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***REVIEW ARTICLE***

# Regenerative Medicine Approaches for the Treatment of Pediatric Physeal Injuries

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The physis, or growth plate, is a cartilaginous region at the end of children's long bones that serves as the primary center for longitudinal growth and characterizes the immature skeleton. Musculoskeletal injury, including fracture, infection, malignancy, or iatrogenic damage, has risk of physeal damage. Physeal injuries account for 30% of pediatric fractures and may result in impaired bone growth. Once damaged, cartilage tissue within the physis is often replaced by unwanted bony tissue, forming a ''bony bar'' that can lead to complications such as complete growth arrest, angular or rotational deformities, and altered joint mechanics. Children with a bony bar occupying <50% of the physis usually undergo bony bar resection and insertion of an interpositional material, such as a fat graft, to prevent recurrence and allow the surrounding uninjured physeal tissue to restore longitudinal bone growth. Clinical success for this procedure is <35% and often the bony bar and associated growth impairments return. Children who are not candidates for bony bar resection due to a physeal bar occupying >50% of their physis undergo corrective osteotomy or bone lengthening procedures. These approaches are complex and have variable success rates. As such, there is a critical need for regenerative approaches to not only prevent initial bony bar formation but also regenerate healthy physeal cartilage following injury. This review describes physeal anatomy, mechanisms of physeal injury, and current treatment options with associated limitations. Furthermore, we provide an overview of the current research using cell-based therapies, growth factors, and biomaterials in the different animal models of injury along with strategic directions for modulating intrinsic injury pathways to inhibit bony bar formation and/or promote physeal tissue formation. Pediatric physeal injuries constitute a unique niche within regenerative medicine for which there is a critical need for research to decrease child morbidity related to this injurious process.

**Keywords:** physis, growth plate, stem cells, biomaterials, bony bar, bone growth

## Introduction

Injuries incurred by skeletally immature patients are unique both in their causes and gravity of their consequences. Physes, or growth plates, are cartilaginous regions at the ends of children's long bones that function as primary sites of bone elongation. Physeal injury may result from trauma, infection, metabolic abnormalities, or malignancy.

The major concern with physeal injury is that damaged cartilage within the physis can be replaced by bony repair tissue, forming a ''bony bar'' or ''physeal bar''. Depending on the size and location of the injury within the physis, the bony bar may cause asymmetric growth arrest with subsequent angular deformity or complete cessation of longitudinal growth. The latter is a devastating outcome for children that have not yet reached their full height. Current treatment involves surgical resection of the bar and replacement with an interpositional material to preserve normal growth in the remaining physis. Bar reformation and additional growth effects, however, remain major complications of bar excision.

A critical need exists for developing effective treatments for children with physeal injuries, which not only prevent

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bony bar formation but also regenerate physiologic physeal cartilage and restore normal bone elongation. This review describes physeal anatomy, mechanisms of physeal injury, and current surgical therapies to treat complications resulting from physeal injuries. Furthermore, it discusses ongoing research efforts for physeal injury repair, including stem cell- and biomaterial-based tissue engineering strategies, as well as potential new avenues for physeal cartilage regeneration.

## Anatomy, Physiology, and Injury of the Physis

The physis is a complex cartilaginous structure composed of peaks and valleys that lies between the epiphysis and metaphysis at both proximal and distal ends of long bones (Fig. 1). Longitudinal growth occurs in the physis through endochondral ossification, beginning *in utero* and continuing until the end of puberty.[1] Chondrocyte proliferation then slows until the entire physis has undergone ossification, defined as skeletal maturity.[2]

Chondrocytes exist within three distinct zones in the physis—the resting zone, the proliferating zone, and the hypertrophic zone (Fig. 1). Closest to the epiphysis, resting zone chondrocytes are hyaline cartilage cells, believed to be the progenitor cell population for the growth plate. Proliferative zone chondrocytes undergo rapid mitosis, forming vertical stacks of chondrocytes which form the basis for longitudinal growth.[3] Hypertrophic zone chondrocytes exit the cell cycle, swell in size, and overproduce glycogen to increase extracellular matrix (ECM) volume. The hypertrophic state has long been thought to be the endpoint of chondrocyte differentiation.[4]

Following hypertrophy, chondrocytes undergo apoptosis leaving a network of calcified matrix for osteoblasts to invade and begin forming bone. Recent lineage-tracing experiments provide evidence that transdifferentiation of hypertrophic chondrocytes to osteoblasts also occurs in addition to apoptosis.[5–7] The ECM is then mineralized to form mature bone, a process called ossification, which contributes to longitudinal expansion of the pediatric skeleton.

