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An in-depth examination of bone avascular necrosis and early-stage treatment modalities utilizing regenerative medicine

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ABSTRACT

Femoral head avascular necrosis (AVN) represents a debilitating, progressive pathology characterized by ischemic osteocyte loss, impaired osseous remodeling, and subsequent subchondral fracture, frequently resulting in early osteoarthritis among younger patients. Despite an array of clinical interventions, a gold-standard therapy capable of consistently restoring vascular perfusion and structural integrity while avoiding total joint replacement remains unavailable. This review offers a rigorous and focused evaluation of regenerative medicine modalities for early-phase AVN, emphasizing tissue engineering innovations. We develop a conceptual model that links pathophysiological mechanisms (ischemia-driven necrosis) to specific therapeutic targets (angiogenesis, bone formation, and mechanical stability), and evaluate strategies such as stem cell transplantation, growth factor signaling, and advanced 3D scaffolds or hydrogels. Diverging from standard descriptive summaries, we provide a critical analysis of current field-wide challenges, including the limited translational success of animal studies and the lack of standardized clinical guidelines. We conclude that injectable hydrogel platforms functionalized with progenitor cells or angiogenic signals, offer the most compelling minimally invasive solution, provided that issues regarding mechanical durability and sustained delivery are resolved. Advancing the field will necessitate stage-specific randomized trials and a more precise alignment between experimental models and human clinical reality.

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Introduction

Avascular necrosis (AVN), sometimes called osteonecrosis (ON), is a progressive pathological condition involving the loss of bone tissue. This condition is defined by the death of cells and cellular elements inside the bone after an interruption in the blood supply to the subchondral region (1, 2). This condition is sometimes called aseptic necrosis and ischemic bone necrosis (3). Deprivation of blood flow to bone tissue reduces bone cell function, thereby leading to bone degradation (4). Impaired local blood distribution is widely regarded to be the primary cause of this condition. In instances without traumatic injury, the bone's vascular network remains anatomically intact, but there is a notable alteration in the physiological distribution of blood.

While AVN can manifest across various skeletal regions, the femoral head remains the predominant site of clinical involvement, representing roughly 75.9% of all diagnosed cases. Although other anatomical locations, including the humeral head, lunate, and talus, may be affected, they fall outside the central scope of this review. These sites are addressed only tangentially when necessary to elucidate broader physiological principles or shared regenerative mechanisms (5).

Symptoms experienced by patients in later stages of the condition often include localized discomfort in the afflicted joint region, limited range of motion, and edema. It is well-accepted that the early phases of AVN often lack noticeable symptoms, particularly in joints that do not bear weight. Consequently, many patients are referred to orthopedic surgeons after the condition has already progressed to an

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advanced stage, resulting in permanent damage to the bone and articular surface (6). Consequently, it is essential to identify and treat osteonecrosis promptly.

Osteoarthritis predominantly results from reduced blood flow to the subchondral region, often due to trauma. Other contributing factors include autoimmune diseases, hematological issues, and lifestyle choices such as drug use, smoking, and alcohol consumption. The precise mechanisms are not completely understood (7). AVN is associated with both traumatic and non-traumatic etiologies, driven by a range of factors from medications such as steroids and radiation to conditions like sickle cell disease, diabetes, and autoimmune disorders (Figure 1). The primary concern remains the inadequate blood supply, resulting in cellular death, tissue deterioration, and complex repair processes (8, 9).

The natural progression of osteonecrosis varies with the size and location of the ischemic area. While adults and children share underlying mechanisms beginning with ischemic events, young adults typically exhibit better prognoses due to differences in cartilage development and regenerative capabilities (10). The ischemia phase of AVN involves diminished blood supply, leading to necrosis of mesenchymal stem cells, followed by apoptosis of osteoblasts and subsequent death of osteocytes, with necrotic changes observable within hours to days (11, 12). Following this, capillary revascularization occurs in the outskirts of the necrotic region, heralding a cycle of bone resorption and formation, characterized by new bone formation over necrotic tissue (13).

This process creates an imbalance between bone resorption and new bone formation, leading to mechanical deficiencies and potential subchondral fractures due to loss of trabecular architecture. The ongoing stress on a compromised bone architecture can result in significant and lasting alterations (14).

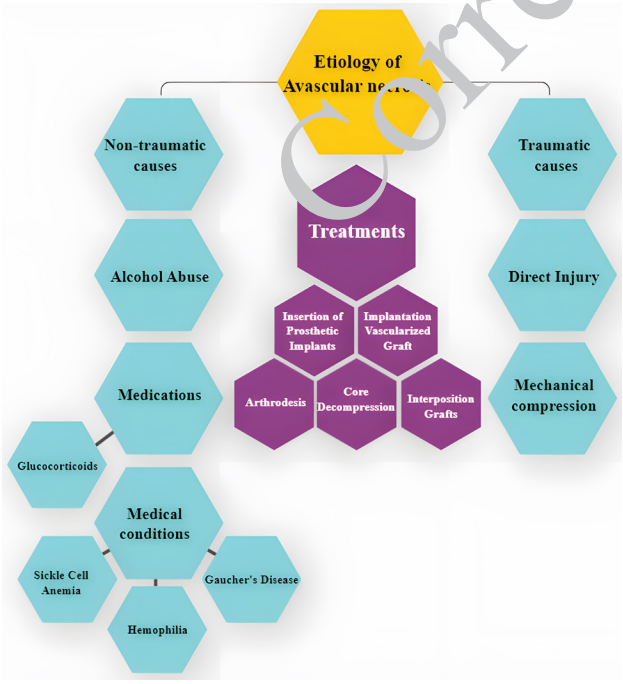

Figure 1. Etiology, risk factors, and treatment associated with avascular necrosis (AVN)
There are a number of potential causes of AVN, but the most well-known is trauma

Radiographic symptoms of AVN typically emerge in advanced stages, as initial stages often show no abnormalities. A key indicator, the crescent sign, reflects subchondral collapse due to insufficient healing (15). While bone scintigraphy can reveal changes related to osteoblast activity in AVN, its limitations, such as low resolution and specificity, complicate the differentiation from stress fractures and osteoporosis. In primary case series, AVN identification depended on strong radiographic indicators or alternative methods, such as radionuclide bone scans, bone marrow pressure measurements, or histological analysis, when radiographs were normal. The incidence of bone collapse varied widely, from 32% to 79%. MRI has become the preferred method for early detection of AVN, offering detailed quantification, staging, and evaluation of lesions not visible on standard radiographs, highlighting the metabolic changes in bone marrow cells (16). It effectively illustrates osteosclerotic changes resulting from reduced bone resorption linked to reduced osteoclast activity. Diffuse bone marrow edema may indicate early AVN changes, appearing as decreased signal intensity on T1 and increased signal intensity on T2-weighted images. MRI's high specificity for AVN makes it essential for monitoring high-risk patients. In acute AVN phases, MRI may reveal edema with high T2 signal, whereas chronic phases can show a fibrotic, sclerotic pattern with consistently low signal across sequences. Additionally, T2-weighted imaging can detect joint effusion, a common occurrence associated with AVN (17).

Departing from conventional descriptive summaries, this review is structured around a hypothesis-oriented framework that bridges the gap between underlying pathophysiology and targeted therapeutic interventions for early-stage femoral head AVN. We posit that a viable tissue engineering strategy must concurrently address three synergistic objectives: (1) the restoration of vascular perfusion within necrotic bone, (2) the induction of osteogenesis to facilitate the replacement of non-viable tissue, and (3) the provision of structural reinforcement to forestall subchondral collapse. Each regenerative modality examined herein, ranging from cell-based therapies and bioactive signaling to structural scaffolds, is rigorously appraised through the lens of these requirements, with a focused critique of their inherent limitations and unresolved clinical challenges (18).

Method of this article

In this study, data were collected through a literature review. Relevant articles were identified through comprehensive searches of major scientific databases, including PubMed, Scopus, and Web of Science. The search strategy incorporated predefined keywords such as "avascular necrosis," "osteonecrosis," "regenerative medicine," "stem cell therapy," and "core decompression," combined using Boolean operators to ensure both sensitivity and specificity. Inclusion criteria were established to select peer-reviewed English-language articles, primarily focusing on early-stage avascular necrosis and regenerative treatment modalities. Both clinical studies and high-quality review articles were considered to provide a comprehensive understanding of current therapeutic approaches. Studies involving advanced-stage disease without regenerative intervention were excluded to maintain focus on early intervention

strategies. The screening process was conducted in multiple stages. First, titles and abstracts were reviewed to eliminate irrelevant studies. Subsequently, full-text articles were assessed for eligibility based on the predefined criteria. Data were then extracted systematically, including information on study design, patient population, type of regenerative intervention, and reported clinical outcomes. To ensure the reliability of the findings, preference was given to studies with higher levels of evidence, such as randomized controlled trials and meta-analyses. Additionally, efforts were made to include the most recent publications to reflect current advances in the field (Figure 2).

Avascular necrosis classifications

Avascular necrosis of the humeral head

AVN of the humeral head (ANHH) is characterized by bone cell death, disrupting reparative processes and potentially resulting in structural collapse, joint pain, and functional loss (19). Initially theorized by Phemister in 1934, vascular abnormalities such as thrombosis are associated with ANHH. Chandler later drew parallels between ANHH and myocardial infarction, noting anatomical similarities and limited vascular anastomoses in both areas, suggesting that vascular obstruction can lead to degeneration in both bone and myocardium, influenced by collateral circulation and the extent of obstruction (20). Factors contributing to focal thrombosis and ANHH include long-term glucocorticoid treatment and heavy alcohol consumption. While glucocorticoids increase ANHH risk, not all patients on high-dose steroids develop it, and similarly, not all heavy drinkers show symptoms, complicating causation. Patients with underlying diseases, such as systemic lupus erythematosus or acute leukemia, often receive glucocorticoids, leading to ANHH development either before or after treatment begins. Additionally, ANHH has been observed in patients post-chemotherapy or radiation therapy. Early stages of ANHH show the absence of superior retinacular arteries, crucial for supplying the proximal humeral head, leading to limited revascularization and radiographic signs of necrosis (21). Although revascularization improved angiographic findings, the collapse of the humoral head continued. According to these researchers, microscopic stress fractures and eventual propagation of subchondral fracture lines caused interruptions in revascularization in the humoral heads of the affected hips. ANHH has spontaneously resolved in some cases between the earliest and middle stages (22). There is still a lot of uncertainty about the factors contributing to the formation of the disease in some patients and the factors contributing to its resolution in others. A key role in the progression of the disease may be played by "regional endothelium dysfunction" (RED). An endothelial cell monolayer is responsible for maintaining the blood vessel's homeostasis and serves as the inner lining of its walls (23). Because of its unique location, the endothelium is constantly exposed to inflammatory cells and circulating factors, resulting in endothelial activation and/or damage.

Avascular necrosis of the distal radius: the lunate and scaphoid bone

The lunate bone, or semilunar bone, is a crescent-shaped carpal bone located in the wrist, nestled between the ulna, radius and hand. It is positioned centrally in the proximal row of carpal bones, bordered laterally by the scaphoid

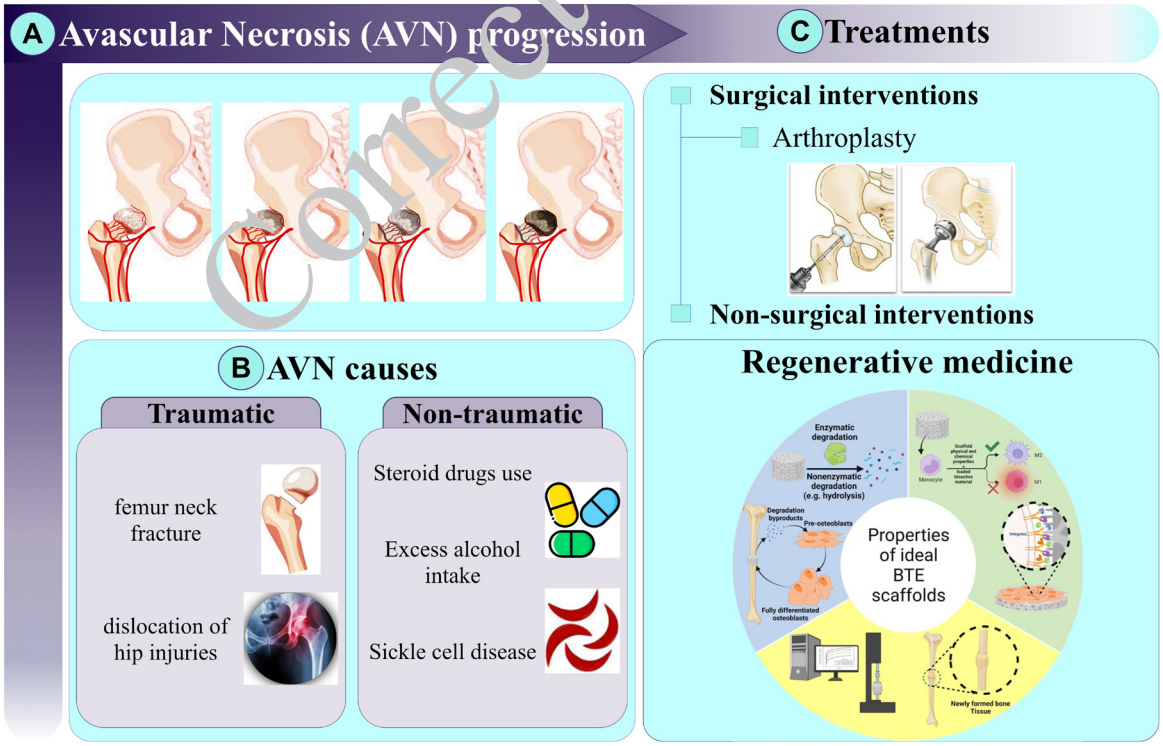

Figure 2. Overview of avascular necrosis (AVN) of the femoral head with a focus on regenerative strategies
A) Schematic illustration of AVN progression from early ischemia to bone collapse. B) Major causes of AVN, including traumatic factors (e.g., femoral neck fracture and hip dislocation) and non-traumatic factors (e.g., corticosteroid use, excessive alcohol consumption, and sickle cell disease). C) Therapeutic approaches with particular emphasis on regenerative medicine and tissue engineering. Emerging strategies involve the design of biomimetic scaffolds that support cell adhesion, proliferation, and differentiation, enable controlled biodegradation, and promote vascularization and new bone formation

