous bone transplants serve as an effective osteogenic scaffold for tissue regeneration, and biological adjuncts can enhance their effectiveness. Autografts containing hyaluronic acid resulted in greater healing than grafts alone [21]. These findings establish the strong in vivo bone-healing potential of autografts and strengthen their potential for therapeutic bone regeneration procedures [21].

Similarly, autologous cancellous bone grafts showed the highest histological grades of bone healing among all tested groups in an observational rabbit model designed for evaluating various bone graft materials. In this study, autograft, allograft, Demineralized Bone Matrix (DBM), bovine

xenograft, and synthetic alternatives were employed to treat New Zealand white rabbits with standardized femoral condyle lesions [22]. The animals were evaluated at 1, 3, and 6 months after implantation. According to histological analysis, the cancellous autograft outperformed all other graft materials in terms of new bone formation and integration, revealing its substantial in vivo osteogenic and osteoconductive potential. The results support autologous grafts' biological advantage and translational significance for clinical bone healing by indicating through experimentation that they perform better than alternative materials in promoting trabecular bone regeneration in animal models [22].

2.2 Allografts

Allografts are produced from individuals of the same species. Following comprehensive screening, these grafts are carefully selected, processed, and stored in bone banks. Allografts can be derived from living donors or cadaveric bone material treated to reduce immunological reactions and avoid the transmission of infectious illnesses. They come in many shapes and sizes, such as cortical, cancellous, or cortico-cancellous grafts [23]. Allografts may be considered in adult and older patients when avoidance of donor-site morbidity is desirable and larger graft volumes or structural support are required [24].

Allogeneic materials are rich in type I collagen and morphogenetic proteins (BMPs), which provide osteoinductive properties. However, although derived from humans, they have distinct genetic compositions, raising concerns about immunological rejection, blood compatibility, and disease or tumour cell transmission [25-27]. They are osteoinductive and osteoconductive, but lack osteogenic qualities, and their processing reduces their biological and mechanical properties [25-27]. Though allogeneic materials have some advantages similar to autogenous bone, such as higher availability, they are more expensive to manufacture [25-27].

Furthermore, a retrospective 10-year study conducted by Galagali DA et al. (2023) at an Indian tertiary tissue bank noted an increasing clinical adoption of allografts, with 2,776 grafts distributed primarily for acute fractures with bone loss or severe comminution. This descriptive study analysed 10 years of data from 2012 to 2022 [28]. This study analysed the number of grafts procured and used. Indications for tissue allografting were classified based on the surgical speciality utilizing the graft: orthopaedics, neurosurgery, ophthalmology, oromaxillofacial surgery, otorhinolaryngology, urology, and wound care. Special emphasis was placed on the use of bone allografts by orthopaedics and neurosurgery specialities. This study gives an outlook on the utilization of bone grafts in a tertiary healthcare facility [28]. The major findings were a recent development and different from the classical belief that bone grafting is mainly used for non-unions and arthrodesis. The proximal tibia emerged as the most common site for graft placement, highlighting the utility of allografts in diverse clinical scenarios. This was mainly due to the increased awareness and availability of tissue banking [28].

2.2.1 Recent Evidence and Clinical Outcomes of Allografts

A retrospective clinical trial assessed the efficacy of allograft bone grafts in restoring bone defects caused by surgical curettage of benign bone cancers in human patients. The study involved 55 patients (aged 10-61 years) who were treated between 2020 and 2022 and observed for 12-30 months after surgery. Patients were separated into two groups: those who received only allograft

bone and those who received allograft bone along with calcium phosphate bone powder [29]. All patients had effective primary incision healing with no problems such as graft non-union, postoperative fractures, or tumour recurrence. At 6 months, the group that received allograft bone combined with calcium phosphate had significantly shorter healing durations and higher Karnofsky Performance Status (KPS) scores than the allograft-only group. While 12-month healing rates and limb function were statistically similar between groups, the combined graft group revealed faster early recovery and functional improvement [29].

Furthermore, a retrospective study conducted by de Paula et al. (2024) with 147 adult patients undergoing cervical and lumbar spinal fusion procedures found that structural bone allografts generated from human cadaver donors had consistently high long-term fusion rates across a wide range of pathological indications, including degenerative conditions, traumatic fractures, and neoplastic involvement [30]. Over a mean follow-up duration of more than 12 years, adequate spinal fusion was accomplished in 98.9% of cervical cases and 97.9% of lumbar cases, with minimal graft subsidence and a low rate of side effects. Structural allografts also proved cost-effective, minimized the morbidity associated with autologous bone harvesting, and provided a viable alternative when anatomical constraints prevented the use of interbody cages or other devices. Overall, this evidence supports the clinical efficacy, safety, and long-term dependability of human structural bone allografts in spinal arthrodesis procedures [30].

In addition to long-bone and tumour reconstruction, clinical data from spinal surgery support the effectiveness of allografts in human patients. In a prospective semi- randomized study comparing autologous iliac crest bone grafts to cadaveric allografts in patients undergoing instrumented Anterior Cervical Discectomy and Fusion (ACDF), both graft materials achieved comparable fusion rates and satisfactory clinical results [31]. Radiographic examination revealed no statistically significant variations in the course or quality of spinal fusion between the two groups over the follow-up period.

Importantly, patients who received autografts had a higher incidence of donor- site-related morbidity, such as postoperative pain and discomfort at the iliac crest harvest site, whereas the allograft group remained unaffected by these problems. Graft collapse, subsidence, and implant-related problems occurred at equal rates in both groups, showing that allografts provide adequate structural support and biological integration in cervical spinal fusion [31]. These results suggest that using allografts as a reliable substitute for autografts in spinal reconstructive surgeries is clinically feasible, especially when reducing surgical time and donor-site morbidity is the primary concern [31].

2.2.2 Translational Evidence from Preclinical Allograft Models

In addition to autografts, researchers have conducted significant preclinical studies using well-established animal models to evaluate the efficacy of bone allografts. In a preclinical study using a sheep segmental tibial lesion model, researchers compared the biological performance of cortical bone allografts alone to allografts seeded with autologous Bone Marrow Stromal Cells (BMSCs) [32]. The study found that both groups developed new bone at the defect sites; however, the defects treated with cellularized allografts (allograft + BMSCs) indicated faster and more complete bone union than non-cellularized controls. Histological study revealed improved grafted bone remodelling and integration into host tissue, with cellularized allografts showing marrow space

restoration and osteogenic activity by 18 weeks post-implant [32]. This signifies that allografts have osteoconductive properties and that progenitor cell augmentation may significantly enhance their biological performance, providing valuable translational understanding for therapeutic bone reconstruction procedures [32].

Furthermore, the regenerative capabilities of human allogeneic bone grafts were tested in a controlled rabbit calvarial lesion model, alone and in combination with Mesenchymal Stem Cell-Conditioned Media (MSC-CM), to examine the impact of biological augmentation on the recovery of bone [33]. Eight rabbits were given bilateral 8 mm calvarial lesions, with one side treated with allograft alone (HB group) and the other treated with allograft mixed with MSC-CM (HB +GF group). At 2 and 8 weeks post-operatively, both groups had new bone production; however, the HB + GF group showed considerably better bone regeneration than the allograft-only group ($p < 0.05$). Notably, in the MSC-CM group, new bone extended into the centre of the lesion and showed greater extent of graft resorption and marrow formation by 8 weeks, indicating improved remodelling and osteogenic activity [33]. This shows that allograft bone naturally promotes bone regeneration; paracrine substances delivered via conditioned media can significantly enhance its osteogenic performance, providing preclinical support for adjunctive strategies in bone graft applications [33].