In addition to unique zonal cellular morphology, the composition of the ECM and mechanical properties change across the physis. For example, the resting zone is predominately made of horizontally aligned collagen II fibers and a low cell:ECM ratio, the proliferative zone has vertically aligned collagen II fibers and a moderate cell:ECM ratio, and the hypertrophic zone is composed predominately of collagen X and a high cell:ECM ratio. These structural properties, as well as others, lead to varying mechanical properties across the physis and have been reviewed elsewhere.[8] Briefly, the resting zone is stiffer and more impermeable than the other zones,[9,10] and mechanical properties are further influenced by loading, zonal height, and age.[11]

While both physeal and articular cartilages are variants of hyaline cartilage, they differ in structure and function. These

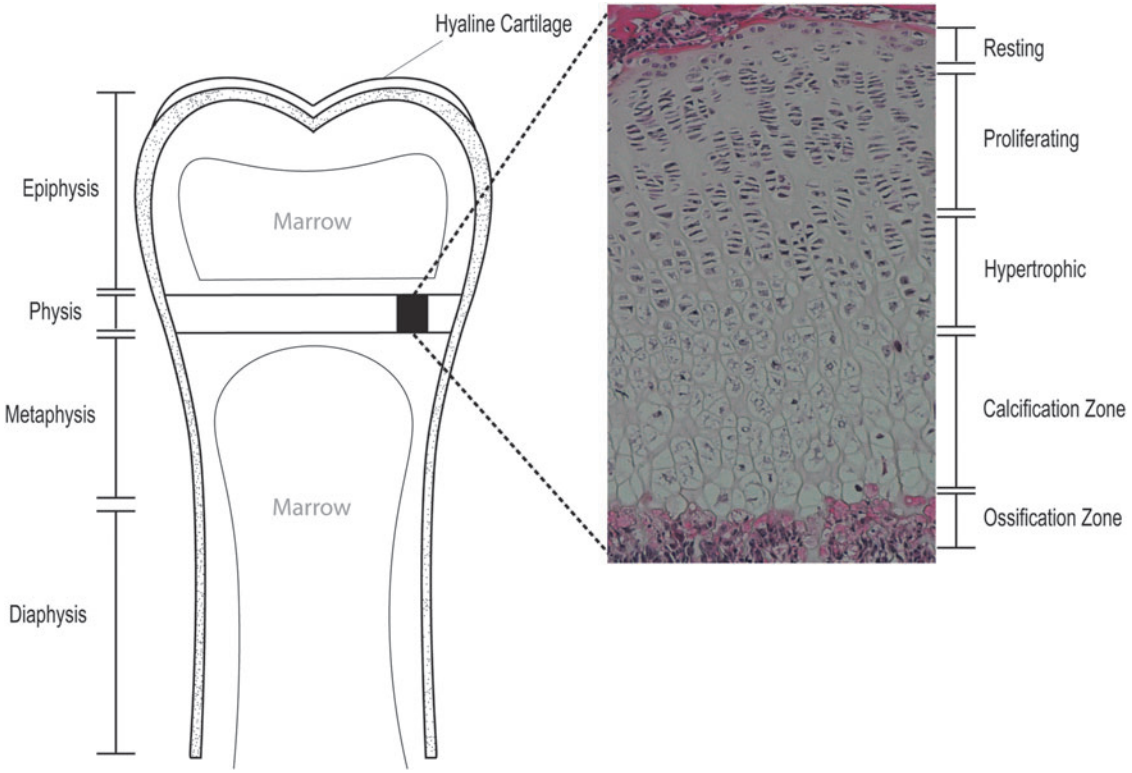


**FIG. 1.** Anatomic location of the physis (or growth plate) in long bones. The physis is a cartilaginous region located between the epiphysis and the metaphysis at each end of long bones. Marrow compartments serve as a source of nutrition with vessels feeding the growth plate cartilage. The juxtaposition of hard cortical bone against relatively softer cartilage forms a weak point in the pediatric skeleton. (Expanded section): Five cartilaginous zones of the physis. Resting zone chondrocytes give way into rapidly proliferating chondrocytes that form vertical stacks in the proliferating zone. Cells in the hypertrophic zone enlarge by producing glycogen. In the calcification zone, chondrocytes undergo apoptosis and the extracellular matrix calcifies forming a network for osteoblasts to invade and form new bone in the ossification zone.

differences are due, in part, to their differing developmental origins: physeal cartilage arises from limb mesenchyme condensations, while articular cartilage comes from the interzone, a thin mesenchyme lining on the ends of future limbs.