bone and medially by the triquetral bone (24). The lunate articulates with several bones: laterally with the scaphoid, medially with the triquetral, and distally with the capitate and hamate bones. Stabilized by ligaments connecting it to the surrounding bones, its proximal surface articulates smoothly with the radius, and its lateral surface has a flat facet for the scaphoid. The bone features rough palmar and dorsal surfaces, with a broad dorsal surface and a deep concave distal surface. Blood supply is provided by dorsal and palmar branches (25). The dorsal carpal arch (dorsal carpal network, posterior carpal arch) is an anatomical term for the combination (anastomosis) of the dorsal carpal branch of the radial artery and the dorsal carpal branch of the ulnar artery near the back of the wrist. It is made up of the dorsal carpal branches of both the ulnar and radial arteries. It also anastomoses with the anterior and posterior interosseous arteries (26).

The dorsal carpal arch, formed by the anastomosis of the dorsal carpal branches from the radial and ulnar arteries at the wrist, also connects with the anterior and posterior interosseous arteries. It gives rise to three dorsal metacarpal arteries. The lunate bone, which is integral to wrist movement, displays variable features, with about one-third lacking a medial facet, thus not linking to the hamate bone. Additionally, in approximately 20% of individuals, the blood supply may exclusively come from palmar vessels. Ossification of the lunate begins between 18 months and 4 years of age. Commonly dislocated, the lunate's name originates from its crescent shape, while in amphibians and reptiles, it is called the intermedium due to its position among proximal carpals (27).

Kienböck disease develops over time in the wrist. It causes severe pain and limited mobility by damaging the lunate bone. In extreme cases, carpal collapse ensues, marked by capitate proximal migration and scaphoid rotatory subluxation. Degenerative changes occur in the intercarpal and radiocarpal joints. Medication choice depends on illness severity (28). For significant lunate collapse, there is no ideal treatment. Advanced Kienböck sickness is treated to maintain wrist alignment and reduce instability. These include intercarpal arthrodesis, proximal row carpectomy, and vascularized grafts. Tendon interposition grafts and prosthetic implants have had poor success in directly replacing bone. Wrist problems may be treated by cell-based tissue engineering. MSCs in bone marrow, adipose tissue, and synovium can differentiate into multiple cell types (29).

Numerous studies have assessed treatment options for Kienböck disease to replace the carpal bones (30). Huang *et al.* evaluated the effectiveness of autologous cartilage constructions from mesenchymal stem cells derived from bone marrow. They extracted lunate bones from twenty-seven White rabbits and categorized them into three groups: Group 1 underwent excision only; Group 2 received a gelatin-hyaluronan scaffold post-excision; and Group 3 received a scaffold with mesenchymal stem cells and a cartilage-growing medium. After six weeks, Group 1 was euthanized, while Groups 2 and 3 were euthanized at six or twelve weeks. The study concluded that using autologous mesenchymal stem cells with a biodegradable scaffold can effectively reduce carpal collapse when employed as cartilaginous implants (29).

A study investigated the ability of canine bone marrow stromal cells (cBMSCs) to regenerate bone in scapholunate cavities that had been treated with liquid nitrogen. Autologous BMSCs were cultured to increase their quantity. The study found that BMSCs could cure Kienböck disease, as the inability to regenerate bone tissue in the scapholunate joint led to collapse and abnormalities (31). The study on treating osteochondral lesions in the lunate cavity involved transplanting bone marrow mesenchymal stromal cells from New Zealand white rabbits onto scaffolds after removing the lunate bone. A 12-week radiographic analysis showed all subjects demonstrated ossification, with the formation of cartilage-like cells and new bone tissue. It was concluded that the development of new blood vessels is crucial for bone tissue regeneration with cell-loaded scaffolds (32).

Scientists investigated variously sized 3D printed lunate prostheses for treating advanced Kienböck's disease. The prostheses were evaluated using the visual analog scale, wrist mobility, strength, and Mayo Modified Wrist Score. The findings suggest this approach may correct carpal tunnel anatomy, reduce discomfort, and enhance wrist mobility in severe cases. Additionally, patient satisfaction and follow-up results were noted (1). Xie *et al.* documented a successful reconstructive procedure using 3D printing technology to create a lunate prosthesis for a patient afflicted with Kienböck's Disease. The prosthesis was fabricated with tomographic image processing and segmentation, leading to the full range of motion restoration within a span of 12 months. The research showcased the capacity of 3D printing to restore the patient's functionality (33).

Avascular necrosis of the femoral head Bone

Severe necrosis leads to complete deterioration of the articular cartilage of the femoral head due to underlying subchondral bone necrosis. This occurs before revascularization and is hindered by the loss of structural stiffness due to osteoclasts' resorption. The consequence is early-onset osteoarthritis (OA) in the hip joint. While several factors may contribute to osteonecrosis of the femoral head (ONFH), the specific pathophysiological mechanisms are not fully understood. Non-surgical management approaches exist, but surgical interventions remain the primary treatment option (34).

One key indicator of AVN is discomfort in the pelvic or groin area, noticeable when rising, climbing stairs, or walking up inclines, which may hinder mobility. Traumatic causes of AVN frequently include femoral neck fractures and hip dislocations, disrupting blood flow to the femoral head. A fracture can lead to detachment or damage to the retinacular and synovial capillaries, causing necrosis due to reduced blood supply, potential increases in intra-articular pressure, and complications from constrained movement during fixation (35).

Corticosteroids were identified as the leading cause of AVN in a 2021 cross-sectional study, accounting for approximately 32% of cases, particularly among women. Trauma was the second most common cause, responsible for about 28% of cases, with a notable prevalence in males. Pathologically, the necrotic femoral head has a characteristic dense, wedge-shaped appearance and displays disruptions in the bone trabecular structure, along with granulation tissue in cystic lesion areas due to bone resorption. Histological analysis revealed disorganized bone structure, bone marrow necrosis, and empty lacunae, along with revascularization and neofibrous tissue at the disease periphery (36).

AVN of the femur occurs in two stages: initial ischemia in the hip region followed by a regenerative process. X-ray and MRI are insensitive to early changes due to the lack of association between ischemia and mineral composition alterations, whereas bone scintigraphy effectively detects AVN by showing reduced blood flow in the femoral head. Histology remains the definitive method for confirming tissue necrosis, allowing for assessment of cellular mortality in bone marrow cells, osteoblasts, and osteocytes (34, 37).

Biological plasticity allows the femoral head to regain its original form and height, requiring acetabulum coverage and confinement therapy. Excessive revascularization can enhance anabolic activity, creating an endochondral callus (38, 39). Hip arthroplasty is a therapeutic modality for advanced stages of joint degeneration (40). Noninvasive therapies such as shockwave stimulation, hypertension oxygenation therapy, pharmacological interventions, exercise-based rehabilitation, and restorative pharmaceutical agents offer some improvement, but they often lack substantial efficacy (35). Surgical methods like core decompression, osteotomy, vascular grafts, and arthroplasty have limitations, including limited applicability and frequent revisions, which need to be addressed in clinical settings (41). Various non-surgical techniques, including bone-marrow-derived stem cells, have been developed to slow AVN progression and prevent total hip replacement (42). However, these interventions show only partial effectiveness and provide only temporary pain-management benefits, making their overall effectiveness uncertain (20).

Animal models of AVN

AVN is a significant contributor to both pain and disability, often impacting young individuals during their most productive years. Preclinical studies are crucial in the search for more effective therapies because they use animal models that mimic human AVN. Creating cell models to investigate AVN also aids in understanding the diseases complex origins and pathogenesis. The efficacy of several methods developed for the prevention and treatment of VNN has not been well evaluated using preclinical animal and cell models (43).

Over the years, quadrupeds such as mice, rats, rabbits, dogs, pigs, sheep, goats, and horses have all been used to create AVN animal models. However, many models lack human-like physiological and metabolic details. The success rates and features of animal models of human AVN, as revealed by various approaches, vary substantially. Femoral head necrosis produced by surgical vascular deprivation, steroid-induced ONFH models, frozen femoral head damage, and alcohol-induced ONFH models are the present *in vivo* animal models of ONFH (44, 45).

Rat model

Experimental rat models of femoral head AVN have been systematically developed using diverse surgical and pharmacological approaches. One notably effective technique involves femoral neck ligation, in which non-absorbable sutures are placed circumferentially around the neck to definitively occlude the vascular supply to the femoral head epiphysis, thereby inducing ischemic necrosis (46, 47). To ensure the induction of highly reproducible osteonecrosis, this surgical technique is frequently augmented by the transection of the ligamentum teres and concurrent hip dislocation (48). Furthermore, thermally mediated injury via electrocoagulation targeting the femoral neck region has been employed as an alternative method to successfully induce AVN (49). To facilitate rigorous comparative analysis, sham-operated control groups subjected to identical surgical exposure but omitting femoral neck ligation or capsular transection are systematically incorporated into the experimental design (50). To simulate chronic ethanol exposure, researchers frequently leverage the Lieber-DeCarli liquid diet formulation, which incorporates an ethanol concentration of 5-8% (w/v). In this nutritional model, alcohol accounts for approximately 35-36% of the total daily caloric intake, with administration periods typically spanning from 1 to 24 weeks to evaluate dose- and time-dependent effects on bone metabolism (51, 52). To ensure nutritional consistency, isocaloric control groups are maintained by substituting ethanol with dextran-maltose (52). Within this experimental framework, Muscone has demonstrated significant therapeutic potential, exerting potent protective effects against the development and progression of alcohol-induced osteonecrosis (51).

Rabbit model

Diverse rabbit-based experimental models have been established to investigate the progression of femoral head osteonecrosis. Specifically, the intraosseous administration of anhydrous alcohol has emerged as a reliable methodology for precipitating necrotic transformations within a four-week induction period (53). A localized model of femoral head osteonecrosis has been established in rabbits via a surgical approach involving the creation of a targeted intraosseous tunnel. This methodology utilizes multiple, systematic freeze-thaw cycles, typically five consecutive repetitions using liquid nitrogen to induce precise thermaly-mediated necrotic damage to the femoral head (44, 54). In a seminal study by Motomura *et al.*, a robust model of corticosteroid-induced osteonecrosis was established in rabbits by intramuscular administration of methylprednisolone (MPSL) at varying doses. The successful induction of necrotic lesions was subsequently validated through detailed histological assessment conducted four weeks post-injection (54). Furthermore, a synergistic model employing concomitant administrations of horse serum and methylprednisolone has been utilized in rabbits to effectively simulate the multifactorial pathogenesis of AVN. This dual-stimulus approach aims to replicate the complex interplay between immune-mediated vasculitis and steroid-induced metabolic disturbances (55). Similarly, a combination of dexamethasone and horse serum has been used to induce osteonecrosis in rabbits over a six-week protocol (56). To simulate the complex etiology of AVN, Wang *et al.* developed a sophisticated hormone-allogeneic serum rabbit model. This protocol integrates an initial immune-sensitization phase, facilitated by repeated exposure to horse serum, followed by escalating doses of methylprednisolone acetate. Executed over a six-week longitudinal period, this synergistic approach ensures the robust manifestation of the disease's key pathophysiological hallmarks (56).

Pig and sheep

Large animal models offer superior translational utility,

as their biomechanical profiles more closely approximate human weight-bearing conditions. In porcine models, femoral head ischemia is meticulously induced via a combined surgical approach involving femoral neck ligation and transection of the ligamentum teres (57). To isolate the effects of ischemia and prevent confounding femoral head distortions, certain protocols incorporate above-knee amputation to eliminate weight-bearing, thereby simulating clinical joint-unloading strategies; the contralateral limb remains intact to serve as an internal control (58). Parallelly, cryo-insult methodologies have gained prominence in ovine models. This technique involves the targeted application of liquid nitrogen to the femoral head through a pre-drilled osseous tunnel, typically executed via three systematic freeze-thaw cycles (59, 60). By utilizing the contralateral non-surgical hip as a control, this approach yields highly reproducible necrotic lesions while maintaining the structural integrity of the surrounding anatomical landscape.