Moreover, in a large-animal sheep model of severe femoral condyle cancellous bone defects, researchers examined the biologic performance of cancellous allografts versus untreated defects and synthetic alternatives [34]. At 4, 8, and 12 weeks after implantation, the cancellous allograft group revealed increasing bone volume density (BV/TV) and significant new bone ingrowth inside the defect site using micro-computed tomography and histological studies.

The allograft group showed successful osteoconduction and graft integration, with a significantly greater bone volume fraction at each time point relative to baseline. Histology showed that the allograft scaffold was connected by host bone, with cellular activity, trabecular remodelling, and vascular invasion consistent with typical bone healing processes [34].

A comparative study used the rat femoral critical-size lesion model to assess the bone-healing capacity of allografts, autografts, and artificial bone substitutes. In this work, a standardized segmental lesion was generated in rats' femoral diaphysis and then treated with autogenous bone, allograft bone, or synthetic graft material [35]. Radiographic and histological examinations at 4 and 8 weeks post-implantation determined that, while autografts achieved the fastest and most complete defect healing, defects treated with allograft bone still showed significant new bone formation and acceptable graft integration when compared to synthetic controls. Histomorphometric analysis showed significant increases in new bone percentage in the allograft group over time ($p < 0.05$), confirming the osteoconductive capacity of allografts to promote bone regeneration [35]. Although the rate and amount of restoration were slightly lower than with autografts, the allograft group consistently outperformed synthetic alternatives, establishing its biological relevance in preclinical bone healing models [35].

2.3 Synthetic Grafts

The purpose of synthetic bone materials is to stimulate bone repair. Synthetic materials have several advantages over the surgical method used to obtain autogenous material, including biocompatibility, osteoconduction, injectability, moldability, easy manipulation, minimally invasive procedure, scar reduction (because only the affected area is surgically treated and only one surgery

is required), and a lower risk of infection and other complications [36-38]. Another benefit is that the material may be easily made in large quantities, unlike autogenous, allogeneic, or xenogeneic materials. When autologous bone is insufficient, or an osteoconductive substitute is required, synthetic bone grafts may be considered across a broad age range, including adults and older adults [3]. As an alternative, several bioceramic materials have been developed, and when applied to medium and small bone defects, periodontal disease-related bone defects, maxillary sinus elevation, and increasing the bone crest for implant placement, several clinical and laboratory investigations have shown the osteoconductive properties of these materials [39-43]. Since calcium phosphate-based materials are similar to real bone in composition and have great biocompatibility, they are widely researched and employed as bone grafts within the ceramics group. These materials can be worked into a paste in addition to their granular state, which shortens the time needed for application and primarily increases the defect's moldability [38, 42, 44, 45]. The lack of osteoinductivity of calcium phosphates hinders their usage for larger defects; therefore, several kinds of studies are being conducted to meet this need [46].

It is essential to highlight that synthetic biomaterials lack osteoinductive qualities, which are considered to be favorable for the production of new bone. As a result, these materials still have specific disadvantages when utilizing these materials in critical-sized bone lesions, which encourages ongoing studies and the addition of other elements to enhance their functionality. To enhance the osteogenic qualities of scaffolds of synthetic or xenogeneic origin, additional materials can be added. To improve the quantity and quality of newly formed bone and boost biological performance, many different elements have been studied and combined into these materials, such as growth factors, cellular content, autogenous bone, and medicinal compounds. This field of research is known as tissue bioengineering [25, 47-50].

Graft selection is determined by the biological properties and surgical requirements of the defect site, and bone grafting strategies are required for osseous lesion regeneration and healing. Autografts, allografts, and synthetic grafts are the three main varieties, and each has distinct osteogenic, osteoinductive, and osteoconductive characteristics. Mechanical stability, availability, and immunogenicity further influence graft selection. Table 1 presents the origins, inherent biological characteristics, therapeutic applications, limits, and most recent developments that promote graft efficacy and integration.

Table 1 A Scientific Overview of Bone Graft Modalities and Their Clinical Relevance.

Feature	Autografts	Allografts	Synthetic Grafts
Source	Same individual (autologous)	Same species (human donors - living or cadaveric)	Artificial materials (e.g., calcium phosphate ceramics)
Intrinsic Properties	Osteogenic, osteoinductive, and osteoconductive	Osteoinductive and osteoconductive	Osteoconductive (some efforts to enhance osteoinductivity)
Age Group/ Clinical Population	Pediatric, adolescent, and adult patients; particularly when sufficient donor bone is available.	Adolescents and adults, including older patients, particularly when larger graft volume or structural support is required.	Broad age range; considered when autologous bone is insufficient or an osteoconductive substitute is appropriate.

Biological Components	Osteoblasts, osteoprogenitor cells, BMPs, growth factors.	Type I collagen, BMPs (processed).	No cellular components; may include HA, β-TCP, or be combined with bioactive agents.
Advantages	Gold standard. No immune rejection. High regenerative capacity.	Readily available. No donor site morbidity. Suitable for large defects.	Easy to manufacture. No disease transmission. Moldable, injectable, minimally invasive.
Shortcomings	Limited harvest volume. Donor-site morbidity, including chronic pain (14.2%) and infection (5.9%) [51]. Surgical risks, including neuropraxia, numbness, hematoma, and iatrogenic fracture [51].	-Disease-transmission risk is extremely low with modern screening and processing; estimated risk of HIV or hepatitis B/C transmission is approximately 1 in 1.6 million [52]. -Immunogenic responses may occur because allografts contain donor-derived antigens, although processing and preservation reduce immunogenicity [52]. -Processing and sterilization may reduce mechanical and biological properties of the graft [52].	Lacks osteoinductivity. Limited use as standalone grafts for critical-sized/ segmental bone defects because of insufficient biological activity. Limited or variable biological integration, particularly when adequate vascularization and osteogenic stimulation are lacking [53].
Clinical Uses	Fracture healing, fusion, non-union, trauma, congenital or post-infection/tumor defects	Acute fractures, bone loss, comminution (e.g., proximal tibia, as per tissue bank study)	Small/medium defects, implant placement, periodontal defects, sinus lifts
Recent Advances	BMAC, PRP, stem cells, minimally invasive harvest techniques	Tissue banking and processing improvements	Tissue bioengineering: adding growth factors, cells, therapeutic elements to improve performance
Cost & Availability	Limited and costly (due to harvest)	More available, but processing is expensive	Cost-effective and scalable.

2.3.1 Recent Evidence and Clinical Outcomes of Synthetic Grafts

A prospective, multicenter randomized controlled trial evaluated the clinical and radiographic effectiveness of a new Biphasic Calcium Phosphate (BCP) bone graft with 40% β-TCP and 60% HA compared with Demineralised Bovine Bone Mineral (DBBM) in the reconstruction of peri-implant dehiscence defects. After 18 weeks of recovery, both groups showed significant improvements in clinical and radiographic markers ($p < 0.001$ for within-group changes). The buccal dehiscence faults were fully resolved in 59.3% of the BCP group and 42.3% of the DBBM group. The mean resolution rate was 71.3% in the BCP group versus 57.9% in the control group [54]. Mean horizontal bone thickness did not differ significantly between groups (BCP: -0.50 ± 0.66 mm vs. DBBM: -0.66 ± 0.83 mm; $p = 0.133$), but both grafts demonstrated favorable clinical and radiographic outcomes with no severe complications or significant differences in postoperative discomfort or early wound healing. These outcomes provide evidence that synthetic BCP grafts can achieve equivalent results to conventional xenografts, hence supporting their use as effective synthetic alternatives in peri-implant reconstruction of bones [54].

