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General

Revolutionizing Nonunion Treatment: The Expanding Role of Local Biological Therapies

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The diagnosis and treatment of long bone nonunion remains a historical challenge in the field of orthopedics. Bone nonunion management encompasses surgical and non-surgical options. This review discusses the potential use of untraditional methods in their treatment such as orthobiologics. The cornerstone of local biological therapies lies in their ability to directly stimulate the biological processes essential for bone repair. This precision targeting ensures that bone healing pathways are activated specifically where needed, enhancing both the rate and quality of union. It particularly addresses the use of biologic agents and the possibility of their combination with biomaterials to enhance their stability, delivery, and osteoconductive properties. The aim of this review is to obtain an updated point of view regarding the management of nonunion of the bone as well as expanding on the latest innovations aiming to create a shift from passive biologic supplementation to active regenerative engineering.

INTRODUCTION

Bone nonunion remains one of the most complex challenges in orthopedic medicine, potentially resulting in devastating consequences. Fracture nonunion is the inability of the fracture to heal and unite within a minimum of six to nine months from the time of injury.[1,2] Around 5%–10% of patients with fractures in the USA suffer from problematic fracture healing and nonunion.[3] In fact, not only is nonunion difficult to treat, but it also imposes an important financial burden in terms of direct and indirect costs such as loss of productivity, impairment of function, increased need for medical aids, higher rates of early retirement and unemployment, substantial decrease in quality of life, as well as emotional and mental health problems.[1,4,5] Two requirements should be met for the successful treatment of nonunion: mechanical stability, and enhancement of the biological milieu with bone grafting.[1,6] Despite significant advances in surgical techniques and mechanical stabilization, some fractures fail to heal due to a lack of adequate biological stimuli. Local biological therapies have emerged as a powerful solution, transforming the landscape of nonunion management through regenerative and personalized approaches. In this review, we aim to shed light on the new therapies that can potentially and substantially revolutionize the treatment of nonunion of the bone.

I. TARGETED STIMULATION OF HEALING

When combined with a stable mechanical fixation, the nonunion site can be biologically optimized by local therapies and their ability to directly stimulate the biological processes essential for bone repair.

1. BONE MORPHOGENETIC PROTEINS (BMPS)

BMP, a transforming growth factor beta in the extracellular bone matrix with osteogenic properties, was first discovered by Marshall R. Urist.[7] BMP acts by transforming mesenchymal progenitor cells into osteoblasts, thus inducing bone growth. In a diabetic rat segmental defect model, treatment with recombinant human BMP-2 (rhBMP-2) significantly enhanced bone formation on histological analysis at both 3 and 6 weeks, improved radiographic healing at 6 weeks, and promoted greater angiogenesis compared to control groups.[8]

Gagnon et al, in a systematic review, reported multiple studies that show promising results of BMP as an adjunct, even alternative, therapy to autologous bone grafts in the treatment of nonunion.[9] A retrospective case-control study by Haubruck et al found that BMP-2 was significantly superior to BMP-7 when comparing each of their combinations with autologous bone grafts in the treatment of lower limb nonunion.[9]

A prospective case series by Choi et al assessing rhBMP-2 demonstrated the effectiveness and safety of a combination therapy of autologous bone grafting (ABG) with rhBMP-2 in the management of nonunion after long

bone fractures. Their results showed efficacy as well as an acceleration of bone healing, where all the patients achieved union within 3 to 6 months.[7] Calori et al compared PRP with recombinant BMP-7 (rhBMP-7), finding higher success and faster healing in the BMP-7 group.[8]

Some studies suggest that when used in high doses, BMP may be associated with certain malignancies among other complications.[8] Although basic research links BMP-2 expression with certain cancers, most clinical studies have not confirmed a relationship between BMP-2 use and cancer development.[9] BMPs are also associated with ectopic bone growth by converting various cell types into bone cells; however, recent studies indicate that this does not negatively affect the outcome in long-bone nonunion.[9] Choi et al showed that the use of rhBMP-2 in combination with ABG did not induce antibody development and is therefore well tolerated.[7]