Dog

Canine models serve as a pivotal platform for investigating the pathogenesis of femoral head osteonecrosis and validating the efficacy of regenerative interventions. The predominant induction technique involves surgical hip dislocation followed by localized cryo-ablation of the femoral head. For instance, Jin *et al.* demonstrated this by inducing necrosis via hip dislocation and an incision of the ligamentum teres, then applying 100-150 ml of liquid nitrogen for approximately 3 minutes, followed by controlled thawing in warm saline (61). Similarly, Gao *et al.* refined this thermal insult by utilizing a specialized freezing cannon to deliver 100 ml of liquid nitrogen over an extended eight-minute duration (62). Beyond cryo-insult, core decompression models have been established in dogs to optimize surgical techniques and scaffold biocompatibility. These models typically employ image-guided navigation for the precise placement of a Steinmann pin, creating a standardized intraosseous tunnel (63). Due to their comparable femoral head dimensions and axial loading patterns to humans, canine models are exceptionally valuable for evaluating post-operative weight-bearing recovery and the long-term structural integration of tissue-engineered constructs.

Comparative analysis of AVN animal models

Each animal model offers a unique set of advantages and inherent limitations within the context of translational research. Steroid-induced models (typically rabbits and rats) are highly valued for their ability to simulate non-traumatic AVN and facilitate the study of dose-dependent pharmacological effects; however, they are often constrained by prolonged induction protocols (4-6 weeks) and inconsistent necrosis rates (56). Conversely, surgical models, such as femoral neck or circumflex artery ligation, yield rapid and highly reproducible ischemic insults, yet they may fail to fully recapitulate the insidious and progressive vascular compromise characteristic of human non-traumatic pathogenesis (49). Furthermore, while cryo-insult models (liquid-nitrogen freezing) provide exceptional precision in lesion localization and are gold standards for large-animal studies (sheep, dogs), the mechanism of thermally mediated injury remains pathophysiologically distinct from the chronic ischemic cascade observed in clinical AVN (60, 62). A comparative summary of the four main AVN models, steroid-induced, surgical ligation, cryo-insult, and alcohol-induced, including their advantages, limitations, and translational relevance, is provided in Table 1.

Regenerative medicine strategies for AVN treatment

The existing therapeutic approaches for AVN may be categorized into two main groups: non-surgical interventions and surgical procedures. The available information suggests that non-operative approaches may effectively control early-stage illness, specifically through protective weight-bearing strategies (64). The potential enhancement of results from incorporating extracorporeal shock wave treatment remains inconclusive due to insufficient data. Restricted weight-bearing, acupuncture therapy, hyperbaric oxygen therapy, and extracorporeal shock wave therapy are examples of physical therapy. At the same time, bisphosphonates, anticoagulants, vasodilators, statins, and traditional Chinese medicine are considered effective treatments for AVN patients' clinical symptoms (65).

According to reports, a significant majority of patients who do not undergo surgical procedures are likely to experience femoral head collapse, necessitating the need for total hip arthroplasty. Therefore, non-operative treatment is not a suitable alternative for patients in the early stages who are seeking to maintain the integrity of the original joint (66). Also, surgical treatment methods of the femoral head AVN, such as metallic implants for hip arthroplasty, are considered aggressive treatments. Metallic implants (e.g., titanium and tantalum) provide the mechanical support that is missing in non-structural bone grafting, making them a candidate to address pre- and even post-collapse lesions in AVN. Several metals are used in these implants, chosen for properties such as biocompatibility, strength, and elasticity (34).

Table 1. Comparative analysis of animal models for femoral head avascular necrosis (AVN)

Model	Species	Induction method	Time to necrosis	Advantages	Limitations	Translational relevance
Steroid-induced	Rat, Rabbit	High-dose corticosteroids (MPSL, dexamethasone)±horse serum	4-6 weeks	Mimics non-traumatic AVN; dose-dependent	Variable necrosis rate; prolonged induction	Best for studying steroid-induced ONFH
Surgical ligation	Rat, Rabbit, Pig	Femoral neck ligation+ligamentum teres transection	2-4 weeks	Rapid, reproducible ischemia	Does not recapitulate progressive vascular compromise	Testing rapid vascular regeneration
Cryo-insult	Rabbit, Dog, Sheep	Liquid nitrogen application (multiple freeze-thaw cycles)	2-4 weeks	Precise lesion localization; reproducible	Freeze injury differs from ischemic pathophysiology	Testing load-bearing scaffolds
Alcohol-induced	Rat	Lieber-DeCarli liquid diet (5-8% ethanol, 35-36% calories)	4-24 weeks	Mimics alcohol-associated AVN	Long induction period; variable compliance	Studying alcohol-induced osteonecrosis

Currently, there are no specific molecular targets or therapeutic interventions to treat and control the condition. The ineffectiveness of pharmacological drugs and AVN of the femoral head, particularly in advanced stages of the condition, has prompted a shift towards tissue engineering approaches. According to Figure 3, these efforts aim to enhance therapy choices and improve patient clinical outcomes (67).

Tissue engineering or regenerative medicine approaches promise to regenerate the vasculature in the femoral head, a primary contributor to the condition, and to regenerate necrotic bone tissue (68). The integration of biomaterials, stem cells, and growth factors in tissue engineering approaches presents an exciting prospect for circumventing the demand for metallic implants and invasive surgical procedures (69). The use of structural, bioactive biomaterial platforms has the potential to contribute to the stabilization of the femoral head and facilitate osteogenic differentiation for bone regeneration. Additionally, these platforms may provide angiogenic signals to promote vascular recovery within the femoral head (70).

Clinicians have attempted to combine surgical techniques with autografts, vascular implants, and metallic implants; however, these hybrid therapies are only partially effective because they do not induce bone tissue regeneration to replace the necrotic core and revascularize it. Advancements in biotechnology and materials science have enabled the development of several biomaterials for bone replacement, effectively addressing the challenges associated with bone grafting procedures (71).

Stem-cell therapy

The procedure for treating early bone death in the hip without a blood supply is still a topic of debate among medical professionals. Our approach involves drilling the affected bone and injecting concentrated stem cells derived from the patient's own bone marrow. Preliminary results from similar methods have shown a lower risk of collapse than traditional core decompression techniques. This technique is minimally invasive, requires only an overnight hospital stay, does not necessitate a period of non-weight-bearing, and is generally well tolerated by patients (64).

The application of precursor cells with osteogenic or angiogenic properties (or both) in combination with or without supporting growth factors is an attractive prospect for enhancing osseous regeneration. Among the different types of cells, adult stem cells, called MSCs, which can differentiate into multiple mesenchymal tissue types, are particularly promising for therapeutic purposes (72). These cells are found in specific areas of the human body and are crucial for maintaining the health of various tissues such as bone, skin, and blood. MSCs can undergo multiple cell divisions without losing their unique properties and can differentiate into various mesenchymal cell types, including bone, cartilage, and fat cells (73). The use of MSCs has been shown to promote tissue regeneration following transplantation in a dog model of AVN. However, whether this effect is due to the osteogenic differentiation of the transplanted cells or is mediated through their trophic activities is uncertain. Most tissues in the body require adequate vascularization for viability and function. Without sufficient blood supply, cells suffer from hypoxia, nutrient deprivation, and the accumulation of waste products, which disrupts tissue homeostasis and impedes tissue regeneration. Endothelial progenitor cells (EPCs) can differentiate into mature endothelial cells and can be isolated from bone

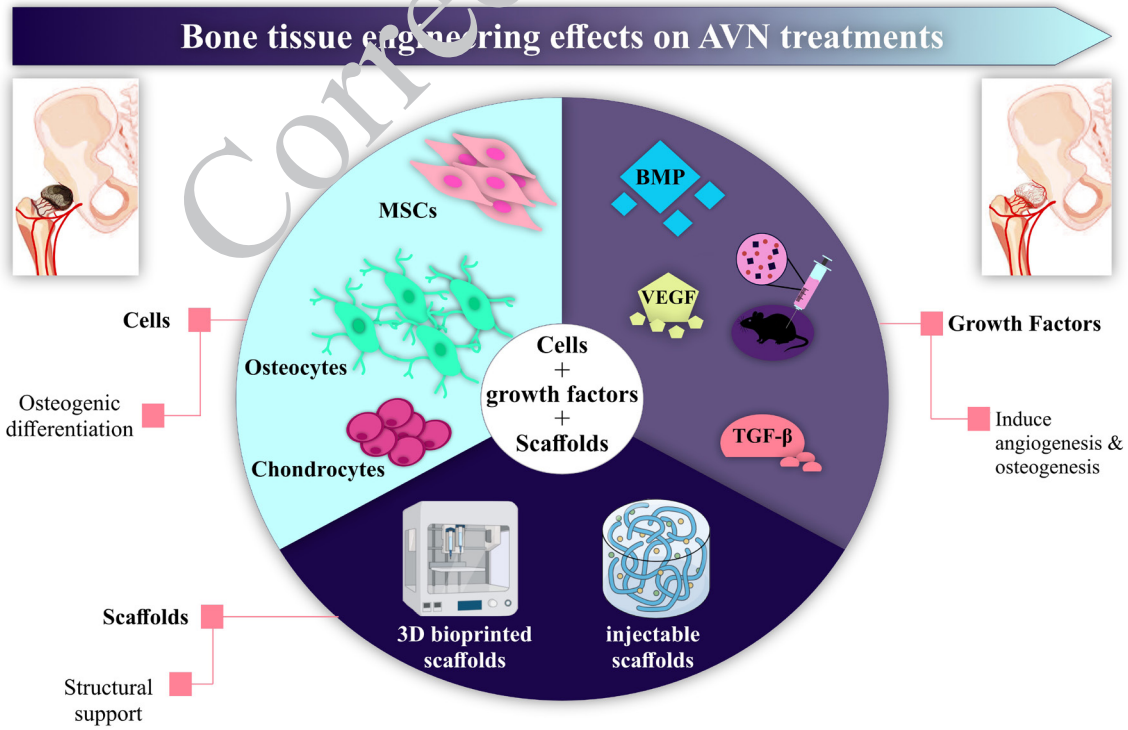

Figure 3. Role of bone tissue engineering in the treatment of avascular necrosis

Schematic illustration of the synergistic interplay between cells, growth factors, and biomaterial scaffolds in promoting bone regeneration. Mesenchymal stem cells (MSCs) and bone cells contribute to osteogenic differentiation, while growth factors stimulate angiogenesis and new bone formation. Advanced scaffold systems, including injectable scaffolds, provide structural support and a conducive microenvironment for cell attachment and proliferation. The integration of these components enhances vascularization, accelerates tissue repair, and improves functional recovery in necrotic bone lesions

marrow and peripheral blood (66). In general, growth factors promote both the differentiation and proliferation of MSCs into bone-forming cells (osteoblasts) and cartilage-forming cells (chondroblasts). One of the benefits of using growth factors to treat AVN of the femoral head is that many of these factors can be injected without additional surgical intervention (Figure 4).

They can also be administered in combination with surgical procedures and tissue-engineered grafts/scaffolds. The study by Luo *et al.* demonstrated that BMSCs seeded onto LiCPP scaffolds supplemented with various growth factors were effective in treating AVN owing to their osteogenic and angiogenic properties (48). In contrast to a previous study, researchers have successfully created a scaffold of beta-tricalcium phosphate (β-TCP) modified with a peptide that attracts BMSCs via adsorption and freeze-drying. After core decompression, this scaffold was implanted in rabbits with early AVN. The results showed that the scaffolds have a strong affinity for BMSCs. Compared with control groups, the scaffolds led to greater bone regeneration, indicating a promising advancement in surgical interventions for AVN. Despite promising preclinical results, clinical translation of stem cell therapy for AVN faces several challenges: (1) lack of standardized isolation and expansion protocols for MSCs, (2) heterogeneous clinical outcomes with variable rates of femoral head collapse (ranging from 20–60% across studies), (3) uncertainty regarding whether benefits arise from direct differentiation or paracrine effects, and (4) optimal cell dosage and delivery route remain undefined (74).

Drug delivery and growth factor

3D scaffolds can be enhanced by incorporating growth factors or other small molecules that facilitate osteoinductive stem cell differentiation, angiogenesis, cell proliferation, and bone regeneration. These properties are crucial in treating osteonecrosis, improving patient outcomes, and quality of life (75). Various growth factors, including vascular endothelial growth factors (VEGF), bone morphogenic protein (BMPs) belonging to (TGF-β), hepatocyte growth factor (HGF), and fibroblast growth factors (bFGF), are commonly used along with 3D scaffolds. In tissue engineering, VEGF is another commonly used growth factor (GF). VEGF plays a significant role in regulating angiogenesis during bone regeneration (76-81).