Furthermore, a network meta-analysis of controlled studies examining Bone Substitute Materials (BSM) in sinus floor augmentation and dental implant operations analyzed data from 362 sinus augmentation specimens from 283 participants [55]. The study evaluated autografts (AB), Allografts (AL), BCP, β-TCP, and xenografts. The research results revealed that autografts produced 12.33% more new bone than BCP (95% CI: 10.74, 13.93), whereas allografts produced 5.14% more than BCP (95% CI: 3.33, 6.95). BCP led to 4.14% larger new bone growth than Xenografts (95% CI: -6.38, -1.9). Although autografts and allografts were the most effective for osteogenesis, BCP consistently outperformed xenografts and β-TCP. This shows that biphasic calcium phosphate offers adequate new bone synthesis in clinical settings and represents a promising synthetic option for bone augmentation therapy. Residual graft material was also minimal in xenografts, showing performance discrepancies [55].

Additionally, a clinical case series analyzed the utilization of Polylactic Glycolic Acid (PLGA)-coated β-TCP as a synthetic substitute for bone for retaining the alveolar ridge after tooth extraction. During the course of this investigation, patients treated with the β-TCP graft showed excellent new bone production and maintained alveolar ridge height during the early healing phase. At 8 weeks after transplantation, radiographic study revealed the mean vertical ridge height loss measured at 0.87 ± 0.35 mm, indicating successful maintenance of alveolar bone dimensions [56]. A histomorphometric analysis of biopsies taken during implant placement revealed a large proportion of healthy bone (mean ~33-38%), with residual β-TCP particles embedded in a calcified matrix. In practice, all implant sites in the study indicated primary stability at placement, with no adverse reactions, significant inflammation, or graft-related issues reported throughout follow-up. These results indicate that β-TCP-based synthetic grafts can effectively support alveolar bone growth and dimensional stability, providing an alternative to autografts in dental and maxillofacial bone restoration [56]. Overall, Studies indicate that synthetic bone grafts, especially those incorporating β-tricalcium phosphate and biphasic calcium phosphate, exhibit osteoconductive properties and promote bone regeneration in humans.

2.3.2 Translational Evidence from Preclinical Synthetic Models

According to preclinical trials in animal models, synthetic bone graft substitutes such as HA and β-TCP have been investigated to enhance bone regeneration and integration in critical-size bone defects. In an ovine scapular model, the synthetic HA graft was fabricated by preparing a homogeneous water-based HA slurry, coating a combustible polyurethane foam template, and then drying at 50°C and sintering at 1000°C to generate a highly porous, open-cellular structure [57]. The sintered material was subsequently crushed and sieved to obtain granules of 1000-2000 μm. The β-TCP graft was produced using a porogen-based fabrication approach followed by pressing and sintering at 1000°C [54]. β-TCP exhibited significantly higher early new bone formation compared with both synthetic HA and bovine-derived HA (3 months: 55.65 ± 2.03% vs. 49.05 ± 3.84% and 47.59 ± 1.97%, respectively, $p \leq 0.03$; 6 months: 62.03 ± 1.58% vs. 55.83 ± 2.59% and 53.44 ± 0.78%, respectively, $p \leq 0.04$). All graft materials maintained osteoconductivity and long-term integration, achieving comparable rates of new bone growth and bone-particle contact by 12-18 months. Notably, no harmful or inflammatory tissue reactions were discovered, confirming these synthetic substitutes' biocompatibility. In addition to promoting early osteogenesis, these grafts allow gradual scaffold disintegration and host bone replacement, which is equivalent to conventional bone regeneration. By modifying porosity, particle size, and content, researchers can optimize mechanical stability and osteoconductive properties. This makes them viable alternatives in instances where autograft harvesting is limited or not allowed [57].

Furthermore, a controlled preclinical study examined the impact of various HA coating intensities on α-Tricalcium Phosphate (α-TCP) bone graft substitutes in critical-size calvarial lesions in 24 New Zealand white rabbits. The α-TCP core was prepared by mixing calcium- and phosphate-containing precursor powders in appropriate proportions, followed by high-temperature sintering, after which the surface of the sintered TCP was converted into a biomimetic nanocrystalline HA coating [58]. Four experimental groups were evaluated based on the composition of biomimetic HA/α-TCP granules with coating ratios of 3%:97% (BBCP1), 12%:88% (BBCP2), and 23%:77% (BBCP3), along with sham-treated controls. At 3 weeks, all groups had early ingrowth of structured bone from defect margins. After 12 weeks of healing, specimens showed lamellar bone production in peripheral regions and a combination of woven and lamellar bone in the centre, indicating advanced maturation. Quantitative analysis revealed that the BBCP1 group (lowest HA coating) had the highest new bone area and bone volume compared to other compositions and controls, as well as much reduced residual biomaterial, showing faster scaffold disintegration and remodeling. These findings demonstrate that biomaterial resorption and osteogenesis are influenced by the degree of HA coating, with faster bone formation and faster fracture repair being associated with lower HA contents in the composite [58].

In a controlled canine mandibular extraction socket model, three Hydroxyapatite-β-Tricalcium Phosphate (HA-β-TCP) collagen composite grafts containing different HA/β-TCP ratios have been evaluated for alveolar ridge preservation performance. The HA-β-TCP collagen composites were commercially available graft materials in which collagen was incorporated as a coating or binder; HBC28 contained approximately 10 wt.% bovine collagen, while HBC37 and HPC64 contained approximately 4-6 wt.% collagen, with bovine-derived type I collagen in HBC37 and porcine-derived type I collagen in HPC64 [59]. Eight mongrel dogs underwent bilateral premolar extractions, with sockets randomly grafted with HBC28 (20% HA/80% β-TCP with bovine collagen), HBC37 (30%

HA/70% β-TCP with bovine collagen), or HPC64 (60% HA/40% β-TCP with porcine collagen). All groups demonstrated comparable new bone development as well as effective socket maintenance at 12 weeks, revealing the synthetic composites' overall osteoconductive potential [59]. Graft composition has an essential impact on resorption kinetics and remodeling behavior, as proven by the statistically significant differences in residual graft volume and bone volume fraction between graft types identified by histological and histomorphometric analyses: HBC28 had a higher residual graft volume, while HPC64 had more extensive graft resorption. All analyzed HA-β-TCP collagen composites were biocompatible, and no group showed adverse tissue responses or inflammation. This shows that synthetic HA-β-TCP collagen grafts are suitable for extraction socket preservation, with varying resorption and integration profiles depending on material composition [59].

3. Clinical Applications and Outcomes of Bone Grafts in Orthopaedic Surgery

Intended to manage critical-sized one-bone defects, nonunions, spinal fusions, joint reconstructions, and subsequent arthroplasties, modern orthopaedic surgery relies extensively on bone grafting. Osteointegration, osteogenesis, osteoconduction, and osteoinduction are the four primary biological features that are essential for carrying out this function [60, 61]. Additionally, several other factors will affect a bone graft's success percentage. These consist of, but are not restricted to, compressive strength, cost, ease of handling, plasticity, sterility, structural integrity, biocompatibility, bioresorbability, and adequate porosity for vascular ingrowth [62]. These characteristics work together to support their use, provide host tissues with sufficient long-term tolerance, and enhance the likelihood that osteo-regenerative processes will be successful [62]. According to a meta-analysis by Azi ML et al. (2016), 91% of patients had a primary bone union rate, meaning most recovered without additional therapies. Furthermore, the secondary union rate increased to 98% when follow-up procedures, including a second procedure or re-grafting, were incorporated [17].