2. PLATELET-RICH PLASMA (PRP)

Platelet-rich plasma (PRP) promotes a regenerative microenvironment by releasing growth factors that regulate the inflammatory process and cell anabolism.[10] PRP contains platelets, plasma proteins, leukocytes, and a small amount of red blood cells. The alpha (α)-granules of platelets contain growth factors—vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-β1), insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), hepatocyte growth factor (HGF), platelet-derived epidermal growth factor (PDEGF), and platelet-derived growth factor (PDGF); hemostatic factors—fibrinogen and factor V; angiogenic factors—VEGF; and metalloproteases—MMP-2, MMP-9, and cytokines.[10] The gamma (γ)-granules contain serotonin, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and calcium. These factors and molecules aid in the regeneration and healing of soft tissues and fractures.[10] Ghandi and colleagues observed that local PRP injections at fracture sites in diabetic rats led to a fourfold rise in PDGF, a threefold increase in TGF-β1 and VEGF, and a 1.5-fold elevation in IGF-I expression.[1]

Although there is extensive preclinical research supporting the role of PRP in bone healing, clinical evidence for its effectiveness in treating acute fractures and long bone delayed or nonunions remains scarce.[11] Two systematic reviews by Griffin et al and Roffi et al determined that the current limited and heterogeneous data prevent definitive conclusions regarding PRP's efficacy in bone healing.[11] In the systematic review by Jamal et al, thirteen comparative and noncomparative studies describing the role of PRP in management of delayed and nonunion mostly support PRP's effectiveness in bone healing, although with mixed results.[11] In a meta-analysis, Zhu et al revealed that PRP is effective not only in healing rate, but also in healing time of nonunions and delayed unions.[12] Impieri et al reported several studies that showed good healing rates with PRP injections, with minimal complications.[8] Yang et al reported no side effects or complications from the use of PRP therapy.[12]

Although PRP shows potential benefits in promoting bone healing, inconsistencies in PRP preparation techniques and the absence of standardized protocols likely contribute to the heterogeneity of available studies, affecting the results of its efficacy in the management of delayed and nonunions.[11,12]

3. CONCENTRATED BONE MARROW ASPIRATE (CBMA)

cBMA is a promising, FDA-compliant modality for cartilage repair, with the iliac crest providing the most favorable source of osteogenic progenitor cells.[10] Although the concentration of stem cells within cBMA is relatively low after extraction and processing, its therapeutic benefits are largely attributed to its rich reservoir of growth factors including PDGF, TGF-β, and BMP-2 and BMP-7, which contribute to its pronounced anabolic and anti-inflammatory properties.[10] These factors play crucial roles in modulating the local microenvironment, enhancing tissue repair, and reducing inflammation, thereby supporting the application of cBMA in orthopedic and cartilage repair strategies.

Hernigou et al showed that cBMA significantly improved tibial nonunion healing, with outcomes closely linked to progenitor cell concentration.[10] Similarly, Jäger et al found that combining cBMA with bovine hydroxyapatite accelerated bone regeneration compared to collagen scaffolds.[10] Schottel et al reported that the success rates of BMA for tibial nonunions range from 75% to 90%, with similar outcomes for nonunions of the femur, humerus, and ulna.[13] While studies report favorable results, no randomized controlled trials have directly compared BMA to other treatments like intramedullary nailing or autologous bone grafting.[13] Retrospective comparisons suggest BMA performs similarly to these methods, but large-scale studies are needed to definitively determine its optimal use in nonunion management.[13] Canton et al reported a 100% union rate using cBMA-augmented allografts, with an average healing time of 6.5 months and no complications.[8]

Although cBMA is largely safe, potential risks include vascular and nerve injuries during iliac crest aspiration, chronic harvest site pain, hemorrhage (especially with anticoagulant use), infection, and fat embolism.[10,13]

II. ENHANCED EFFICACY WITH FEWER SYSTEMIC EFFECTS

The application of orthobiologics in orthopedic interventions offers a significant advantage over systemic therapies by permitting the delivery of bioactive agents directly to the site of injury, thereby enhancing therapeutic effect while concurrently decreasing the risk of systemic toxicity.

The delivery system of various biological agents to the injury site has been a critical aspect of these types of therapies. At the moment, a variety of methods exist depending on the agent to be delivered. Direct injection for PRP and stem cells is the current preferred method for concentrated application, while scaffold systems are currently being explored for sustained release of certain active bioagents enhancing the regenerative process.[14] The minimally invasive nature of delivery has created an attractive option for en-

hancing bone healing, augmenting soft tissue repair, and allowing cartilage regeneration and preservation.