Yan *et al.* investigated the osteogenic effects of polylactide-polyethylene glycol-polylactide-microscopically modified bone-defect scaffolds containing VEGF and rat mesenchymal stem cells (MSCs) *in vitro* over 46 days. They observed adequate GF release from the scaffolds during this time. Additionally, they found that the scaffolds significantly increased osteoblast differentiation, indicating the potential of this method to treat additional bone defects. In a similar study, hydroxyapatite collagen scaffolds were combined with VEGF, and the resulting GF release was observed both *in vitro* and *in vivo*. The researchers observed sufficient GF release *in vitro* and osteogenesis and osteoblast differentiation in stem cells. They also noted vascularization, which they attributed to VEGF and the scaffold (82).

According to studies, BMP subsets 2, 6, and 7 are particularly effective in AVN of the femoral head. In

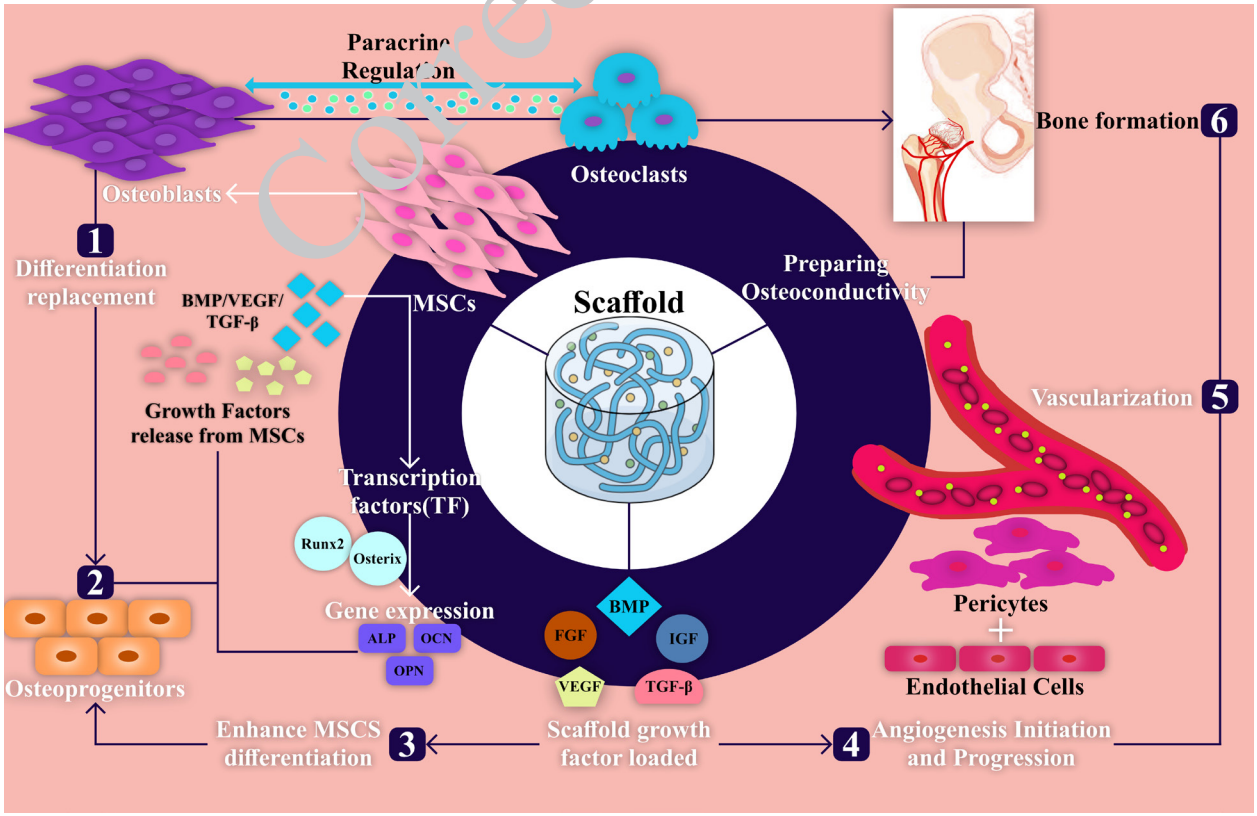

Figure 4. Schematic representation of the cellular and molecular processes involved in scaffold-mediated bone regeneration in avascular necrosis repair
Mesenchymal stem cells (MSCs), osteoblasts, osteoclasts, and endothelial cells interact with biomaterial scaffolds and undergo osteogenic differentiation through the activation of key transcription factors and gene expression markers. Paracrine signaling and growth factor release regulate osteoblast and osteoclast activity, enhance angiogenesis, and support endothelial cell migration, proliferation, and new bone formation, ultimately leading to structural restoration of necrotic bone tissue

addition to promoting osteogenesis and cell differentiation, BMPs promote angiogenesis in the human body. In bone regeneration studies, BMP-2 is still the most frequently used protein. Researchers used human BMSCs combined with BMP-2 in a hydrogel scaffold for 56 days to observe effects in severe combined immunodeficiency mice (83). In addition, the study revealed that the trabecular bone structure formed in the mice by this treatment was conducive to bone regeneration due to its favorable vascularization for BMP-2 production. Similarly, Xia *et al.* used BMP-2 in a PLGA/gelatin microsphere scaffold in a rabbit model over 12 weeks, demonstrating that scaffolds can serve as reservoirs for GFs and prolong their release. They observed that BMP-2 scaffolds increased osteogenesis and BMSC proliferation *in vitro* (33). Furthermore, evidence indicates that BMP-2-containing scaffolds exhibit greater osteogenic potential and sustained GF release *in vivo* (55). BMPs are frequently employed in combination with VEGF, a growth factor that promotes angiogenesis, to facilitate vascularization (84-86).

In an *in vitro* study carried out by Liao *et al*, BMSCs (2×10^5/mL) from rats were seeded onto plates and transfected with BMP-6 and VEGF growth factors. They were then seeded onto PLAGA (poly lactic-co-glycolic) scaffolds, and the angiogenesis and bone regeneration were observed *in vivo*. The study revealed that incorporating growth factors led to significantly greater advances in bone and vascular development. In their experiment with AVN-affected rats, Wang and colleagues used Colla Cornus Cervi and BMSCs genetically modified to express BMP-7. CCC is derived from deer antler glue and is believed to have osteogenic properties. The results demonstrated substantial cell proliferation and osteogenesis in the experimental group. The substance known as hepatocyte growth factor (HGF) can promote blood vessel growth, similar to VEGF. However, studies have shown that HGF is a more effective stimulator of cell differentiation than VEGF (87). In a study by Wen *et al.*, it was found that HGF (hepatocyte growth factor) at high concentrations is highly effective at promoting osteogenic differentiation of MSCs and aiding tissue repair in rabbit models. To test this, HGF was combined with fibrin glue, a material that supports cell differentiation, and transfected into rabbit-derived MSCs. These cells were then observed *in vivo* in 30 rabbit models, with both randomized and controlled groups. The results showed that HGF significantly promoted cell differentiation and vasculogenesis, while fibrin glue supported the differentiation and regeneration of femoral head necrosis. Other growth factors used in similar studies include platelet-derived growth factors. Ultimately, FGFs promote osteoblast differentiation, bone formation, and wound healing. Among them, FGF-2 is particularly renowned for its capabilities in tissue engineering. In a canine model, Momose and colleagues utilized FGF-2 in collagen hydrogel scaffolds to examine its effects on periodontal healing over a four-week period. They observed increased cell and tissue ingrowth, as well as vessel formation, in the scaffolds. As a result of the scaffold, alveolar bone was also regenerated (72).

According to Yao and colleagues, deferoxamine was released slowly over 10 days in a laboratory setting. Additionally, deferoxamine was found to augment BMP-2's differentiation-inducing effects on scaffolds, resulting in improved angiogenic and osteogenic properties (88). Simvastatin has also been used with scaffolds to promote angiogenesis. Simvastatin was incorporated into a poly (beta-amino ester) hydrogel matrix and examined *in vitro*. The scaffold released a total amount of 162 mg of simvastatin over a period of 20 days while simultaneously promoting the activity of preosteoblasts. It was found that simvastatin was gradually released from both PLGA and biphasic ceramic scaffolds containing simvastatin additions without affecting the chemical or mechanical properties of the scaffolds over 40 days (89). A study investigated the diffusion of dexamethasone *in vitro* when combined with silk fibroin/PLGA scaffolds. The results showed that BMSC growth and differentiation were promoted by the rapid release of the drug from a three-dimensional scaffold, as reported in study (90). In another study, dexamethasone was paired with porous lactic acid scaffolds in a lab setting. The results showed a steady release of the drug for a month. When this dexamethasone-containing scaffold was tested *in vivo*, it promoted bone and blood vessel growth without altering the drug's chemical composition (91).

Growth factor–based therapies for AVN present several important limitations that warrant careful consideration. One major concern is the occurrence of dose-dependent adverse effects. For example, BMP-2 has a relatively narrow therapeutic window; concentrations below about 0.5 mg/ml tend to produce inadequate bone formation, whereas levels above 2 mg/ml may paradoxically stimulate osteoclast activity and increase the risk of cyst formation and inflammatory responses. Likewise, VEGF can induce dose-related complications, as supraphysiological concentrations are associated with vascular leakage and edema (82, 83).

The risk of heterotopic ossification is a major safety concern, especially with BMP-2. Clinical studies have reported ectopic bone formation in approximately 10–30% of cases within the surrounding soft tissues, such as muscle, tendons, and the joint capsule, after BMP-2 administration. This complication may result in joint stiffness, pain, and reduced range of motion. Moreover, the likelihood of heterotopic bone formation increases when BMP-2 diffuses or leaks beyond the intended target area, and it can be further modulated by the characteristics of the delivery vehicle (84, 92).

Challenges related to delivery and burstrelease behavior further hinder the clinical translation of growth factor therapies. Achieving therapeutic efficacy requires that these factors be delivered in a sustained and localized manner to the necrotic region of the femoral head. Systemic administration is largely ineffective due to rapid clearance, and direct local injection without an appropriate carrier results in rapid diffusion away from the target site within hours. Many conventional scaffold systems release a large proportion, often 40-70%, of their growth factor cargo within the first 24-48 hr. This initial burst can generate supraphysiological peak concentrations that, instead of promoting repair, may impair osteogenesis or provoke undesirable side effects. An optimal delivery platform would therefore provide controlled, sustained release over approximately 2-4 weeks, aligning more closely with the natural timeline of bone healing (88, 92).

The clinical translation of growth factor-based therapies faces several formidable challenges: (1) Pharmacokinetic limitations, specifically rapid *in vivo* degradation and premature burst release, which diminish therapeutic

efficacy; (2) safety concerns, primarily the risk of ectopic bone formation in non-target tissues; (3) economic barriers, including the prohibitive costs associated with recombinant growth factor production; and (4) methodological uncertainties, as the optimal dosage and synergistic combination strategies (e.g., the co-delivery of BMP-2 and VEGF) have yet to be standardized. A comprehensive comparative synthesis of growth factors utilized in AVN management, delineating their primary mechanisms, delivery modalities, clinical/preclinical outcomes, and associated risks, is summarized in Table 2.

Platelet-rich plasma (PRP)

Platelet-derived growth factor, transforming growth factor, basic fibroblast growth factor, endothelial growth factor, insulin-like growth factor, and VEGF are all regenerative cytokines in PRP (93). An early-stage hormonal ONFH model was alleviated by PRP, according to Karakaplan *et al.* (94). A study conducted *in vitro* combined PRP with scaffolds to improve bone formation and blood vessel growth. Additionally, research has shown that a PRP-based hydrogel scaffold promotes bone formation and blood vessel growth. These scaffolds effectively release PRP, and osteoblasts proliferate, remain alive, and attach well to them (95). The study's findings imply that various additives can be incorporated to facilitate bone formation or blood vessel growth. In addition to drugs that promote angiogenesis, growth factors are commonly used to promote bone development. In a study by Houdek *et al.*, HSS in 22 patient with ONFH (35 hips) increased from 57 to 85 points over a 3-year period. 93% of patients showed no progression or complications (96). The use of PRP in conjunction with CD and stem cell transplantation or bone grafting can induce osteogenic activity and stimulate stem cell differentiation in ARCO stage I and II patients (97). According to these results, PRP may be a promising non-invasive therapy for reversing ONFH progression, improving functional outcomes, and reducing the need for more invasive treatments. In addition, patients with ARCO stage I and II ONFH can benefit from PRP combined with stem cell transplantation or bone grafting. ONFH symptoms can be alleviated by PRP by the following mechanisms, according to previous studies (98, 99): 1) the stimulation of angiogenesis and osteogenesis, 2) the inhibition of the inflammation response in necrotic lesions, 3) the suppression of GC-induced apoptosis, and 4) the activation of osteogenesis and autophagy (99). PRP has been shown to be effective in combination therapies. However, prospective randomized trials are required to identify the optimal concentration of PRP and the proportion of stem cells. Furthermore, platelet lysates can also reduce bone resorption and cell apoptosis in femoral head necrosis (100).

Standardization challenges remain a major limitation in the clinical application of PRP, as no universally accepted preparation protocol exists. Key variables differ widely across studies, including centrifugation speed (typically between 1,200 and 3,200 rpm), the choice between single- and double-spin processing, and the method of activation (such as thrombin, calcium chloride, or no activation at all). These methodological differences result in substantial variability in the final platelet concentration, which has been reported to range from approximately 2-fold to 8-fold above baseline levels (100).