Clinical applications differ by anatomical location: bone grafting with augmentation (e.g., calcium phosphate cements or hybrid allograft materials) promotes early mobilization and minimizes implant failure in proximal femur fractures, particularly in the elderly [63, 64]; Autografts and bone graft extenders, including demineralized bone matrix (DBM), are widely employed to promote arthrodesis in spine surgery; structural grafts assist in restoring anatomy and maintain alignment in distal radius and wrist fractures, especially in comminuted intra-articular patterns [65, 66]. A recent meta-analysis by Tavares WM et al. (2022) has demonstrated that autologous and alternative grafting materials are both very successful in lumbar fusion procedures [67]. Fusion rates can exceed 85% when equipped. Local bone grafts are a suitable autograft alternative with reduced donor site morbidity, with the highest reported fusion success rate (95.3%). The results suggest that patients who received bone grafts had lower reoperation, improved mobility ratings, and greater pain relief—all of which affected functional recovery [67]. The effectiveness of the injectable bone substitute CERAMENT™ in tibial plateau fractures with significant subchondral voids was assessed in the prospective experiment by Iundusi R et al. (2015) [68]. Three years following CERAMENT™ augmentation and open reduction and internal fixation (ORIF), subjects reported promising outcomes. CERAMENT™ was totally resorbed in an average of five months of use, and radiologically, the fracture reduction was sustained with minimal loss. Patients recovered functionally more quickly, achieving early mobilization and being able to bear weight. The study discovered that

CERAMENTTM reduced postoperative collapse and increased rehabilitation periods as a suitable alternative to traditional bone transplants [68]. Additionally, the introduction of biologics such as platelet-rich plasma (PRP), BMPs, and MSCs to grafts has significantly increased the regenerative potential for bone grafting techniques. This comprehensive analysis included data from 203 patients participating in six observational studies, with 117 receiving recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) [69]. Randomized controlled studies were not practicable due to the intervention's practical limitations. Only two studies used blinded radiological assessment of fusion, which increases the risk of bias. At 24 months, the fusion outcomes were reviewed uniformly. Autologous Bone Grafts (ABG) have limited applications in revision and multi-level spinal fusions due to inadequate quantity and poor bone quality. On the other hand, rhBMP-2 showed a high overall fusion rate of 92.3% and a statistically significant reduction in the duration of fusion when compared to bone Grafts [69]. Though rhBMP-2 did not outperform ABG in terms of fusion rates, it ultimately resulted in faster fusion as well as less postoperative pain. Even in high-risk patients who had previously failed fusions, rhBMP-2 achieved an 88.1% fusion rate. Despite safety issues, including retrograde ejaculation, radiculitis, and potential carcinogenicity at high dosages, rhBMP-2 remains an effective graft alternative in complicated, revision, and multi-level spinal fusion cases, particularly where autograft or allograft possibilities are limited [69]. Therefore, bone grafting remains crucial in orthopaedic surgery. Recent developments in physiologically active scaffolds, nanotechnology-enhanced materials, and patient-specific grafting techniques are improving both immediate and long-term clinical outcomes [69].

4. Emerging Technologies

4.1 Nanomedicine

Infections, cancers, and bone loss are among the bone conditions driving demand for bone regeneration [70]. Natural bone regeneration is complicated and can be delayed by conditions such as osteoporosis or diabetes, major deformities, and poor vascularization [71].

Although autografts offer advantages such as osteoinductive potential and biocompatibility, they also have disadvantages like infection risk, limited supply, and donor site morbidity [72]. Although synthetic alternatives offer structural support, they often lack the required biological signals. Innovative strategies like Tissue Engineering (TE), which involve the recruitment, differentiation, and production of bone matrix by osteoprogenitor cells supported by porous biodegradable scaffolds, are required to address such problems [72-74]. By replicating the natural architecture of bone, advances in scaffold materials seek to promote osteogenesis and angiogenesis [75, 76]. Since most biological molecules exist and function at the nanoscale, nanomedicine, or the application of nanotechnology in medicine, aims to address disease-related issues at this scale [7]. Polymeric nanofiber networks may resemble the structure of normal human tissue at the nanoscale. Nanomedicine for cartilage and bone restoration may provide better therapeutic benefits by replacing damaged osteochondral tissue with native biological tissue, potentially restoring joint function, due to the high surface-to-volume ratio of nanoparticles [77]. For example, targeted labelling technology based on nanoparticles can quickly evaluate bone quality and spot early cartilage abnormalities that are difficult to identify with current monitoring techniques [78].

NPs enable targeted, minimally invasive therapy by delivering bioactive chemicals, modifying the immune system, and providing scaffolding for bone formation [79, 80]. They are a revolutionary tool

in TE and Regenerative Medicine (TERM) because of their low toxicity, flexibility, and potential for modified delivery [81, 82]. These features enable precise control of biological processes and real-time monitoring, which enhances the quality of synthetic tissues and overcomes challenges in TE and TERM [83]. Furthermore, NPs can mitigate the negative aspects of bioactive chemicals, such as poor solubility, unstable bioactivity, and short circulation half-life. They are therefore perfect for managing and observing bioactive compounds [84].

Therefore, one current therapy for osteoporosis is the development of osteoporotic bone-targeting drug delivery systems with enhanced therapeutic efficacy but fewer non-targeted side effects. Since they have a strong tendency to bind hydroxyapatite, bisphosphonates, one of the medications that target bone, can serve as a linker between NPs and bone minerals [85].

Using NPs to deliver osteoclast inhibitors or osteoblast activators is an alternative way to treat osteoporosis by modifying the cellular and molecular function of bone. To encourage bone development, small-molecule medications, growth factors, or gene-targeted chemicals have been delivered to specific bone locations in the clinic using PLGA, hyaluronic acid, or chitosan nanoparticles [85]. Furthermore, co-delivering several medications (such as osteoclast inhibitors and osteoblast activators) in nanocarriers through various functionalizations will serve as a single, multipurpose method for adjusting the equilibrium of bone remodeling [86].

Nanomaterials with biomimetic characteristics and superior physicochemical qualities have become more and more popular in orthopedic clinical trials and applications as the bone extracellular matrix is primarily composed of highly organized collagen nanofibers and nanocrystalline HA [87].

By using 3D porous scaffolds for regulated, sustained drug delivery, nanomedicine promotes tissue regeneration. Chemical vapor deposition, anodization, and micelle lithography are techniques used to produce drug-loaded nanoparticles. By facilitating growth factor release, these NPs aid in tissue growth and repair. Certain NPs have demonstrated efficacy in bone regeneration and nerve growth, including graphene oxide composites and iron oxide-gold [88].

NPs, including gold NPs, cerium oxide NPs, and superparamagnetic microspheres, are being used to treat wounds and heart tissue. Combining nanomedicine with TE aims to develop advanced scaffolds for regenerating different tissues, including the heart, skeletal muscles, bones, and neurons. This combination results in innovative and efficient scaffolds for effective tissue regeneration and repair [88].