This has become particularly interesting for special populations such as athletes, high-risk individuals, elderly, and pediatric patients. The use of orthobiologics in the athletic population has recently gained support with research being led by top academic institutions worldwide, particularly with the use of PRP in ulnar collateral ligament injuries, hamstring injuries, patellar tendon injuries, and plantar fasciitis. Multiple studies have shown higher injury prevention, decreased healing time, and faster return to sport, showcasing the efficacy of these agents and their safe use.[15] The National Basketball Association has even gone one step further by releasing a consensus statement that orthobiologics may be used by team physicians on a case-by-case basis.[16] Orthobiologics are specifically important in high-risk individuals and the elderly who may suffer from multiple comorbidities. Systemic drugs such as nonsteroidal anti-inflammatory medication may be contraindicated in patients with cardiovascular or renal dysfunction, allowing orthobiologics to play a key role in treatment.[17] A meta-analysis by Xiong et al showed significant improvement in functional outcome of patients suffering from knee osteoarthritis and degenerative temporomandibular joint disorders with the use of PRP.[14] A systematic review performed by Everts et al focused on chronic wounds and diabetic ulcers. They found that PRP injection compared to no PRP therapy increased the proportion of complete wound closure, decreased the time to complete wound closure, and reduced wound area and depth. No adverse effects attributed to PRP could be identified, further enhancing the efficacy and safety profile of this agent.[18] Navani et al performed a randomized controlled trial (RCT) to assess the safety and effectiveness of PRP or bone marrow concentrate (BMC) intradiscal injection compared to control groups in discogenic chronic low back pain. Both PRP and BMC were found to be statistically significant in decreasing pain and increasing functionality, with no adverse effects identified.[19] The pediatric population remains an area of growing interest with a few case studies reported in literature. This is mainly due to the unknown incidence of adverse effects from these agents. There is a lack of large RCT studies available, making it difficult to draw conclusions regarding safety and efficacy.[15]

While current literature and research seem encouraging, large RCTs are required to truly reach definitive guidelines regarding the use of many of these agents such as BMPs, cBMA, and PRP. One of the main drawbacks in this rapidly advancing field remains: a multitude of questions are still to be answered regarding optimal dosage, delivery methods, and treatment combinations, in addition to determining the right agent for the right condition as well as timing and frequency.[14] As some of these orthobiologics become more popular, such as BMPs, DBM, and PRP, it is important to note that literature has pointed out certain cases of postoperative inflammation, ectopic bone formation, bone resorption, and a possible role in tumorigenesis.[9]

I. INTEGRATION WITH ADVANCED BIOMATERIALS

Biologic agents are often combined with biomaterials to enhance their stability, delivery, and osteoconductive properties.

1. CALCIUM BASED PHOPHATES

Calcium phosphate (CaP) materials are key components of bone matrixes. They vary in bioactivity based on their chemical species and osteogenic signaling.[20] Common forms like hydroxyapatite (HAP), tricalcium phosphate (TCP), and amorphous calcium phosphate (ACP) support bone regeneration by influencing osteoblast function and local microenvironment conditions.[21] The size of CaP particles influences biological responses, enhancing osteoblast proliferation, activity, and survival compared to microscale particles. CaP materials have long been used in bone tissue engineering due to their strong osteoconductive and osteoinductive properties. Despite their clinical success, the exact molecular mechanisms by which CaPs promote bone regeneration remain unclear.[22,23]

2. COLLAGEN MATRICES

Collagen is one of the main proteins in soft tissues and bone; it is the most abundant protein in the human body.[24] Collagen-based matrices offer an excellent scaffold for cells to regenerate soft tissue defects in a well-organized manner. It mimics the extracellular matrix and has low immunogenicity. Multiple methods are used to harvest acellularized dermal matrices. They can be harvested from human donors, animals (porcine), and plants (seaweed). There is also the possibility to generate it from recombinant bacteria or yeast.[25,26]

3. SYNTHETIC POLYMERS

Biodegradable polymers, both natural and synthetic, have been extensively explored for scaffold fabrication in tissue engineering, especially for bone substitution.[27] While natural polymers offer advantages like degradability and low toxicity, they lack the control of customizable degradation rates provided by polylactic-co-glycolic acid (PLGA)-based polymers, which can be controlled by adjusting the levels of glycolic to lactic acid.[28] PLGA scaffolds are now being studied for multiple uses:

1. Double-layer PLGA scaffolds + bone marrow-derived mesenchymal stem cells (BM-MSCs): Enhanced osteogenesis and simultaneous osteochondral repair in a rabbit model.[29]
2. Dai et al developed PLGA scaffolds with directional pores, improving cell migration, uniform cell distribution, and stronger bone-cartilage regeneration.[30]
3. Liu et al used 3D printing to optimize scaffold structure; scaffolds printed at a 0°/90° angle showed superior mechanical strength and supported better osteoblast adhesion, proliferation, and differentiation.[31]

III. GROWING EVIDENCE AND TAILORED THERAPY

It is important to highlight that with the rise of supporting data and personalized approaches, treatment choices are now more frequently influenced by both clinical evidence and detailed evaluations of nonunion features. Thus, the different types of nonunion should be recalled. Historically, the classification of aseptic nonunions based on radiologic and biological features was first introduced by Judet and Judet in 1958, followed by further refinement in the 1970s by Weber and Cech. The Judet brothers categorized nonunions into hypertrophic (or hypervascular) and atrophic (or avascular) types. Building on this, Weber and Cech expanded the classification to include hypertrophic, oligotrophic, and atrophic forms, noting that while hypertrophic and oligotrophic nonunions exhibit vascularity, atrophic types lack it. Despite the potential overlap in radiological presentation complicating diagnosis, the Weber-Cech classification remains the cornerstone for therapeutic decision-making.[32,33] Current treatment strategies typically focus on enhancing mechanical stability in hypertrophic nonunion, while atrophic nonunion often requires fibrous tissue debridement, reosteosynthesis, and biological enhancement through grafting and growth factor application.[32] Therefore, nonunions characterized by poor biological activity, such as atrophic types, show marked improvement with osteoinductive treatments like bone morphogenetic proteins (BMPs) and concentrated bone marrow aspirate (cBMA). On the other hand, hypertrophic nonunions, which typically have adequate biological potential, tend to heal more effectively when mechanical stability is enhanced.[34]

The established optimal approach for treating atrophic nonunion involves revising the fixation and incorporating osteoconductive elements, such as scaffolds and a supportive mechanical setting, along with osteoinductive strategies like bone grafts, bone morphogenetic proteins, and enhanced vascularization.[32]

Numerous studies have indicated that bone healing tends to be slower in individuals who have risk factors. Conditions such as smoking, diabetes, poor nutrition, and infections are recognized for their negative impact on the healing process and may require adjustments in the choice or dosage of biological treatments.[35] For example, the amount of bone morphogenetic proteins (BMPs), especially BMP-2 and BMP-7, used to treat nonunion fractures depends on individual patient risk factors. These factors, impacting bone healing, may require modifications to the BMP dosage to improve results and reduce potential complications.[36]

Animal research has demonstrated that nicotine negatively impacts bone healing. Although human studies did not show a strong link between smoking and the number of osteogenic precursor cells, nicotine has been found to increase osteoclast activity, which may contribute to impaired bone regeneration. Additionally, nicotine can reduce microvascular blood flow and tissue oxygenation, potentially leading to platelet clumping and the formation of microthrombi.[37] Moreover, individuals with diabetes exhibit a clear reduction in collagen production within the bone callus, along with a significant decline in the number of cells essential for tissue repair. To note that those undergoing insulin treatments and maintaining good glycemic control face a reduced risk.[1]

Concerning the nutritional status, deficiencies in certain amino acids may disrupt normal bone regeneration. However, the primary effect of malnutrition on bone healing is largely due to vitamin D deficiency and the development of osteoporosis. Calcitriol, the active form of vitamin D, plays a crucial role in regulating bone calcium levels. Additionally, both vitamin D and calcium are considered vital for ongoing bone remodeling and the repair of fractured bones.[38] In a study conducted by Haubruck et al. (2018), they evaluated the factors affecting the success of non-union treatment. They found that risk factors negatively influencing the outcome in the total collective were identified as active smoking, atrophic biology, higher BMI, and larger defect size.[38]