[12] Articular cartilage also has unique zonal organization similar in nature to the physis but with noticeable differences; for example, superficial zone chondrocytes are flattened and produce lubricin while deep zone chondrocytes form columns with vertically aligned collagen fibers that withstand compressive loading. The important difference between these two cartilages is function: the physis is a transient tissue that undergoes endochondral ossification to elongate long bones, while articular cartilage is a permanent tissue designed to protect joint surfaces. Articular cartilage does not calcify, except under pathological conditions.

The physis is vulnerable to injury in that the juxtaposition of relatively soft cartilage against hard bone serves as a weak point in the pediatric skeleton. Complications of physeal damage can range from inconsequential to the generation of a bony bar. The latter occurs when layers of physeal chondrocytes are damaged such that bony repair tissue forms and connects metaphyseal to epiphyseal bone. Lateral or medial physeal bony bar formation may result in asymmetric growth arrest, generating angular limb deformities.[13] In severe cases, the bony bar results in complete growth arrest. Classically, injuries resulting in bony bar formation must undergo surgical correction to remove the bony bar and minimize further effects on the limb's growth potential.

### Physeal Fractures and the Salter–Harris Classification System

Fractures are one of the most common pediatric traumas, occurring in one in two males and one in three-to-four females.[14,15] Of those, between 18% and 30% will involve the physis.[16,17] The Salter–Harris (SH) Classification System classifies physeal fractures into five distinct patterns (types I-V) of physeal involvement (Fig. 2A).[18] Fracture prognosis and predicting bony bar formation are somewhat dependent on this classification. Compression type fractures (SH type V) are the most likely fracture pattern to result in growth arrest. In the upper extremity, these are followed by fractures that cross the epiphyseal plate (SH types III, IV),