4.2 3D Printing of Personalized Bone Scaffolds

Bone is a strong tissue that can repair itself. However, the body cannot repair a significant bone defect, known as a Critical-Sized Defect (CSD) [89, 90]. The skeletal region implicated and the condition of the soft tissue surrounding it can affect the magnitude of CSDs [91, 92]. For instance, a defect with a diameter of 3 cm is considered a CSD for the radius and ulna, but a defect with a diameter of 5 cm is considered a CSD for the femur and tibia [93, 94]. To restore the bone's function in these situations, surgical procedures such as bone grafts are required [95]. Traditional treatment methods, including autografts, allografts, and xenografts, have been limited due to related problems such as a shortage of donors and donor sites, the need for additional surgery, the possibility of disease transmission, and the immune response following implantation. To restore,

maintain, and/or enhance bone function, researchers have developed new constructs using bone tissue engineering (BTE) [96, 97].

3D printing processes can produce highly porous materials with connected pores has led to their increasing popularity in the scaffolding industry. Using various biomaterials (natural and synthetic), cells (such as cell lines and stem cells), and growth factors (such as osteogenic and angiogenic), bioprinting also makes it possible to create biomimetic scaffolds [98]. Figure 3 illustrates the complete workflow of 3D-printed bone scaffold fabrication and implantation.

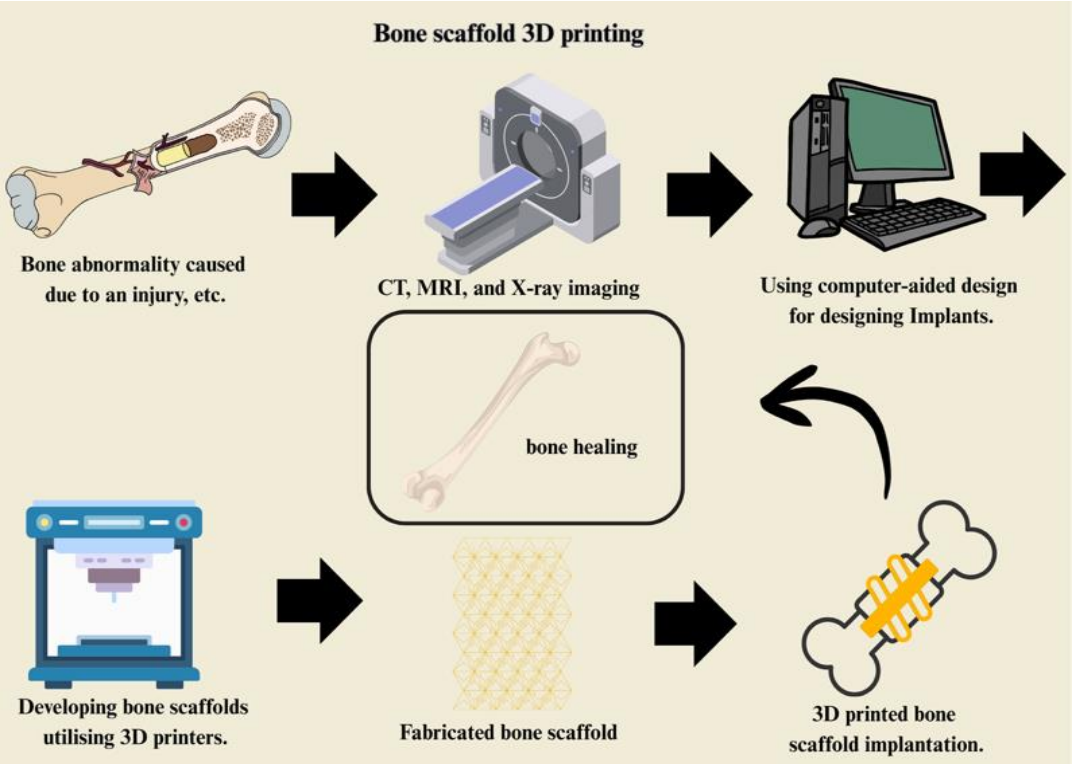


Figure 3 Workflow of 3D-printed bone scaffold fabrication and implantation. The image was created manually using Canva.

To date, several studies have attempted to develop a scaffold that resembles genuine bone in terms of its mechanical and osteoconductive characteristics. Ren et al. (2024) developed HA-hybrid bioinks using a one-pot synthesis strategy and fabricated scaffolds using Digital Light Processing (DLP) 3D printing. (Gelatin Methacryloyl)/Alginate Methacryloyl (GelMA/AlgMA) and GelMA/AlgMA/HA bioinks demonstrated good printability, stability, and improved mechanical properties [98]. The HA-containing 3D-printed scaffolds enhanced osteogenic differentiation in vitro. They demonstrated improved bone-repair capability in a rat calvarial defect model compared with pure organic bioinks, showing the potential of DLP printing for bone tissue engineering [98]. Likewise, Song et al. (2024) utilized DLP 3D printing to fabricate hierarchical biomimetic GelMA/Silk Fibroin Methacryloyl (SilMA)/HAp scaffolds 100. After preparing GelMA and SilMA, the authors generated biomimetic micropores and then added hydroxyapatite (HAp) to the bioink. The produced scaffolds were tested for vascular regeneration, osteogenic differentiation, cell adhesion, and proliferation. After 12 weeks of implantation, the scaffolds promoted tissue integration and bone regeneration, achieving a bone-volume fraction of 42.39%, demonstrating their potential for repairing critical bone defects [99]. The performance of 3D-printed bone scaffolds depends on

multiple interconnected design requirements, including architecture, porosity, mechanical properties, degradation, and vascularization [100]. Figure 4 depicts the fundamental design parameters and their influence on scaffold performance and bone regeneration.

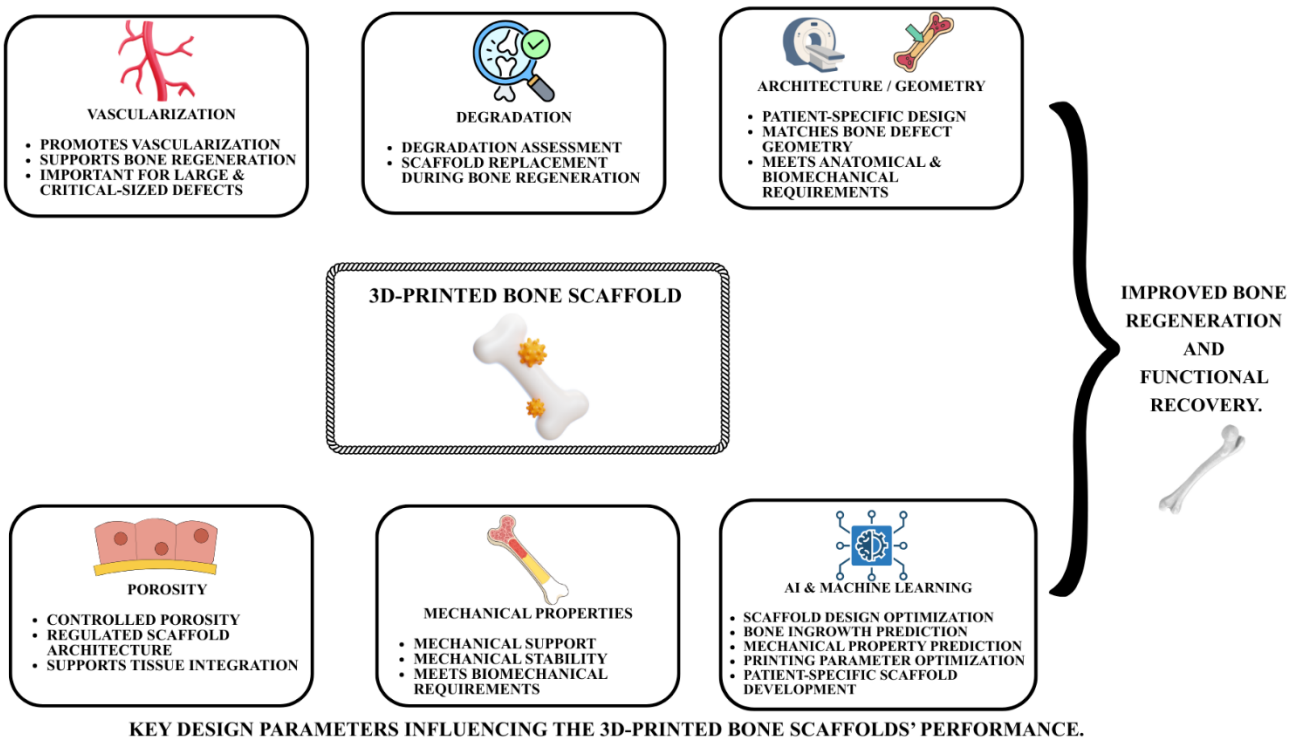


Figure 4 Key design parameters influencing the 3D-printed bone scaffolds' performance. The image was created manually using Canva.