Variability in preparation methods further complicates the clinical use of PRP. The absence of standardized protocols extends to several critical factors, including the type of blood collection tube and anticoagulant used, storage conditions, and the interval between preparation and administration, which can range from immediate application to delays of up to 4 hr. In addition to these technical inconsistencies, substantial inter-individual variability exists in PRP composition. Patient-specific factors such as age, comorbidities (including diabetes or chronic steroid use), and the activity of the underlying disease can markedly influence platelet content, growth factor levels, and overall biologic potency (98, 99).

Clinical outcomes reported in the literature remain inconsistent. A systematic review by Han et al. found that, among 12 included studies, 8 demonstrated beneficial effects of PRP, whereas 4 reported no statistically significant advantage compared with core decompression alone. These conflicting findings may be attributed to several factors, including variations in patient selection (for example, ARCO stage I versus stage II disease), differences in PRP preparation protocols, and the use of additional combination therapies (98).

Table 2. Comparison of growth factors and delivery strategies for avascular necrosis (AVN) treatment

Growth factor	Primary mechanism	Delivery strategy	Scaffold/hydrogel combination	Reported effects	Major risks	Key reference
BMP-2	Osteoinduction (MSC → osteoblast)	Scaffold-incorporated, hydrogel, microspheres	PLGA/gelatin, collagen, β-TCP	↑ Osteogenesis, ↑ bone volume	Ectopic bone formation; high cost; burst release	(54, 85, 93)
VEGF	Angiogenesis (endothelial proliferation)	Microsphere-loaded, hydrogel-encapsulated	PLGA-mPEG, gelatin-tyramine, HA-collagen	↑ Vascularization, ↑ capillary density	Edema; leaky vessels; short half-life	(54, 80, 84)
BMP-6	Osteoinduction	Gene transfection (with VEGF)	PLAGA scaffold	↑ Bone formation, ↑ osteoblast differentiation	Similar to BMP-2; less studied	(94)
BMP-7	Osteoinduction	Genetically modified BMSCs	Colla Cornus Cervi (CCC)	↑ Cell proliferation, ↑ osteogenesis	Similar to BMP-2	(95)
HGF	Angiogenesis+osteogenesis	Fibrin glue, gene transfection	Fibrin glue with MSCs	↑ Vasculogenesis, ↑ osteogenic differentiation	High concentration required	(76)
FGF-2	Osteoblast differentiation+wound healing	Collagen hydrogel	Collagen scaffold	↑ Cell ingrowth, ↑ vessel formation	Short half-life; rapid degradation	(87)
Deferoxamine	Angiogenesis (HIF-1α stabilization)	Slow-release scaffold	Gelatin-methacryloyl+black phosphorus	↑ VEGF expression, ↑ angiogenesis	Iron chelation side effects	(88, 96)
Simvastatin	Angiogenesis+osteogenesis	Hydrogel-incorporated, scaffold-loaded	Poly(beta-amino ester) hydrogel, PLGA	↑ Preosteoblast activity, ↑ bone formation	Dose-dependent effects; systemic concerns	(89, 90)

Despite encouraging therapeutic outcomes, the clinical integration of PRP in AVN management is significantly hindered by several critical factors: (1) methodological heterogeneity, characterized by a lack of standardized preparation protocols, including inconsistencies in centrifugation parameters, activation modalities, and final platelet concentrations; (2) pharmacological ambiguity, specifically the absence of consensus-based dosing regimens; (3) methodological gaps in clinical evidence, primarily the scarcity of high-quality, large-scale prospective randomized controlled trials (RCTs); and (4) synergistic uncertainty, as the optimal integration of PRP with mesenchymal stem cells (MSCs) or bio-scaffolds remains poorly defined.

Comparative analysis of regenerative strategies

A rigorous comparative analysis of current regenerative paradigms highlights a series of technical trade-offs. Stem cell-based therapies capitalize on dual osteogenic and paracrine mechanisms; however, their clinical translation is impeded by logistical hurdles in cell sourcing, non-standardized expansion protocols, and significant inter-study heterogeneity in outcomes (66, 72). Growth factor interventions (e.g., BMP-2, VEGF) deliver potent osteoinductive and angiogenic cues but are clinically constrained by unfavorable pharmacokinetics, notably rapid *in vivo* degradation and premature burst release, as well as prohibitive manufacturing costs (75, 83). While Platelet-Rich Plasma (PRP) offers an autologous, cost-efficient alternative, its utility is undermined by a lack of methodological standardization and inconsistent therapeutic efficacy (100). Furthermore, injectable hydrogels enable minimally invasive administration and controlled payload release, yet they often lack the biomechanical properties to prevent subchondral collapse under physiological loading (58). Consequently, while multimodal combination strategies (integrating cells, scaffolds, and signaling molecules) demonstrate superior synergistic potential in preclinical models, they introduce substantial regulatory hurdles and manufacturing complexities.

Challenges in cell-based therapies for AVN

Despite promising preclinical data, the transition of cell-based interventions for femoral head osteonecrosis (ONFH) into routine clinical practice is obstructed by several physiological and logistical bottlenecks (58).

Survival within the hostile necrotic microenvironment

The ischemic femoral head constitutes a pathophysiologically "hostile" niche, characterized by chronic hypoxia, localized acidosis, nutrient depletion, and a surge in pro-inflammatory cytokines and reactive oxygen species (ROS). These conditions severely compromise the fitness of transplanted cells; longitudinal studies indicate that viability often plummets to below 5-10% within the first 14 days post-transplantation. To counteract this "engraftment-survival gap," current research focuses on cytoprotective priming, including hypoxic preconditioning, genetic engineering (e.g., HIF-1α stabilization), and the co-administration of anti-apoptotic agents or biomimetic extracellular matrix (ECM) components (58, 72).

Inefficient engraftment and integration

Beyond initial survival, the stable integration of cells into the dense, devitalized necrotic matrix remains a formidable challenge. Traceable MSC studies reveal that long-term engraftment rates rarely exceed 10-30%, as the lack of functional vasculature impedes cell homing and migration. To address this, scaffold-mediated delivery systems (e.g., biofunctionalized hydrogels or osteoconductive ceramics) are being used to provide a protective "synthetic niche," thereby enhancing cell retention and spatial organization at the defect site (65).

The autologous vs. allogeneic dilemma

While autologous MSCs eliminate the risk of immunogenicity, they are burdened by invasive harvesting, time-intensive *ex vivo* expansion, and substantial patient-to-patient variability. Critically, the regenerative potency of autologous cells may be diminished in patients with underlying comorbidities such as chronic steroid use or alcoholism. Conversely, allogeneic MSCs offer a standardized "off-the-shelf" solution with superior quality control. Although MSCs exhibit low baseline immunogenicity, concerns persist regarding potential allo-sensitization, disease transmission, and the long-term safety of repetitive dosing (74).

The rise of exosome-based "cell-free" therapies

To circumvent the risks associated with live-cell transplantation, such as tumorigenicity and embolic events, MSC-derived exosomes (30-150 nm vesicles) have emerged as a potent therapeutic alternative. These nanovesicles encapsulate a rich cargo of bioactive proteins and RNAs that modulate angiogenesis (e.g., via miR-126) and osteogenesis (via BMP-2/RUNX2 signaling). Recent evidence in steroid-induced models demonstrates that systemic exosome delivery significantly attenuates bone necrosis and enhances microvascular density. However, standardizing isolation techniques and determining the optimal therapeutic window remain essential for clinical translation (74).

Paracrine immunomodulation: A key mechanism

The therapeutic efficacy of MSCs is increasingly attributed to their immunomodulatory secretome rather than direct differentiation. By orchestrating a phenotypic shift in macrophages from a pro-inflammatory (M1) to a pro-resolving (M2) state and suppressing T-cell proliferation, MSCs effectively dampen the chronic inflammatory cascade. This systemic modulation is particularly vital in non-traumatic AVN, where donor-derived cells may exert profound regenerative effects through paracrine signaling even if they fail to achieve long-term engraftment (101).

AVN treatment based on tissue engineering

To repair AVN, various materials are used for regeneration. These materials, along with cells and growth factors, are the three main components of bone tissue engineering for treating AVN. Natural and synthetic polymers, ceramics, and metals are among the materials used (68, 102). Ceramic and polymeric structures can provide initial mechanical support to the deteriorating hip joint due to AVN and serve as a tissue engineering therapy. These scaffolds can replicate the environment in which cells grow and facilitate blood vessel growth and cell differentiation (103). The exploration of natural polymers such as alginate, chitosan, and peptide chain hydrogels in osteonecrosis research highlights their

superior biological properties, including enhanced cell proliferation and hydrophobicity, due to their extracellular matrix structure. In contrast, synthetic polymers such as polylactic-co-glycolide (PLGA), polycaprolactone (PCL), and polylactic acid (PLA) exhibit lower biological performance but offer improved mechanical strength, controllable degradation rates, and superior processing capabilities.

Utilizing tissue engineering scaffolds can enhance bone healing by supporting cell migration and osteogenic differentiation. Engineered three-dimensional scaffolds with appropriate architecture and osteoconductive properties have advanced bone tissue regeneration. For effective implantation, scaffolds must be biocompatible, allowing stem or osteoinductive cells to adhere without experiencing cytotoxicity or immunogenicity. Materials such as polylactic acid (PLA), polylactic-co-glycolic acid (PLGA), alginate, and tantalum are noted for their biocompatibility (100). Scaffolds in bone tissue regeneration must be biodegradable to facilitate the growth of native tissue as they dissolve. They should allow the release of stem cells or drugs and degrade easily, which is critical for addressing osteonecrosis of the femoral head. Surgical removal of scaffolds can result in damage and adverse effects. Additionally, scaffolds must not induce an immune response during degradation. Biocompatible options such as PLA, PLGA, alginate, and tantalum metal support cellular function and promote the adhesion of osteoinductive cells without cytotoxicity or immunogenicity, making them essential for preventing complications (104).

The biocompatibility and degradability of alginate, PLGA and PCL have been analyzed in 3D scaffolds. Previous studies have used multiple materials in the construction of tissue engineering scaffolds to capitalize on their advantages (105). For example, a biocompatible porous scaffold made of lactate-co-glycolide/β-tricalcium phosphate (PLGA/TCP) was fabricated by adding magnesium, which increased bone formation and strength and optimized the immune response (106).

As the scaffolds degraded *in vivo*, their volume also decreased. Magnesium enhanced biocompatibility and degradation compared to the standard PLGA/TCP scaffold. To prevent femoral head collapse in the emu model, Qin *et al.* used a novel PLGA/TCP scaffold containing icaritin as a bone filler. They found that the scaffold promotes MSC migration and prevents undesirable cell differentiation at implant sites. When icaritin was added to the scaffold, calcium deposition and osteogenic gene expression were enhanced. The scaffold was not affected in terms of degradability (107).

Porous selenium and silicon dioxide nanocomposites were used in the rat model. Inflammatory responses were not observed in this study owing to the material's good biocompatibility. In addition to preventing steroid-induced osteonecrosis, the scaffolds reduced oxidative stress. Trans-cinnamaldehyde (TCA) has been found to inhibit cartilage damage *in vitro* and has anti-inflammatory properties *in vitro*. An osteoblast proliferation and differentiation increase was observed over 12 weeks on a porous titanium alloy scaffold produced via TCA by Gao *et al.* (62). According to Kawai *et al.*, a functionally graded PCL/b-TCP scaffold implanted in rabbits became more biocompatible after 8 weeks as bone ingrowth increased. The 3D scaffold also degraded efficiently *in vivo* (75).

Further comparative investigations have highlighted how distinct material strategies can enhance scaffold bioactivity. Shen *et al.* demonstrated that PCL-PEG-PCL scaffolds incorporating MgO exhibited superior cytocompatibility, proliferation, and osteogenic differentiation of MC3T3-E1 pre-osteoblasts relative to PLGA controls, with the PEG component conferring a favorable degradation profile and magnesium oxide promoting both *in vitro* and *in vivo* bone formation (108). In a complementary approach, Guillaume *et al.* leveraged the inherent biocompatibility and controlled degradability of poly-trimethylene carbonate (PTMC), showing that surface enrichment with hydroxyapatite nanoparticles and bone marrow stem cells significantly enhanced osteogenic development in rabbit models over six weeks (109). These findings collectively illustrate that the strategic combination of materials, whether through ionic doping with MgO or nanoparticle functionalization with HA, can yield significant additive improvements in scaffold performance for AVN repair (110).