Added to that, progress in molecular profiling could enable clinicians to customize biologic treatments according to each patient's unique genetic or cellular characteristics, paving the way for personalized regenerative orthopedics.[39] Orthopedic treatments have traditionally followed generalized approaches. However, with the rise of personalized orthopedic care, treatment plans based on each patient's unique genetic makeup, lifestyle, and biomarker profile can now be customized. A key advantage of using advanced biomarkers is the capability to monitor treatment effectiveness in real time. Frequent biomarker assessments can reveal how well a patient is responding to therapies for chronic conditions such as osteoporosis or osteoarthritis. If certain markers suggest that the treatment isn't producing the expected results, adjustments can be made promptly.[39]

For example, the use of microRNAs (miRNAs), a type of small non-coding RNA that controls gene expression after transcription by promoting the formation of gene-silencing complexes, in clinical diagnostics holds significant potential. In a study conducted by Dai et al. (2021), a systematic analysis of miRNA expression patterns in infected long bone non-unions to improve diagnostic accuracy and understanding of disease mechanisms was done. Through microarray analysis, researchers discovered 20 miRNAs with altered expression and confirmed six key miRNAs that play important roles in bone formation, immune regulation, and inflammation; an essential process in fracture repair and the development of infections.[40]

IV. ADJUNCT TO SURGICAL INNOVATION

The application of local biologics as an adjunct to surgical innovation is particularly impactful in the management of nonunion, where biological deficits often underlie the failure of fracture healing. Since nonunion sites are often characterized by poor vascularity, chronic inflammation, and a lack of osteogenic and osteoinductive signaling, a particularly innovative approach involves fixation-integrated delivery systems—implants coated or loaded with biologics,

such as BMP-2-eluting nails or locking compression plates (LCPs) with platelet-rich plasma (PRP) reservoirs, enabling simultaneous stabilization and biological stimulation. While research on the exact combination of LCPs with PRP reservoirs is still in the early stages, there is growing evidence supporting the use of PRP to enhance bone healing in conjunction with mechanical stabilization. Though specific studies combining LCPs and PRP reservoirs are still limited, there is substantial research on the individual components and their potential synergistic effects.[41]

In a preclinical study, Singh et al. conducted a study on 19 adult dogs treated for a fracture in the diaphyseal region of three long bones. The results of studies on Guided Tissue Regeneration (GTR) with β-Tricalcium Phosphate (β-TCP) and PRP for fracture repair using internal fixation such as LCPs indicate that this approach significantly enhances bone healing compared to traditional methods.[42] In similar context, Schmidmaier et al. demonstrated that titanium implants coated with bone morphogenetic protein-2 (BMP-2) significantly improved fracture healing in rats. The BMP-2 coating enhanced biomechanical strength and accelerated bone remodeling, as confirmed by histological and mechanical analysis. Upon implantation, the coating degrades over time, releasing BMP-2 directly to the fracture site. This localized delivery system ensures a high concentration of BMP-2 at the healing site, potentially reducing systemic side effects associated with other delivery methods.[43]

While promising, the clinical application of BMP-2-eluting nails requires careful consideration. A study published by Springer et al. showed a higher incidence of local infections in patients receiving BMP-2 compared to controls, leading to the discontinuation of the study before reaching the planned total of 300 patients. He concluded that, unlike with unreamed nails, the healing of open tibial fractures treated with reamed nailing was not significantly accelerated by the local addition of BMP-2, and there was a trend for a higher unclear incidence of infections.[44]

On the other hand, with the rise of minimally invasive surgical (MIS) techniques, there is an increasing reliance on biologic agents to enhance the healing environment, especially in cases where traditional bone grafting or open reduction may be less feasible. As a matter of fact, traditional orthopedic surgeries can involve significant trauma to the tissues, which can increase the time required for healing and lead to complications such as infection or poor wound healing. Calori et al. suggested that percutaneous injections of PRP or cBMA can be beneficial by improving the conditions for tissue regeneration and reducing the need for extensive surgical intervention. Moreover, by minimizing the disruption of the periosteum with the percutaneous technique, the study suggests that the blood supply to the bone is preserved.[45] One of the major challenges in orthopedic recovery is balancing the need for tissue healing with the need for early mobilization in order to prevent stiffness, muscle atrophy, and other complications from immobilization. By using PRP or cBMA, Calori et al. found that healing times were shortened, allowing for earlier mobilization of the patient, improving joint function, strength, and overall recovery.[45]