However, ongoing discussion remains about the best structural characteristics of bone scaffolds, the manufacturing process, the material preparation method, the cell type, biological assistance, and the implantation site. Therefore, further research is required. Both in vitro and in vivo tests have been conducted as preclinical evaluations to monitor bone regeneration in bone scaffolds. Despite advances in this field, these assessments still present challenges. More specifically, in vitro research cannot fully replicate the in vivo environment. The primary disadvantage of in vivo research is species-specific bone variation, which also makes it expensive and requires careful ethical considerations [101].

4.3 Stem Cell-Based Tissue Engineering

Two distinct categories of stem cells can be defined: (i) adult stem cells and (ii) Embryonic Stem Cells (ESCs) [102]. Mesodermal in origin, MSCs can be extracted from bone marrow, adipose tissue, the periosteum, and the human umbilical cord. They possess the capacity to differentiate toward the osteogenic lineage and develop osteoblast-like cells [103, 104]. In recent years, MSC cell sheets have been used extensively to regenerate a variety of tissues and organs, such as bone, cartilage, meniscus, tendons, teeth, periodontal tissue, and skin. As observed, for instance, with Human PDL stem cells (PDLSCs) and human Jaw Bone Mesenchymal Stem Cells (JBMSCs), cell sheets are thin films with a thickness of around 80- 150 µm that may be produced in two weeks of in vitro growth utilizing MSCs [105, 106]. Importantly, cell sheets can be transplanted directly into tissue without

extra scaffolds because they are rich in cell-to-cell connections and extracellular matrix proteins [107]. Ouyang et al. initially described the use of MSC sheets for osteogenic tissue regeneration in 2006 [108]. In their groundbreaking investigation, the researchers assessed how MSC sheets formed onto demineralized bone grafts using the wrapping method affected the healing of tendon and major bone lesions. The results revealed that the healing of bone defects required the formation of a periosteum-like structure when MSC sheets were incorporated with large allografts [108].

To determine if MSC sheets could potentially be employed for bone regeneration, Chen et al. developed tube-like structures out of MSC sheets and PLGA meshes and implanted them in naked rats. Their investigations revealed that the rats' subcutaneous sites developed dense, mineralized tissue [106].

Additionally, three other investigations demonstrated that MSC sheets produced by comparable wrapping techniques may be used to create viable bone structures [109-111]. In the first investigation, Zou and colleagues found that cortical bone formed in mice when MSC sheets were wrapped onto an allograft and implanted [111]. While Uchihara et al. co-transplanted cell sheets derived from BMSCs with irradiated bone in the rat femur and showed that MSC sheets can be used to facilitate osteogenesis of irradiated bones [110], Long et al. demonstrated in the second study that allografts wrapped with MSC sheets were able to enhance graft-host osteointegration as well as bone callus formation [109].

Significantly, mineralized osteogenic BMS sheets combined with tubular coral scaffolds can produce bone grafts in specific shapes and with a structure similar to the original bone [106]. Other research shows that MSC sheets can be used without scaffolds or grafts [112-114]. Akahane et al. were the first to examine the graft-free use of MSC sheets to obtain bone tissue [114]. Using osteogenic supplements, they produced rat MSC sheets, which they then wrapped into tube-like structures before transplanting them into the rats' subcutaneous locations. Osteogenic MSC sheets can serve as osteogenic implants for bone tissue repair, as shown by ectopic calcification six weeks after sheet transplantation. [114]. Later, the same scientists used a needle to inject MSC sheets made from BMSCs into the rats' dead bone and subcutaneous areas [113]. Their findings confirmed that MSC sheets can be inserted with a needle to repair hard tissue in osteonecrosis and non-union cases without scaffolds or grafts. Thus, tissue-derived MSC sheets can be employed in scaffold/graft-free applications or in conjunction with different scaffolds or grafts to reduce the duration of bone regeneration treatment [113]. Figure 5 highlights the combined use of bone tissue engineering components and organic/inorganic nanoparticles to enhance bone repair and regeneration.

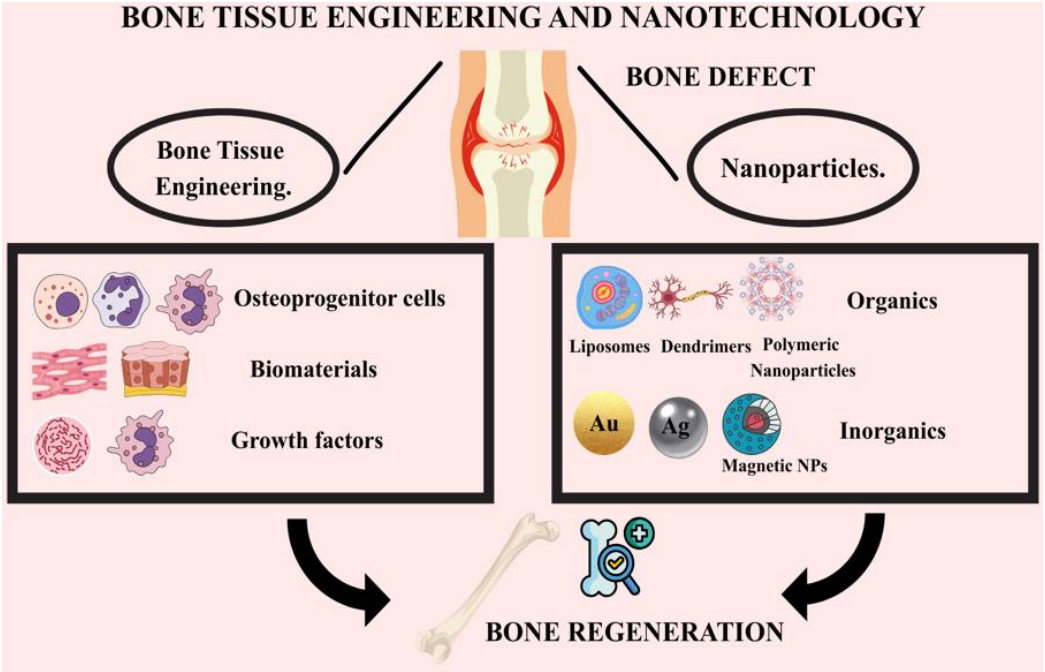


Figure 5 Combined use of bone tissue engineering components and organic/inorganic nanoparticles to enhance bone regeneration. The image was created manually using Canva.

The primary disadvantage of cell sheet technology is the potential for necrosis within the cell sheets due to the lack of vascularization. Therefore, the literature encourages creating 3D vascularized tissues using cell sheet engineering [115].