3D printed & film scaffolds

The successful translation of scaffold-based strategies for femoral head AVN hinges on a precise balance of interrelated material properties, including biocompatibility to prevent adverse immune responses, controlled biodegradability to synchronize scaffold resorption with new tissue formation, and sufficient mechanical strength to withstand physiological loading without premature collapse (111, 112). As comprehensively categorized in Table 3, the materials employed for AVN repair span natural polymers (e.g., alginate, chitosan), synthetic polymers (e.g., PLGA, PCL, PLA), bioceramics (e.g., β-TCP, hydroxyapatite), and metals (e.g., tantalum, titanium alloys), each offering a distinct profile of advantages and inherent trade-offs. Natural polymers typically provide superior bioactivity and cell-interactive moieties but lack mechanical robustness, whereas synthetic polymers afford tunable degradation kinetics and greater structural integrity yet require functionalization to enhance cellular recognition (113). Bioceramics exhibit exceptional osteoconductivity and compressive strength but suffer from brittleness, whereas metallic implants offer unmatched load-bearing capacity at the expense of permanent residency and stress shielding. Informed by these material-level considerations, researchers have increasingly turned to advanced manufacturing techniques, particularly 3D printing and film-based fabrication, to engineer structurally precise, patient-specific scaffolds that address the complex anatomical and biomechanical demands of the femoral head. A detailed comparison of representative 3D-printed and film scaffolds developed specifically for AVN treatment, including their compositional strategies and functional outcomes, is presented in Table 4.

The positive and negative aspects of materials vary with their nature. These characteristics, which include porosity, mechanical support capabilities, biocompatibility, and biodegradability, cannot all be met by a single polymer. A poly (lactic-co-glycolic acid)/β-calcium phosphate/icariin (PLGA/TCP/Icariin, PTI) scaffold was created by Lai *et al.* The phytomolecule icaridin regenerates bone. Following core decompression, these bio-functional scaffolds were implanted in an AVN rabbit model. It was found that the scaffolds improved the tissue's mechanical properties,

Table 3. Use of bioactive substances for the regeneration of the bone and vasculature of the necrotic femoral

Scaffolds materials		Biologically Active Factor	Properties	References
Chitosan	Alginate	BMMSC and EPCs	Porosity, minimal cytotoxicity, superior cell adhesion, and biocompatibility reduced fat production and increased angiogenesis and bone formation	(128)
Alginate (ALG)	—	SMSCs	Osteogenesis, elasticity, injectability, biocompatibility, and biodegradability	(86)
Cervi cornuscolla (CCC)	Deproteinized bone	—	Biocompatibility, cylindrical form, 15 mm diameter, 3.5 mm thickness, pore structure, porosity (72.86 ± 5.45%), 4.45 ± 1.02 MPa compressive strength, 35.81% disintegration rate after 6 weeks, and osteogenesis	(129)
HA	Bisphosphonate CAP	—	Injectability Hydrogel, biocompatibility, biodegradability, osteoconductivity, and bone conductivity promote osteogenic differentiation and bone regeneration	(130)
DBM	—	BFGF, BMP-2	Biocompatibility, enhanced osteogenesis and angiogenesis, cell adhesion	(131)
PLGA	β-TCP	5% Mg 10% Mg 15% Mg	Biocompatibility, biodegradability, pore size PT 423.1±77.0, PT 5 M 418.7±33.4, PT, 10M 392.5±30.2, PT 15M 411.5±26.9, porosity PT 59.1±9.7, PT 5M 59.4±3.1, PT 10M 62.4±5.3, PT 15M 65.8±8.0, the connectivity is 100%, compressive strength, PT 1.5±0.1 MPa, PT 5M 2.9±0.2 MPa, PT 10M 3.1±0.2 MPa, PT 15M 3.7±0.2 MPa, osteogenesis	(115)
PLGA	TCP	Icaritin	Biocompatibility, biodegradability, pore structure, compressive strength 47.03±33.58 N, enhanced bone formation	(116, 132)
PLGA	CPC	BMP, VEGF	Biocompatibility, biodegradability, porosity 62.13±4.28%, compressive strength of 6.60 ± 1.02 MPa, osteogenic, angiogenic	(133)
PCL	TCP	BMMCs	Biocompatibility, pore structure, and porosity near the section is 15%, the middle section is 40%, the far section is 16%, the 8-week degradation rate of the proximal segment is 42.5±14.0%, 5.3±1.9% at the middle segment, 5±5.2% at the distal segment, osteogenic, vascular	(134)
PLA	Nano-hydroxyapatite, collagen	BMMSC	Biocompatibility, biodegradability, pore size of 300±250 μm, porosity of 70-90%, vascularity, osteogenesis	(129)
PPF	CPC	Ginsenoside Rg1	Biocompatibility, biodegradability, pore structure, the compressive strength in C/P = 0, C/P = 1 and C/P = 2 groups are 13.66 3.00 MPa, 15.68	(135)
porous β-TCP granules	----	----	Biocompatibility, porous structure, neovascularization, bone regeneration, the yield strength in normal and treat groups, 18.35% and 31.12%, respectively	(52)
Gelatin	Tyramine	----	Injectable Hydrogel, biocompatibility, biodegradability, revascularization, bone healing	(136)
Gelatin	Tyramine-heparin	BMP2	Injectable Hydrogel, sustained release, biocompatibility, biodegradability, revascularization, bone healing	(137)
Gelatin	Phosphorus nano-sheet	Deferoxamine	Injectable Hydrogel, biocompatibility, biodegradability, osteoconductivity, sustained released, bone conductivity, vascularization	(96)
PCL	Hyaluronic acid	BMSCs, PTH	Injectable Hydrogel, 3D-printed hydrogel, biocompatibility, biodegradability, cartilage regeneration, bone conductivity, vascularization	(138)
PLGA	mPEG	VEGF	Injectable Hydrogel, sustained release, biocompatibility, biodegradability, cell viability, vascularization, bone regeneration of femoral head necrosis	(54)
Silk fibroin	----	Vancomycin	Injectable Hydrogel, *in vitro* and *vivo* biocompatibility, biodegradability, sustained released, reduced bone infection, antibacterial activity	(4)
Silk fibroin	Chitosan	BMP-2, BMSCs	Injectable Hydrogel, thermos-sensitive, biocompatibility, biodegradability, sustained released, neovascularization, bone regeneration	(48)
RADA16 peptide	----	BMP-2	Injectable Hydrogel, sustained released, biocompatible, biodegradable, revascularization, bone healing	(139)
Gelatin	Hydroxypropyl-β-cyclodextrin	BMSCs	Injectable Hydrogel, sustained released, biocompatible, biodegradable, *in vitro* biocompatibility, encapsulation, revascularization, bone healing.	(140)
Gelatine	Hyaluronic acid (HA)	VEGF	Injectable Hydrogel, sustained released, biocompatible, biodegradable, encapsulation, immunomodulatory mechanism, revascularization, bone healing.	(141)
OCMC	CMCS	QK peptide	Injectable Hydrogel, sustained released, biocompatible, biodegradable, revascularization, bone healing	(142)
Sodium Alginate	Polyethylene Glycol	β-tricalcium phosphate/Magnesium oxide	Injectable Composite Hydrogel, sustained released, biocompatible, biodegradable, revascularization, bone healing	(143)

thereby promoting angiogenesis and bone regrowth (114).

In another interesting approach, Peng *et al.* used micro-CT images to fabricate biphasic calcium phosphate (BCP) ceramic scaffolds by gel-lamination, mimicking the cancellous bone microarchitecture of the femoral head. A canine bone defect in the femoral head was implanted with BMSCs seeded on these scaffolds. Several improvements in bone regeneration were observed in the BCP scaffolds after 30 weeks of implantation (115).

Recent studies have used 3D printing to design scaffolds for the treatment of AVN (116). A 3D-printed functionally graded scaffold was created from PCL and β-TCP. It also had the desired degradation rates and mechanical strength properties due to its spatially controlled porous profile. Porosity decreased at the ends while increasing in the middle of the construct. The tissue had the desired mechanical properties. In rabbits with femoral heads and necks, scaffolds were implanted in drilled cavities. After 8 weeks of implantation, the samples were examined. Histological studies showed no bone formation and high mineralization

Table 4. Comparison of biomaterial scaffolds for osteonecrosis of femoral head (ONFH) repair

Biomaterial	Type	Mechanical strength	Degradation rate	Osteoconductivity	Biocompatibility	Key limitation	Key reference
PLGA/TCP	Synthetic/ceramic composite	Moderate	8-12 weeks	Good	Good	Acidic degradation products	(114)
PCL/β-TCP	Synthetic/ceramic composite	High (good load-bearing)	12-24 weeks	Moderate	Good	Slow degradation; hydrophobic	(85)
Alginate	Natural polymer	Low (needs crosslinking)	4-8 weeks	Poor	Excellent	Poor mechanical strength	(86)
Gelatin-based (GelMA, tyramine-gelatin)	Natural-derived	Low to moderate	2-6 weeks	Moderate	Excellent	Rapid degradation; weak mechanical properties	(96, 137)
Hydroxyapatite/ collagen	Ceramic/natural composite	Moderate	8-16 weeks	High	Excellent	Brittle; difficult processing	(84, 119)
Tantalum metal	Metal	Very high (excellent load-bearing)	Non-degradable	Good	Good	Permanent implant; stress shielding	(39)
Silk fibroin	Natural polymer	Moderate to high	4-12 weeks (tunable)	Moderate	Excellent	Batch variability	(54)

on micro-CT, with increased scaffold resorption at both ends (34). In a recent study, Liao *et al.* seeded BMSCs onto PLAGA scaffolds (3D-printed polylactide-coglycolide). *In vivo*, implants showed angiogenesis and bone regeneration. Cell differentiation was supported by the 3D-printed scaffold and growth factors. Wang *et al.* seeded BMSCs onto nHAC/PLA scaffolds in another study. Osteoid tissue formation near the necrotic bone of the femoral head was observed *in vivo*. Polymeric scaffolds were effective in treating femoral head AVN (117).

Using 3D printing technology, Lei H *et al.* created porous Ti6Al4V reconstruction rods integrated with tissues by infusing them with a mercapto-hyaluronic acid hydrogel containing icariin. *In vitro*, results indicated that the reconstruction rod promoted MC3T3-E1 cell adhesion and proliferation. *In vivo* results showed that the reconstruction rod promoted the formation of new bone and blood vessels and effectively integrated with the bone, making it suitable for treating early AVN of the femoral head (118).

Hydrogel

Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb and retain significant amounts of water or biological fluids. Hydrogels are used in a variety of applications. To closely resemble biological tissues, they are characterized by their high water content, soft texture, and occasionally porous structure. These characteristics allow them to imitate organic tissues. Biocompatibility is one of the essential features of hydrogels that makes them suitable for applications involving bone and joints (119). This is because hydrogels are often well accepted by the body, which reduces the risk of adverse reactions. Hydrogels can absorb and retain water or other liquids, sometimes to more than 90 percent of their weight. The swelling properties of these materials include the ability to absorb significant amounts of water, which allows them to mimic the natural conditions to which cartilage and bone are subjected. A network structure is formed by the interconnection of polymer chains, which can occur either physically or chemically. This results in a three-dimensional network. The insertion of hydrophilic groups (such as -OH and -COOH) into the polymer chains makes them more likely to attract and absorb water. This property is referred to as hydrophilicity (120). Biocompatibility, or the capacity to be safe for living tissues, and biodegradability, or the ability to break down within the body, are both characteristics that may be built into hydrogels. The characteristics, such as swelling, mechanical strength, and degradation rate, may be modified by altering the polymer structure and crosslinking processes. Because hydrogels are biocompatible and can deliver drugs and cells directly to injured sites, they are increasingly used in the treatment of bone and joint ailments. These materials are used in bone regeneration and cartilage repair, as well as in the treatment of AVN and osteoarthritis, which opens the door to less intrusive, more individualized treatment. The treatment for bone AVN and osteoarthritis involves hydrogels that deliver anti-inflammatory drugs, cells, and growth factors to the joint in a targeted manner, thereby reducing inflammation and promoting cartilage regeneration. They have the potential to be developed to mimic the properties of trabecular bone and synovial fluid, resulting in improved joint regeneration and lubrication and reduced friction.

Because hydrogel injections provide a less invasive alternative to surgical intervention for some bone and joint conditions, they reduce the pain patients experience and shorten the time it takes for them to resume normal activities (121).

Injectable hydrogel

Scientists are now investigating injectable systems' potential for localized osteonecrosis treatment. These systems involve the direct use of stem cells, growth factors, cytokines, medicines, and hormones at the affected site. These devices may reduce the need for surgical procedures and provide mechanical assistance to the collapsing femoral head (34, 122-124).