The first application of injectable bone marrow for nonunion fractures appeared in the 1980s.[46,47] Connolly et al. successfully treated eight out of ten tibial nonunion by using relatively large amounts of bone marrow (100–150 ml).[48] Healey et al. also evaluated the feasibility of injection of autogeneic bone marrow into the nonunion site in eight patients suffering from nonunion after reconstruction for primary sarcoma. Five of the eight patients received chemotherapy. The authors reported union in five cases (three out of five of whom received chemotherapy).[49]

A definite relationship was apparent between the number of osteoprogenitors injected and the rate of union. Hernigou et al. compared the number of mesenchymal stem cells injected in 53 cases of successful union to seven cases where this technique failed. The united cases received 54,962 ± 17,431 MSCs, while the unsuccessful ones received 19,324 ± 6,843 MSCs. In total, 336 cases of nonunions have been described. The mean age was 38.1 years. The union rate after treatment with percutaneous injection of autologous bone marrow was 88.8%. The mean time to union was 4.8 months.[49]

Ewais et al. highlighted that percutaneous bone marrow injection is a simple, safe, and cost-effective method for treating delayed and non-united fractures. In their study, autologous bone marrow was injected into 21 patients with nonunion of long bone fractures. They achieved successful union in 18 out of 21 cases, with a median healing time of 5.76 months. Bone marrow injection was as effective as open autogenous grafting but with considerably fewer complications, thus providing a reliable source of osteogenic stem cells with numerous advantages compared with standard open grafting.[50]

Garg et al. grafted autogenous bone marrow percutaneously in 20 ununited long bone fractures, all of which were immobilized in a cast. They reported 17 united fractures in 5 months' duration.[51] Similarly, Sim et al. studied the use of autogenous bone marrow injection for the treatment of delayed and nonunion of long bones. There were 10 patients with 11 fractures, and nine fractures were united. The median time to clinical union was 10 weeks, and that for radiological union was 17 weeks (9–29 weeks).[52]

While promising, further research is needed to fully understand the long-term effectiveness and optimal conditions for these treatments.

V. RESEARCH INTO NEXT-GENERATION THERAPIES

While current local biologic therapies have significantly advanced the management of bone nonunion, next-generation strategies are working their way to further revolutionize the topic. These emerging approaches to therapy have focused on actively engineering the regenerative environment, moving beyond passively supplementing the targeted bony nonunion to directly enhance the mechanisms underlying bone repair biologically.[53]