Table 2 summarizes indicative preclinical studies employing MSC sheet-based constructions, which include anatomical regions, experimental models, outcome measures, and significant results that promote the therapeutic effectiveness of this procedure in bone tissue engineering.

Table 2 Preclinical Applications of MSC Sheet-Based Bone Tissue Engineering.

S. No.	Experimental Approach	Model System	Key Findings	Ref.
1	MSC sheets wrapped around demineralized bone grafts	Large bone and tendon defect model	MSC sheets formed a periosteum-like structure and significantly enhanced bone regeneration and integration with allografts.	[108]
2	Tubular constructs created using MSC sheets and PLGA Meshes	Subcutaneous implantation in nude rats	Thick mineralized tissue developed, confirming the potential of MSC sheets for effective boneregenration.	[106]
3	MSC sheets wrapped onto cortical bone allografts	Mouse model	MSC sheets induced cortical bone formation, demonstrating their osteogenic potential in vivo.	[111]

4	MSC sheets applied to allografts to improve bone repair	Rodent model	MSC sheets enhanced graft-host osteointegration and promoted robust bone callus formation.	[110]
5	Co-transplantation of MSC sheets with irradiated bone segments	Rat femur model	MSC sheets supported bone regeneration in irradiated environments, confirming their role in osteogenesis under compromised conditions.	[109]
6	Transplantation of scaffold-free osteogenic MSC sheets formed into tubular shapes	Subcutaneous site in rats	Ectopic bone formation and calcification were observed six weeks post-implantation, validating scaffold-free applications of MSC sheets.	[114]
7	Needle-based injection of MSC sheets into necrotic bone and subcutaneous sites	Rat model	MSC sheets facilitated hard tissue formation in osteonecrosis and non-union without the use of scaffolds or grafts.	[113]
8	Scaffold-free transplantation of MSC sheets	Multiple in vivo models	Studies consistently reported effective bone formation, indicating MSC sheets can be used independently of scaffolds.	[112-114]
9	Mineralized osteogenic MSC sheets combined with coral-based tubular scaffolds	Not specified	Successfully generated bone grafts with defined shapes and structure closely resembling natural bone.	[106]
10	Focus on overcoming necrosis in thick cell sheets by introducing vascularization	Theoretical/ future direction	Lack of vascularization in MSC sheets can cause central necrosis; thus, development of 3D vascularized tissue constructs is a current research priority.	[115]

5. Limitations of 3D-Printed Scaffolds

Although 3D printing facilitates patient-specific scaffold development and precise control over scaffold architecture, several challenges remain that hinder its translation into routine clinical practice. Material selection remains challenging, as an effective scaffold needs sufficient mechanical strength, biocompatibility, controlled degradation, and biological activity [116]. For example,

bioceramics typically exhibit favourable osteogenic properties. Still, they may have relatively poor mechanical performance and slow degradation, whereas polymers can offer flexible degradation and processing characteristics but may have inadequate mechanical strength or osteoinductivity [116]. Another significant limitation, particularly for large and critical-sized bone defects, is inadequate vascularization. Although interconnected pores promote vascular and cellular ingrowth, increasing scaffold porosity may compromise mechanical strength. Inadequate vascularization can limit oxygen and nutrient delivery to the central regions of large scaffolds, thereby compromising cell survival, tissue integration, and bone regeneration. Current vascularization strategies, such as scaffold modification and incorporation of vascular networks, remain technically challenging and require further development [117]. Biological limitations are particularly important for 3D bioprinted scaffolds. Integrating living cells and biological components into printed constructs raises problems related to cell viability, distribution, maturation, self-organisation, and co-culture [116]. Although bioprinting can produce more biologically complex structures, maintaining appropriate cellular organization and maturation after implantation remains challenging [116]. Manufacturing and printing limitations can affect scaffold performance and reproducibility. Factors such as printing resolution, printing speed, material availability, scaffold geometry, and manufacturing consistency can influence the overall structure and mechanical properties of the resulting construct. Furthermore, effectively recreating the complex hierarchical architecture and vascular pathways of native bone remains challenging. Variations between digitally designed and printed scaffolds may affect their clinical performance [116]. Additionally, Clinical translation remains a significant challenge. Despite substantial preclinical research, few 3D-bioprinted bone scaffolds have reached clinical translation, and long-term clinical evidence showing superiority over conventional approaches such as autologous bone grafts remains limited. Producing large-scale, mechanically strong, and adequately vascularized constructs for critical-sized bone defects remains challenging [116, 118]. Furthermore, regulatory requirements, manufacturing standards, reproducibility, and the need for well-designed clinical trials continue to hinder translation into routine clinical practice [116, 119].

6. Machine Learning and Artificial Intelligence

Machine Learning (ML) and artificial intelligence (AI) are predicted to have significant effects on periodontal and bone regeneration. These technologies can optimize treatments by predicting bone growth within scaffolds [120]. Additionally, applying AI and ML to forecast the potential success of bone regeneration for individual patients will assist physicians in making better surgical decisions and enhance patient care. These technologies will probably provide even more accurate forecasts and effective treatment options as they advance [121, 122]. Several recent studies have applied machine learning and artificial intelligence to predict and improve bone regeneration and scaffold performance. Wu et al. analyzed data from a 12-month sheep tibial segmental-defect study to develop a machine-learning-based multiscale model for predicting bone ingrowth within scaffolds. The ML-based predictions demonstrated good accuracy and computational efficiency compared with a conventional multilevel finite-element model, highlighting the potential of machine learning for subject-specific prediction of bone regeneration in scaffold systems [123]. Similarly, Ibrahimi et al. utilized a dataset containing more than 1,000 Triply Periodic Minimal Surface (TPMS) scaffolds to develop machine-learning approaches for designing biomechanically compatible bone tissue-

engineering scaffolds [124]. The mechanical and morphological properties of the scaffolds were characterized using finite-element modelling and image analysis, and three machine-learning models were trained to establish relationships between scaffold design parameters and their properties. The study showed that machine learning could help develop hydroxyapatite-based TPMS scaffolds according to predefined biomechanical, mechanobiological, and technological requirements [124]. More recently, artificial intelligence has been increasingly incorporated into the design and fabrication of 3D-printed bone scaffolds. Quan et al. (2025) explored how nozzle temperature, printing speed, and material feed rate influenced the geometric and mechanical properties of 3D-printed PLA bone scaffolds [125]. Experimental testing was integrated with an Artificial Neural Network (ANN) model to predict scaffold shape and Young's modulus while optimizing printing parameters. The ANN efficiently discovered printing conditions that maximized Young's modulus while adhering to geometric limits, with the resulting mechanical features falling within the typical range of cancellous bone. These results show the potential of AI-assisted optimization for producing patient-specific 3D-printed bone scaffolds [125]. In addition, Bhensdadia et al. (2025) studied an artificial neural network-based inverse design approach for bone tissue engineering scaffolds, aiming to identify suitable scaffold geometries based on predefined target properties [126]. The approach combined artificial neural networks with generative additive models to reduce the computational burden associated with conventional finite-element-based scaffold design [126]. This study demonstrates the potential of AI-assisted inverse design for the rapid development and optimization of scaffolds with desired characteristics [126]. Overall, these outcomes suggest that AI may help predict scaffold performance and optimize scaffold design, facilitating more accurate, patient-specific bone regeneration procedures [126].