Hydrogel scaffolds show great potential as materials for several medical uses, such as tissue-engineering platforms and drug and growth-factor transporters. The encapsulation of cells, growth factors, and bioactive compounds has demonstrated therapeutic benefits for regeneration in peripheral nerves, spinal cord, wounds, bone, and cartilage. Additionally, they are well-suited for rapid, secure bone healing and regrowth (125, 126). Hydrogels may be processed gently and delivered with minimal invasion. These properties make surgery easier and reduce side effects compared to standard procedures. The minimally invasive injection of hydrogel into irregular AVN bone heads provides several research options for AVN therapy (52). Physical support and host penetration from 3D-structured porous hydrogels aid stem cell growth and proliferation. Biomedical researchers

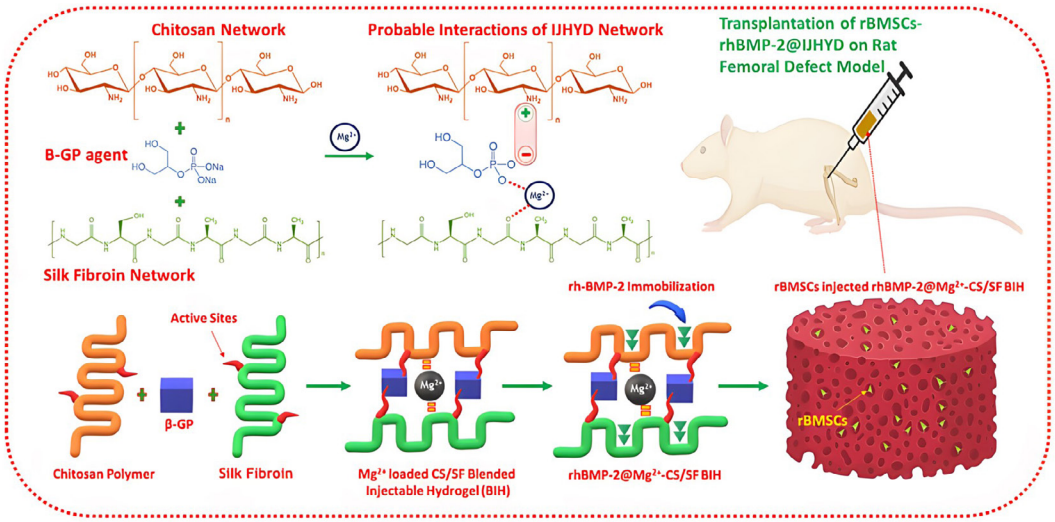

Figure 5. Formation of injectable hydrogel involving magnesium (Mg) loaded chitosan (CS)/silk fibroin (SF) and with physico-chemical reactions
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are interested in injectable hydrogel networks composed of natural polymers such as chitosan, silk fibroin, and cellulose because they break down naturally and are compatible with normal biological processes (127, 128).

Ma *et al.* created an injectable hydrogel composed of tyramine-gelatin microspheres and used a rat model to study ischemic osteonecrosis. Biopolymeric-derived hydrogels have been used in tissue engineering and regenerative medicine. However, challenges such as gelation, swelling, mechanical properties, toxicity, and self-healing capacity may limit their clinical utility. The hydrogels exhibited the ability to enable precise administration of growth factors and stem cells, resulting in enhanced efficiency and compatibility for cell encapsulation (46, 129).

Scientists are creating hydrogel implants that promote bone formation by combining bioactive substances, stem cells, and growth factors that stimulate bone development. Ensuring the effective delivery of these growth factors is vital to fracture regeneration. Selecting appropriate constituents for injectable hydrogels is vital to advancing hard tissue engineering and clinical regenerative medicine. The injectable hydrogels possess favorable attributes due to their appropriate water-loving nature and viscoelastic properties similar to those of tissues (Figure 5). This creates an optimal environment for the introduction of growth factors and stem cells capable of inducing bone formation (46, 130).

Chen *et al.* developed a temperature-sensitive PLGA-mPEG block copolymer to facilitate injection-based vascularization and bone regeneration in femoral head necrosis (Figure 6). The copolymer was impregnated with

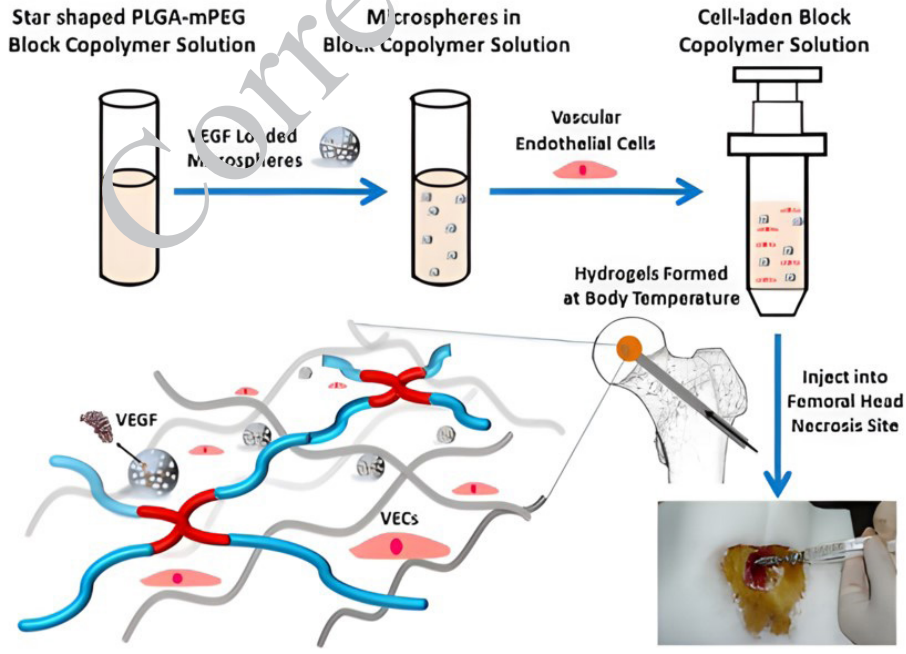

Figure 6. Synthesis of injectable hydrogel with vascular endothelial growth factor-loaded microspheres (VEGF-loaded microspheres) and vascular endothelial cells for vascularization and bone regeneration of femoral head necrosis
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VEGF and vascular endothelial cells (VECs), resulting in hydrogels that may be used for vascularization and bone regeneration (52, 131).

By combining hyaluronic acid with bisphosphonate, Wang *et al.* created HA-BP/Ca-P. Injectability, robust adhesion, and self-healing were among the desired properties exhibited by this modified hyaluronic acid. This material may hasten bone repair in femoral head necrosis by increasing MC3T3-E1 cell proliferation (53). Animal testing for Kim *et al.*'s BMP2-hydrogel treatment for Legg-Calve-Perthes disease or osteonecrosis of the femoral head showed promising results. The hydrogel successfully maintained BMP2 levels for four weeks with its three main ingredients-gelatin, heparin, and tyramine (Figure 7). For adolescents in particular, this quality makes it a potentially life-changing option for treating ONFH (122).

Xu *et al.* investigated the feasibility of treating ischemic tibia bone using a gelatin-methacryloyl hydrogel with a black phosphorus nanosheet and deferoxamine (BPN-DFO). By activating proangiogenic genes, including VEGF, the hydrogel effectively induces angiogenesis, reducing the risk of ischemic bone in the tibia (125).

Biomimetic joints damaged by AVN may be regenerated via 3D printing. Li *et al.* developed a synthetic tissue-like structure to stimulate rabbit humeral head regeneration. Porous materials such as HA/PCL bioinks and stiff supports were used to fabricate this structure. Dynamic mechanical stimulation and parathyroid hormone enhance endochondral ossification, making this method versatile and scalable for large joint repair (44).

Yuan *et al.* formulated an injectable hydrogel using HPβCD-Gel for bone regeneration in cases of femoral head necrosis. The hydrogel was administered in a defect model and paired with BMSC, demonstrating its promise in treating femoral head osteonecrosis (Figure 8). The efficacy of the hydrogel was confirmed in an *in vivo* model (132).

The research by Sharma *et al.* investigated the effectiveness of a gelatine hyaluronic acid injectable hydrogel containing VEGF as a local delivery method. The aim was to sustain vascularization in a mouse maxillary osteonecrosis model treated with bisphosphonates. The findings demonstrated that VEGF facilitates bone regeneration and mitigates medication-induced osteonecrosis (133).

Peyravian *et al.* developed a new injectable hydrogel that combines OCMC-CMCS polymers with an angiogenesis-activator peptide (QK) to heal femoral head AVN in an animal model. Over 21 days, the hydrogel exhibited a favorable swelling ratio, water absorption, and breakdown rate (Figure 9). Additionally, it enhanced the growth, specialization, formation of new blood vessels, and the ability of BM-MSCs and HUVECs to produce bone tissue, thereby avoiding femoral head death and identifying a greater number of blood vessels (134).

The generated hydrogels show potential for treating AVN. They use polymers and bioactive compounds for targeted treatment and optimal effectiveness, notably in the care of osteonecrosis. Zhou *et al.* introduced the SA/β-TCP@PMP system as an injectable composite hydrogel made of sodium alginate (SA) and β-TCP crosslinked with glucono-δ-lactone (GDL). It is reinforced with different amounts of poly (3-hydroxybutyrate-co-4-hydroxybutyrate)/magnesium oxide and polyethylene glycol (P34HB/MgO+PEG; PMP) coaxial electrospun microfibers. The physical characteristics of the composite hydrogel, such as its injectability, surface

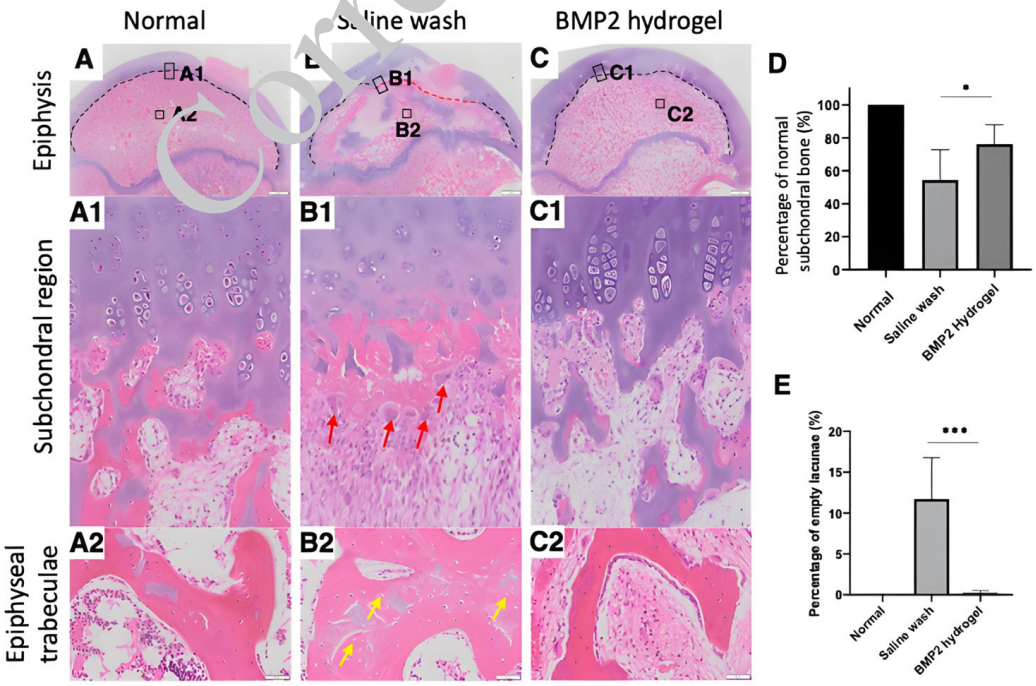

Figure 7. Femoral head's subchondral and trabecular bones were repaired using homodimeric bone morphogenetic protein-2 (BMP2) hydrogel
The femoral heads of the normal, saline wash, and BMP2-hydrogel groups were stained with hematoxylin and eosin (H&E) staining in the photos from A to C. Pictured here are (A1–C1) the subchondral areas of the normal, saline wash, and BMP2-hydrogel groups, as well as (A2-C2) the epiphyseal trabeculae of these same groups. In the normal, saline wash, and BMP2-hydrogel groups, the percentage of the subchondral area exhibiting endochondral ossification was calculated (D), and in the same group, the percentage of empty lacunae was calculated (E). The typical subchondral bone is shown by the black dashed lines. Abnormal subchondral bone is shown by red dashes; osteoclasts are depicted by red arrows; and empty lacunae are depicted by yellow arrows. * Represent $P<0.05$; *** represent $P<0.001$ (Reproduced from Reference 122 under terms of the Creative Commons Attribution License (CC BY 4.0 license))