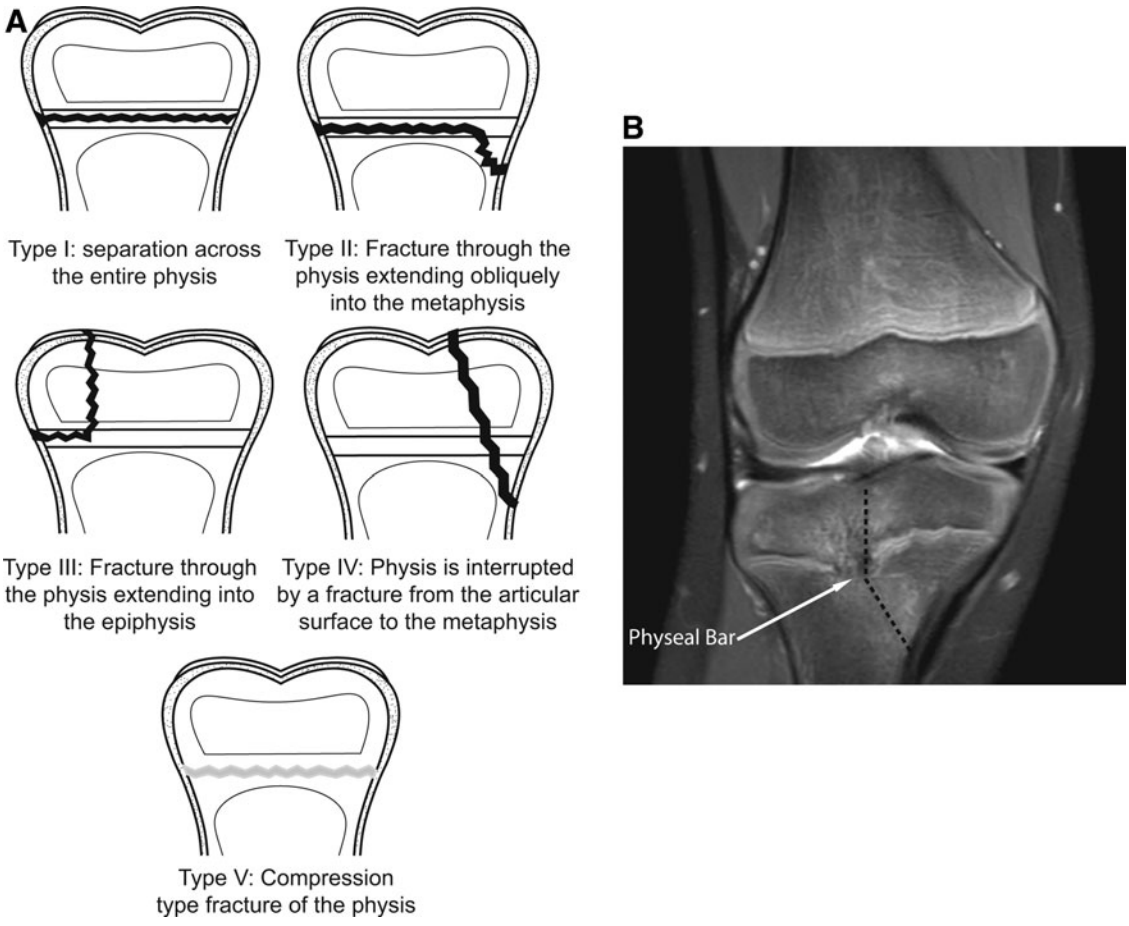


**FIG. 2.** The Salter–Harris classification of growth plate fractures. **(A)** Approximately 5% of physeal fractures are Type I injuries and least likely to cause growth arrest. Type II injuries are the most common, 75% of physeal fractures, and have a moderate potential for arrest. Type III and Type IV each occur in ~10% of fractures and are more likely to lead to growth arrest. Type V injuries are the least common, but the most likely to cause bony bar formation or growth arrest. **(B)** Anteroposterior MRI of a 9-year-old female who suffered a SHIV fracture (*dashed line*) of her right proximal tibial physis 10-months prior. The bony bar (*arrow*) begins central and extends lateral in location, causing significant misalignment of the knee with leg shortening.

which are more likely to demonstrate physeal bar formation (Fig. 2B).[19] In the lower extremity, specifically distal femoral physeal fractures, SH types II, III, and IV are the next most common to result in growth disturbances, especially when the fracture is displaced.[20]

The three most common fracture sites resulting in physeal injury are the wrist, the ankle, and the distal femur. Wrist fractures, specifically distal radius fractures, are one of the most common pediatric fracture types, resulting from high-energy sports trauma.[21] Physeal arrest in distal radial fractures have an estimated incidence of 1–7%.[22] Complications of physeal arrest in the distal radius include limb length discrepancy between the injured and uninjured arms, angular deformities of the radius leading to compromised biomechanics, and the potential to develop subsequent wrist arthritis.

Lower extremity physeal injuries occur primarily at the distal femoral physis (knee) or the distal tibial physis (ankle).[23] Distal femoral fractures for the preadolescent child can be particularly devastating, as growth disturbance occurs in 52–90% of these injuries.[20,24] The distal femoral physis accounts for up to 70% of longitudinal growth of the femur and up to 40% of total longitudinal growth of the lower extremity. Consequences of these injuries are dramatic, including significant leg length discrepancy and angular deformities leading to significant gait disturbances, low back pain, cosmetic deformity, and early-onset arthritis. Complete or partial premature closure of the distal tibial physis is the most common complication of SH types III and IV distal tibial fractures. Resulting growth discrepancies are less significant than those seen in distal femur fractures; however, rotational disturbances and altered ankle joint mechanics can also have long-term consequences for the individual.[25,26]