1. Stem Cell Therapies:
   Mesenchymal stem cells (MSCs), derived from bone marrow, adipose tissue, or umbilical cord sources, have demonstrated potent osteogenic and paracrine abilities, modulating the host's immune system, specifically T cells.[54] These cells can differentiate into osteoblasts and secrete bioactive factors that modulate inflammatory processes, stimulate angiogenesis, and promote bone regeneration and repair. Early clinical investigations, such as the study by Quarto et al. (2011), have shown promising outcomes in patients with critical-sized bone defects treated with expanded autologous MSCs combined with scaffolds.[55]
   The therapeutic potential of MSCs for bone healing is well-established, with various studies demonstrating their ability to regenerate bone in critical defects. For instance, MSCs isolated from human bone marrow have been shown to regenerate normal bone in immunocompromised rat models.[56] Additionally, bone marrow-derived stem cells (BMSCs) have been extensively used in nonunion treatment, with Connolly et al. (1999) reporting a high success rate of bone healing in patients with ununited tibial fractures following autologous marrow injections, with 90% of their patients achieving satisfactory results.[48]
   Hu et al. (2015) reported that MSCs embedded into the porous biphasic calcium phosphate ceramics coated with nano-HA or nano-hydroxyapatite could serve as a firmly established bone substitute for reconstructing bone defects.[57] More recent techniques of fabrication allow for more control over "pore size, porosity, scaffold shape, ease of fabrication, and reliability of physico-mechanical properties," and these include "salt leaching, sponge replica and gas foaming, porogen-based [techniques], and 3D printing."[58]
   The advantages of MSC-based therapies include their multilineage differentiation potential, including chondrocytes, osteocytes, and osteoblasts, and their ability to enhance both the structural and biological aspects of healing, including formation and remodeling.[59] However, limitations remain, including regulatory obstacles, variability in cell quality depending on the donor, and challenges related to cell expansion, viability due to opposing immunities, and delivery.[58] Furthermore, despite their proven efficacy, these therapies still face technical and safety challenges in clinical applications.[60]
2. Exosome-Based Therapies:
   Exosomes, or nano-sized extracellular vesicles secreted by MSCs and other progenitor cells, represent a promising new avenue for cell-free therapy. These vesicles, which are part of a broader category of extracellular vesicles (EVs), carry microRNAs, proteins, and growth factors that play a crucial role in cell communication, angiogenesis, and osteogenesis.[60]
   Derived from various bodily sources, including urine, saliva, synovial fluid, breast milk, and semen, MSC-derived exosomes have demonstrated the potential to enhance bone regeneration by modulating inflammation and promoting vascularized bone tissue formation.[61] Furthermore, Swanson et al. (2020) created a biodegradable scaffold that releases osteogenic exosomes from human dental pulp stem cells (hDPSCs), promoting bone regeneration. This system successfully accelerated bone healing in a mouse calvarial defect model over 8 weeks, without requiring the transplantation of exogenous cells.[62]
   Recent studies have also shown that exosomes can enhance osteogenesis and promote bone healing. For example, BMSC-derived exosomes were found to regulate osteoblast expression by miR-196a, thus improving bone regeneration in rat models and further promoting angiogenesis.[63] Moreover, exosomes from adipose stem cells have been shown to promote osteoblast proliferation and differentiation, as well as to stimulate angiogenesis.[60]
   The advantages of exosome-based therapies include reduced immunogenicity, lower tumorigenic risk compared to cell therapies, and improved storage and handling characteristics. Bone marrow-derived stem cells exosomes have also proven to be diagnostic as well as prognostic biomarkers, effective molecules for delivering drugs, and gene therapy carriers, providing a new insight into the ability to heal tendons and bones.[64] However, challenges persist, including difficulties in standardizing exosome isolation and purification, variability in the content of exosomes, and their relatively short half-life in vivo.[60]
3. Gene Editing and CRISPR:
   Advances in gene editing technologies, particularly CRISPR-Cas9, offer the potential for precise genetic modification to enhance bone healing. CRISPR or Clustered Regularly Interspaced Short Palindromic Repeats and their associated Cas or CRISPR-associated proteins form an adaptive immune system found in many bacteria and archaea. This system allows microorganisms to recognize and defend against invading genetic elements, including bacteriophages, by incorporating short sequences from the invaders into their own genome. These stored sequences then guide Cas proteins to identify and cut foreign DNA during future infections, providing a form of memory to defend themselves.[65]
   Investigative strategies include upregulating osteogenic factors such as bone morphogenetic proteins (BMPs), enhancing angiogenic signaling, or silencing inhibitors of bone regeneration.[66,67] This approach promises a significant leap forward in bone healing, through its ability to directly manipulate the genetic background to optimize regeneration, remodeling, and stimulate the healing processes.
   Gene editing techniques, particularly using the CRISPR-Cas9 system, are also being explored to enhance osteogenesis. For example, MSCs derived from various sources, such as human induced pluripotent stem cells (iPSCs), have been successfully engineered to improve bone healing and osteogenesis. These

cells, when modified using CRISPR technology, can promote the differentiation of iPSCs into osteoblast-like cells and improve bone regeneration in critical-sized defects.[60]

Effective and safe delivery of CRISPR/Cas9 components is critical for achieving successful gene editing outcomes. Current delivery methods include viral vectors, such as adeno-associated viruses (AAV), and non-viral strategies like electroporation, lipid nanoparticles, and exosome-based systems.[53] These methods aim to overcome the technical obstacles associated with gene delivery while ensuring high efficiency and minimal adverse effects.

The advantages of gene editing include targeted, durable modifications that could significantly improve healing outcomes in delayed nonunion. However, significant challenges remain, including ethical concerns across a wide variety of cultures, the risk of off-target effects, and technical barriers related to safe and efficient delivery systems.

CONCLUSION

The evolution of local biological therapies marks a pivotal transformation in nonunion management. By delivering targeted, biologically active agents directly to the fracture site, these therapies stimulate the body's intrinsic healing mechanisms while minimizing systemic risks. Integration with biomaterials and surgical technologies has further enhanced their efficacy. As personalized medicine and bioengineering continue to advance, local biological therapies are poised to become the cornerstone of regenerative orthopedics, turning previously intractable nonunions into opportunities for successful, patient-specific healing.

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