7. Future Directions

Future research in bone tissue engineering should focus on developing multifunctional, patient-specific scaffolds that provide mechanical support while promoting vascularisation, osteogenesis, and tissue integration [127]. Advances in biomaterials and additive manufacturing can enable scaffolds with precisely controlled structures and locally dispersed biological cues customised to the anatomical and biomechanical requirements of particular bone defects [127, 128]. Incorporating osteoconductive materials with bioactive compounds, cells, and controlled-release mechanisms may further enhance the regenerative capability of these constructs [129, 130]. Vascularization should remain a major priority, particularly for treating large and critical-sized bone defects. Future strategies should focus on integrating interconnected vascular networks with osteogenic components through approaches such as prevascularization, angiogenic factor delivery, endothelial-cell-based systems, and advanced bioprinting [131, 132]. Coordinated development of vascular and bone tissues may enhance nutrient and oxygen supply, promote cell survival, and support the long-term integration of engineered constructs. Future developments in 3D bioprinting are projected to improve the biological complexity of manufactured bone structures [131]. Multi-material printing, specialized bioinks, precise distribution of cells and bioactive compounds, and biomimetic hierarchical patterns may enable more accurate replication of the cellular and extracellular conditions of natural bone [133, 134]. However, future research should focus on creating reliable methods to preserve cell viability, enhance cellular maturation, and attain functional tissue organization during fabrication and post-implantation [133, 135]. Standardized,

scalable, and reproducible manufacturing workflows will also be essential for the clinical development of these technologies [133, 135]. Standardized procedures for material characterization, scaffold fabrication, sterilization, mechanical testing, degradation assessment, and biological evaluation will be required for meaningful comparisons between studies and consistent product quality [136, 137]. The implementation of appropriate regulatory frameworks and good manufacturing practices will be equally essential for advancing personalized 3D-printed scaffolds toward clinical application [136, 137]. Personalized bone regeneration represents another important area for future advancement. Combining patient-specific imaging and anatomical data with biomechanical and biological information may facilitate the development of individualized scaffolds that more accurately match individual bone defects [119, 138]. Artificial intelligence and machine learning techniques may support scaffold design, mechanical and biological performance, and optimization of fabrication parameters [138, 139]. Such integration may enhance the precision and efficiency of patient-specific treatment planning [139]. Finally, moving from promising preclinical outcomes to routine clinical application will require robust long-term evidence. Future research should include adequately powered preclinical studies followed by well-designed, long-term clinical trials evaluating safety, mechanical stability, vascularization, bone integration, functional recovery, and long-term outcomes [119, 138, 139]. Demonstrating the safety, durability, biological integration, and therapeutic effectiveness of these constructs will be essential for translating promising laboratory-based developments into routine clinical practice [119, 138, 139]. Figure 6 shows emerging directions in bone regeneration, showing key strategies for patient-specific scaffolds, vascularization, 3D bioprinting, AI and machine learning, standardization, and clinical translation.

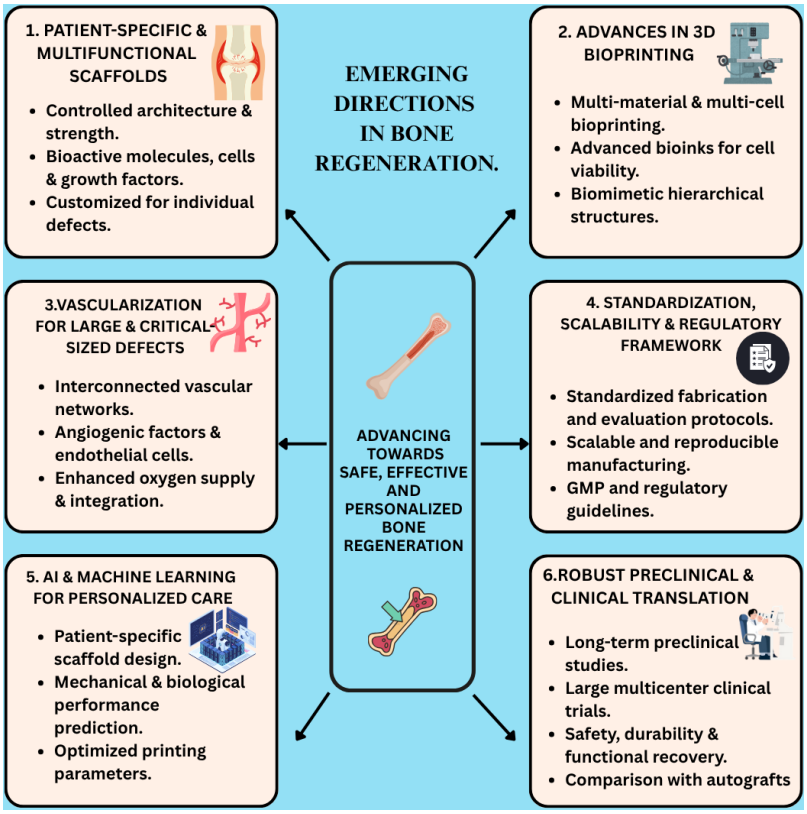


Figure 6 Emerging directions in bone regeneration. The image was created manually using Canva.

8. Conclusion

Bone defect management represents a major clinical challenge, and the selection of an effective grafting strategy is influenced by factors such as defect size, anatomical complexity, mechanical requirements, and graft availability [55, 140]. Autologous bone grafts remain the most widely accepted and clinically effective treatment option due to their osteogenic, osteoinductive, and osteoconductive properties. However, donor-site morbidity and limited accessibility can restrict their use, particularly in large-sized defects [140]. When autograft availability is limited, allografts and xenografts offer alternatives, although concerns about infection, immunological response, and biological integration persist [140].

Synthetic graft materials are more readily available and carry a lower risk of disease transmission, their biological and mechanical properties may not fully replicate those of autologous bone [55, 140]. The development of patient-specific scaffolds with regulated architecture, porosity, mechanical properties, and biological functioning has become feasible because of recent advancements in 3D printing and bioprinting [116, 119]. Therefore, 3D-printed scaffolds may be particularly promising for large, complicated, and patient-specific bone defects because they can provide customized geometries and individualized mechanical and biological properties [116, 119]. However, 3D-bioprinted structures remain in development, with challenges such as vascularization, cell survival, mechanical strength, manufacturing reproducibility, regulatory constraints, and inadequate long-term clinical data [119, 136]. Overall, where appropriate donor bone is available, autologous bone grafting remains the preferred established approach; however, more advanced 3D-printed scaffolds provide an acceptable alternative for complex defects that demand specialized structural and biological properties [55, 119, 140]. Future clinical research comparing advanced 3D-printed and bioprinted structures with conventional grafting procedures is needed to evaluate their long-term safety, effectiveness, and suitability for routine clinical practice [119, 136].

Author Contributions

Suhani Sharma contributed to conceptualization, methodology, data curation, visualization, preparation of the original draft, and review and editing of the manuscript. Dr Sonali Karhana was responsible for conducting the investigation and reviewing the manuscript. Dr Mohd Ashif Khan contributed to the conceptualization and methodology of the study, provided supervision throughout the research process, and validated the findings. All authors reviewed and approved the final version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

AI-Assisted Technologies Statement

Artificial intelligence (AI) tools were used solely for basic grammar correction and language refinement in the preparation of this manuscript. Specifically, ChatGPT (Perplexity AI, Grammarly) was employed to improve grammatical accuracy, linguistic clarity of the English text, style corrections, and to enhance the presentation of trial summaries so that the information could be easily readable and clearly understood. All scientific content, data interpretation, and conclusions

were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the content of the manuscript.

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