### Rare Etiologies of Physeal Damage

Although less common than fracture, the physis may be damaged by infection, malignancy, or by iatrogenic damage, an unintended surgical complication. Infectious etiologies of physeal injury include hematogenous osteomyelitis of metaphyseal bone with extension into the growth plate. As blood flows through the narrow capillaries within the marrow compartment of long bones, low fluid flow rates can promote bacterial stasis and facilitate infection.[27] In severe cases, resulting chondronecrosis and abscess formation resulting from infection lead to bony bar formation and its complications as described above.[28]

Pediatric bone tumors, including osteosarcoma and Ewing's sarcoma, may also lead to physeal damage.[29] Damage may be secondary to the degree of tumor physeal involvement or from tumor resection surgery.[30–32] Radiation therapy and chemotherapy regimens also may potentially cause physeal damage by interfering with normal chondrocyte physiology.[33–37]

During the treatment of pediatric musculoskeletal conditions, unintended physeal damage may occur. Premature physeal closure has occurred secondary to limb lengthening procedures.[38,39] In addition, anterior cruciate ligament reconstruction may result in unintended physeal damage to either the distal femoral or proximal tibial physis, potentially leading to growth arrest.[40,41] To prevent this, surgical techniques to avoid transphyseal instrumentation have been developed to preserve the physis.[42–45]

### Current Therapeutic Techniques Following Physeal Arrest

Growth arrest, angular or rotational deformities, and subsequent altered joint mechanics are feared consequences of physeal injury in the immature skeleton and may develop up to 2 years postinjury. As such, patients with physeal injuries are followed for a longer duration than other musculoskeletal injuries. Depending on injury characteristics, physicians may choose nonoperative therapy, including casting, to ensure anatomic alignment of the limb with close radiologic follow-up for observation of bony bar formation.[46] Severe injuries often require surgical intervention.

When bony bar formation occurs in patients with significant potential growth disturbance, the current gold standard therapy is bony bar resection.[47–49] Typically, patients are younger and have a significant (50–70%) portion of healthy uninvolved physis.[50] Following bony bar resection, the injury site is filled with an interpositional material such as fat, muscle, or silicone rubber to prevent reformation of bony tissue and allow the uninjured physeal cartilage to restore normal growth. Unfortunately, clinical success for resection ranges from 18% to 35%.[51] Avascular fat grafts do not integrate into host tissue. Rather, they break down over time and fail to provide structural stability, leading to collapse of the injured growth plate area and either physeal closure or bony bar recurrence. Other graft materials, such as silicone rubber, are not ideal biomaterials because they do not incorporate within host tissues and may migrate from the surgical site causing subsequent problems. Current interpositional materials offer imperfect solutions and ultimately the bony bar may return and affect growth.[52]

If pronounced angular limb deformities following bony bar formation exist, corrective osteotomy to the affected limb may be performed to improve limb length and joint biomechanics.[53] Osteotomy involves creating a wedge-shaped bone defect, then opening the wedge, lengthening, and correcting the angular deformity.[54] Complications include infection, neurovascular injury, additional physeal damage, or recurrence of the angular deformity.[55,56]

In severe cases, ipsilateral epiphysiodesis, artificial closure of the physis, may be performed following bony bar formation to prevent further limb angulation. Generally, this is performed in cases with minimal residual growth potential, or cases where the bony bar occupies more than 50% of the physeal volume.[57] Closing the injured physis limits the degree of subsequent angular deformity that can occur. In cases where several centimeters of limb growth is anticipated, but the bony bar occupies more than 50% of physeal volume, bilateral epiphysiodesis is performed, tethering both physes to minimize limb length discrepancy.[58] Complications include unpredictable growth arrest leading to continued limb length discrepancies or worsened angular deformities.[59,60]

Successful restoration of growth following bony bar formation is limited with current therapeutic options. Existing interpositional materials are insufficient in restoring longitudinal growth in that they do not integrate into host tissues and they rely on the uninjured physeal cartilage to preserve growth. Surgical techniques are often limited by the extent of physeal injury. Reformation of the bony bar following

resection occurs in up to 15–38% of cases, leading to additional growth disturbance or additional surgeries.[51] The unpredictable nature of surgery coupled with imperfect graft materials results in high rates of bar reformation speaking to the critical need for novel, regenerative treatment methods.