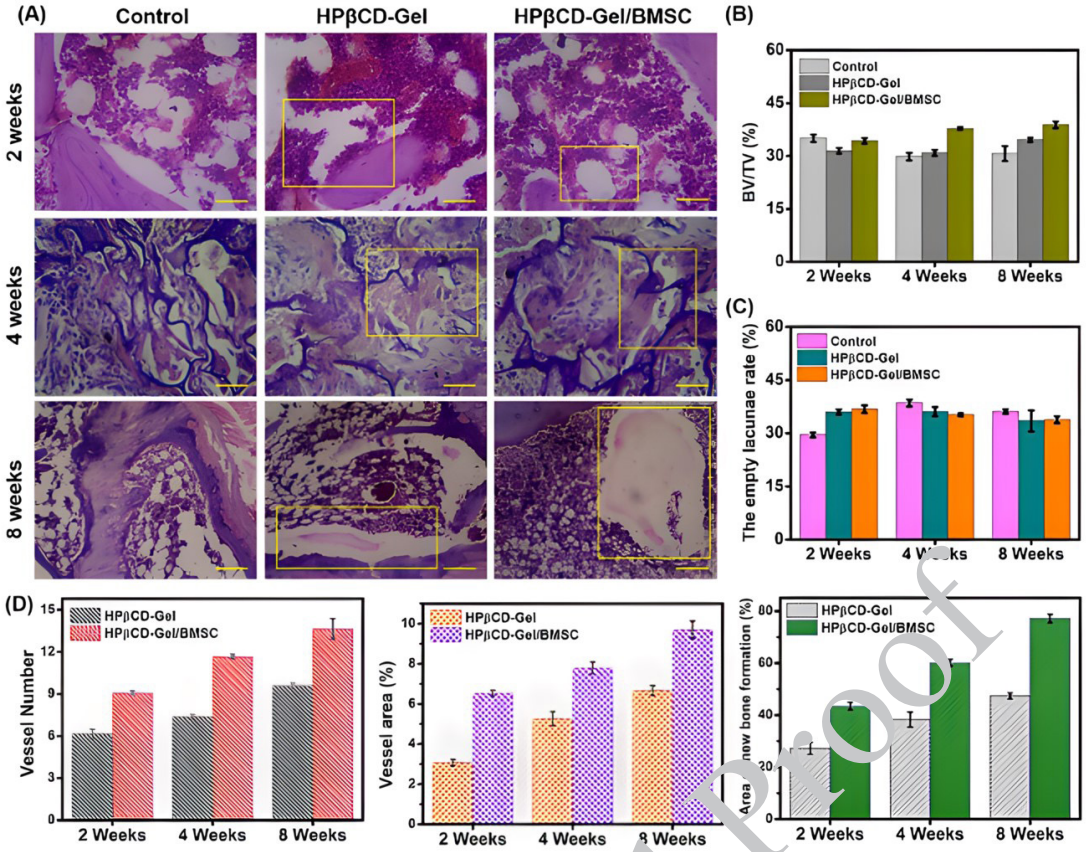

Figure 8. Subchondral and trabecular bones of the femoral head were repaired by the hydroxypropyl-β-cyclodextrin (HPβCD) cross-linking of gelatin (Gel) bone marrow-derived mesenchymal stem (BMSC) hydrogel
(A) The effects of BMSC on the SD rat model of HPβCD-Gel hydrogels-induced onchondrogenic necrosis were examined. Evaluation of osteoclasts in each treatment group at 2, 4, and 8 weeks post-operatively using hematoxylin and eosin (H&E) staining. (B) The ratio of empty lacunae in the control, HPβCD-Gel hydrogels, and HPβCD-Gel hydrogels loaded with BMSC treatment groups at 2, 4, and 8 weeks; and (C) quantitative assessment of bone volume. (D) The total area of newly generated blood vessels, the area of newly produced bone, and the number of new blood vessels in each sector. All panels had a scale bar that read 100 μm. Data are presented as the mean±standard deviation (P<0.05) (Reproduced with permission from Reference 132, Elsevier/Materials Letters, and Copyright © 2022 Elsevier Inc.)

morphology, swelling, degradation, and mechanical properties, as well as the release of magnesium ions, can be precisely tuned by varying the concentration of PMP microfibers. In a rabbit model of steroid-induced ANFH, the hydrogel exhibits the best angiogenic and osteogenic properties and promotes robust new bone formation in irregular bone lesions (135).

Barriers to clinical translation and future directions

While preclinical outcomes have been encouraging, the clinical translation of tissue-engineered interventions for AVN is hindered by substantial obstacles. Primary among these are stringent regulatory and manufacturing requirements; cell-derived therapeutics necessitate specialized Good Manufacturing Practice (GMP) environments, rigorous quality assurance protocols, and complex regulatory pathways (such as those dictated by the FDA or EMA). These prerequisites inevitably escalate production costs and extend development timelines. Furthermore, the clinical viability of scaffold materials is contingent upon proving long-term biocompatibility, ensuring absolute sterility, and maintaining rigorous batch-to-batch consistency during large-scale manufacturing (58).

A major limitation in current research is the discrepancy between evaluation timelines: while the majority of preclinical investigations focus on short-term outcomes (typically 8-12 weeks), human AVN management requires joint stability and function over several decades. Furthermore, the long-term safety profile of these regenerative interventions remains a critical concern. Potential risks that necessitate rigorous long-term monitoring include the biological impact of scaffold degradation byproducts, the possibility of ectopic ossification, inherent immunogenicity, and the potential for tumorigenic transformation in *ex vivo* expanded stem cell populations (136).

From a socioeconomic perspective, cell-mediated interventions entail significantly higher expenditures than conventional core decompression. At present, it remains uncertain whether the incremental therapeutic gains from these advanced therapies justify the substantial additional costs, underscoring the critical need for large-scale pharmacoeconomic evaluations and cost-effectiveness analyses (75).

The existing body of literature is predominantly composed of small-scale case series and non-controlled investigations, which limits the generalizability of current findings. To definitively establish clinical efficacy, determine optimal therapeutic dosages, and refine patient selection criteria, there is an imperative need for high-quality evidence from stage-specific, prospective randomized controlled trials. Such rigorous studies are essential to transition these regenerative strategies from experimental concepts to standardized clinical practice (58).

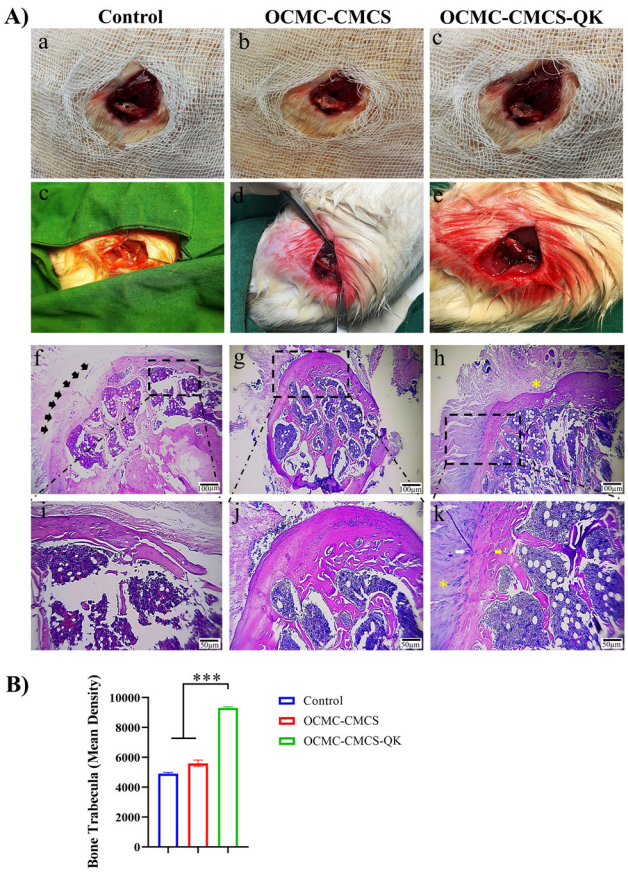

Figure 9. Oxidized carboxymethyl cellulose-carboxymethyl chitosan-QK peptide (OCMC-CMCS-QK) sustained-release hydrogel's biocompatibility in living organisms

(A) Eight weeks later, pictures of chronic osteomyelitis treated with both hydrogels and hematoxylin and eosin (H&E) staining showed that the avascular necrosis (AVN) in the femoral head had healed. Following 8 weeks of hydrogel implantation, (a, d) construct an AVN model and assess its efficacy; (b, e) prepare the AVN site for the placement and injection of free-peptide hydrogel; (c, f) prepare the AVN site for the placement and injection of hydrogel containing QK peptide; and assess its efficacy after 8 weeks. Hydrogel-QK treated the femoral head and prevented further bone loss (g, j). The model group served as a control, as seen by the H&E staining (h, k). The Experimental group (i, l) treated with CMCS-CMCS hydrogel was shown by H&E staining. OCMC-CMCS-QK hydrogel treatment was revealed by H&E staining in the experimental group. Bony trabeculae fragments with regular shapes were seen by H&E staining in hydrogel-QK. Bone marrow cells were seen by the black arrows. Osteoblasts were observed at the yellow arrows. The images are magnified by a range of 100 μm to 50 μm. (B) The overall subchondral bone of the femoral head is measured by the number of bone trabeculae. Data are presented as mean±SD from three independent tests (n=3), $^{****}P<0.0001$ (Reproduced with permission from Reference 134, Elsevier/Materials Letters, and Copyright ©2024 Elsevier B.V.)

Clinical integration: Staging, indications, and outcomes

The clinical applications of regenerative approaches in the management of AVN have become increasingly significant, particularly in the early stages of the disease when structural collapse of the bone has not yet occurred. Within the framework of Regenerative Medicine, the primary objective is to preserve the native joint by restoring biological function rather than replacing damaged tissue with prosthetic components. This shift toward biologically driven therapy is especially important for younger patients, for whom delaying or avoiding joint replacement is a major clinical priority (137).

The ARCO staging system plays an important role in guiding treatment decisions for regenerative interventions. Biologic therapies tend to be most effective in the early stages of the disease, particularly in ARCO stages I and IIA, whereas more advanced stages (IIIB–IV) are generally considered unsuitable for regenerative approaches. Ideal candidates for biologic treatment typically include patients younger than 50 years, with lesions involving less than 30% of the femoral head, no evidence of subchondral fracture on MRI, and inadequate response to conservative management (34).

Core decompression (CD) alone provides clinical success—defined as avoidance of arthroplasty in approximately 60-70% of patients with early-stage AVN. When CD is combined with stem cell augmentation, success rates increase to about 75-85%. A meta-analysis has shown that this combined approach reduces the risk of femoral head collapse by roughly 45% (OR 0.55, 95% CI 0.35-0.86, $P<0.01$). Despite these improvements, treatment failure still occurs in about 15-25% of cases within 3-5 years after regenerative therapy. Factors associated with higher failure rates include lesion size greater than 30%, lateral necrosis, continued corticosteroid use, and patient age over 45 years (20).

One of the most established clinical strategies involves combining regenerative techniques with Core Decompression. This procedure reduces intraosseous pressure and creates a more favorable environment for revascularization. When augmented with mesenchymal stem cells or bioactive agents, it not only alleviates pain but also enhances bone regeneration and improves functional outcomes. Stem cell–based therapies, in particular, have shown strong clinical potential due to their ability to differentiate into osteoblasts and secrete growth factors that promote both osteogenesis and angiogenesis. These mechanisms directly address the underlying pathophysiology of AVN, which is characterized by impaired blood supply and subsequent bone cell death (138).

In addition to stem cells, biologic adjuncts such as platelet-rich plasma contribute to the regenerative process by delivering a concentrated source of growth factors that accelerate tissue repair and vascular formation. More advanced applications, including tissue engineering, integrate scaffolds with cellular components to provide structural support while facilitating new bone formation in necrotic regions. These approaches aim to restore both the architecture and function of bone tissue before irreversible damage occurs. Another important clinical application is therapeutic angiogenesis, in which targeted stimulation of new blood vessel formation helps re-establish perfusion to ischemic bone. By improving oxygen and nutrient delivery, these interventions can halt or significantly slow disease progression. Collectively, these regenerative modalities contribute to pain reduction, functional improvement, and a decreased need for invasive surgical procedures such as total joint replacement. Overall, the integration of regenerative medicine into the clinical management of AVN represents a paradigm shift from symptomatic treatment to disease-modifying therapy, offering more durable and physiologically aligned outcomes for patients in the early stages of the condition (139).

Conclusion and future perspectives

Osteonecrosis is a bone disease where the blood supply is interrupted, leading to bone loss. Early intervention is crucial to prevent joint degradation and arthritis. Current surgery for AVN is ineffective, and increasing dead tissue

complicates treatment. Clinical treatment plans for AVN can only be developed with the help of a material system that, in the early stages, mechanically assists and promotes the recruitment, differentiation, and regeneration of bone tissue and the formation of supporting blood vessels. Incorporating osteogenic and vasculogenic growth factors into biomaterial techniques may help regenerate the femoral head and modify its mechanical properties to support healing.

Investigation into injectable technologies for use in less invasive surgeries is crucial. The restoration of femoral head integrity shows excellent therapeutic potential for joint preservation with injectable tissue-engineering therapies. However, stringent randomized controlled trials targeting the disease's stage-dependent treatment are necessary to thoroughly evaluate these novel regenerative techniques.

We might enhance available therapies by better understanding the basic pathophysiologic processes of ONFH, which involve complex cellular interactions, bioactive substances, and signaling pathways. Stem cell engineering may significantly alter this process. With advancements in materials design and a greater understanding of the mechanisms underlying ONFH, there are strong prospects for developing sophisticated scaffolds to treat ONFH.

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Authors' Contributions

N P conducted formal analysis, reviewed and edited the manuscript, and was responsible for the original draft. Z P contributed to formal analysis, review, editing, and the initial drafting. R N engaged in formal analysis, review, editing, and drafting of the original manuscript. MM K participated in formal analysis, review, editing, and original drafting. SA contributed through formal analysis, review, editing, and drafting of the initial document. SM J was involved in formal analysis, review, editing, and original draft preparation. MJ H participated in formal analysis, review, editing, and drafting. H V contributed to formal analysis and the creation of the original draft. M M engaged in formal analysis, review, editing, and drafting of the original manuscript. PB M was responsible for formal analysis, review, editing, original drafting, and supervision.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration

The authors have not used any AI tools or technologies to prepare this manuscript.

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