### Regenerative Approaches to Treat Physeal Injury

The morbidity and unpredictable nature of physeal injuries coupled with current therapeutic limitations establish a critical need to develop effective treatments for affected children. Successful treatments should prevent bony bar formation and simultaneously regenerate native physeal cartilage, restoring normal bone elongation. Regenerative approaches utilizing stem cells, growth factors, and biomaterials have the potential to overcome the shortcomings of current approaches by restoring physeal cartilage and, thus, may play an important role in the treatment of physeal injuries. An overview of the current animal models of physeal injury and research using cell-based therapies, growth factors, and biomaterials in the different animal models of injury along with strategic directions for modulating intrinsic injury pathways is presented below.

#### *Animal models of physeal injury*

To investigate the different regenerative medicine approaches, animal models of physeal injury have been developed where injury to the physis results in bony repair tissue, mimicking the bony bar formation seen in pediatric patients. In addition to bony bar formation, it has also been shown that tethers can form in the surrounding uninjured physis after injury and are another mechanism of growth dysfunction that should be evaluated.[61,62]

In small animal models, such as mice and rats, therapeutics can be tested immediately after injury to determine whether they prevent bony bar formation and restore bone elongation. Thus, they are a good initial model to test novel therapeutics. However, due to their small size, it is difficult to resect the bony bar that forms and implant a therapeutic material, as would be performed clinically.

Larger animal models such as the rabbit, miniature pig, or sheep have been used for these types of interventions.[63–69] In the larger models, an injury to the physis is created and the bony bar allowed to form. A second intervention is then performed to remove the bony bar and implant a therapeutic material. The desired outcomes are prevention of bony bar reformation, prevention of angular deformities, and restoration of longitudinal growth.

In addition to providing a means to test novel therapeutic strategies, animal models of physeal injury also offer the opportunity to study mechanisms of bony bar formation and identify potential targets for modulation.[61,70–74] The rat model of physeal injury has been widely used to investigate pathophysiology.[61,62,70,71,74–80] A drill-hole defect in the proximal tibial physis creates a bony bar in a predictable and reproducible manner (Fig. 3A). This well-established model has contributed to identifying four phases of injury repair: inflammatory, fibrogenic, osteogenic, and remodeling (Fig. 3B).[70,71,74–81]

During the 3 days following injury, inflammatory cells infiltrate the injured area.[75,78] From days 4 to 7, mesenchymal stem cells (MSCs) migrate from surrounding marrow compartments and go on to express osteogenic markers such as Runx2, alkaline phosphatase, and osteocalcin (Ocn).[74,75,78] Evidence of angiogenesis and formation of mineralized tissue occurs between days 8 and 14.[74] Bony remodeling occurs after 14 days, producing a bony bar by 28 days.[74] Within each

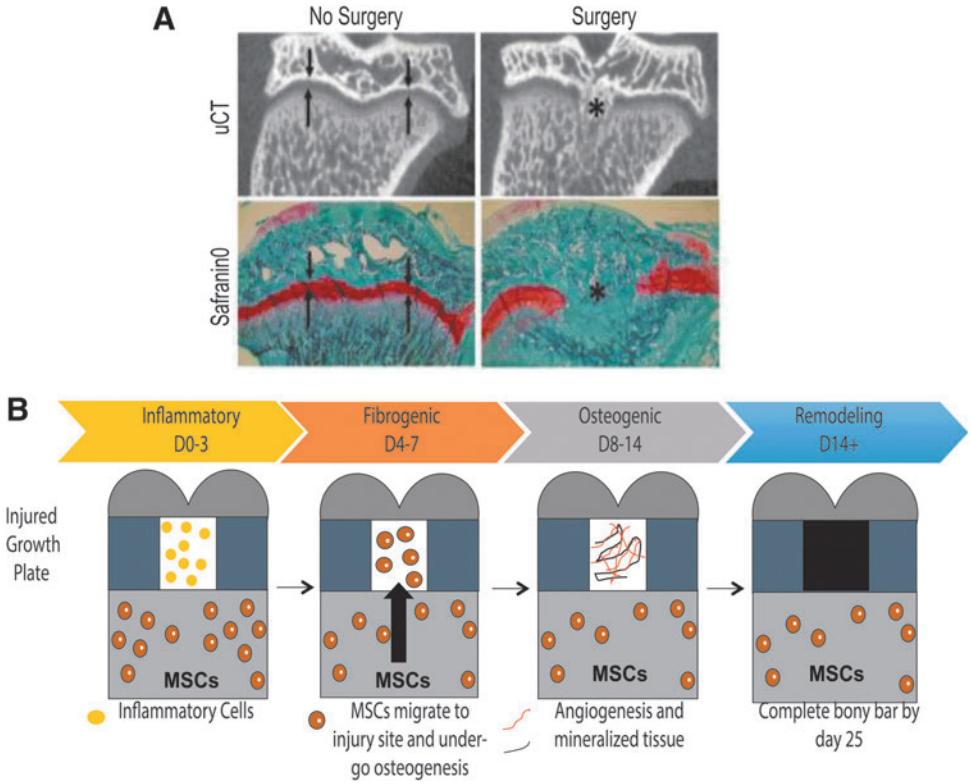


**FIG. 3.** **(A)** μCT and SafraninO/Fast Green images of rat physes 28 days after surgery. Both μCT and SafraninO staining for cartilage tissue (*red*) show an intact growth plate in the No Surgery rat, while the rat that underwent surgery displays bony tissue within the growth plate. *Arrows* show growth plate area. *Shows area of bony bar. **(B)** Schematic of repair phases after physeal injury in the rat model. MSCs, mesenchymal stem cells.

phase, specific cell signaling pathways play central roles, offering opportunities to inhibit osteogenesis or promote chondrogenesis. This injury model also suggests that endogenous MSCs play a central role in bony bar formation and are a potential target for physeal injury therapeutics.

### *Cellular based therapies*

In early studies, implantation of articular cartilage or peripheral physeal cartilage as an interpositional material in a sheep model of growth plate injury inhibited bony bar formation.[82] Despite correction of growth deformities, the transplanted cartilage demonstrated variable levels of apoptosis, and normal physeal cartilage was not regenerated.

To optimize integration of implanted chondrocytes into the injured physis, other groups have isolated and embedded chondrocytes within scaffolds. Rabbits treated with physeal implants composed of cultured chondrocytes embedded within agarose gels exhibited no growth arrest or angular deformity compared to untreated animals.[83] After 2–4 weeks, implants formed columnar and prehypertrophic chondrocytes similar to native physeal cartilage.[83] In pig models, cartilage-like discs generated from autologous articular chondrocytes prevented bony bar formation and growth arrest.[84] Cultured epiphyseal cartilaginous disc implants containing epiphyseal chondrocytes also prevented bony bar formation in a sheep model.[85] On further investigation, these discs integrated into host growth plate cartilage, forming columnar and prehypertrophic zones mimicking native physeal cartilage.[86]

These studies suggest that chondrocytes could be used to promote native-like cartilaginous repair tissue. However, using autologous chondrocytes clinically may be limited by the need to isolate cells from healthy pediatric tissues, thus creating secondary injury sites. This has led to the investigation of alternative cell sources such as MSCs.

MSCs are an attractive cell source for tissue engineering due to their availability, immune privilege, and multipotent differentiation capacity, especially toward the bone and cartilage lineages. MSCs originating from various tissue sources have been investigated for physeal injury treatment. MSCs derived from periosteum, bone marrow (BM-MSC), and adipose tissue (AT-MSC) were compared for their ability to treat partial growth arrest in a rabbit model.[87] Periosteal and BM-MSC implants corrected angular deformities and growth arrest, while AT-MSCs did not. Furthermore, periosteal MSCs and BM-MSCs yielded native-like repair tissue with columnar chondrocyte arrangement and a prehypertrophic zone, while AT-MSCs resulted in irregular arrangement, suggesting that cell source may affect repair potential.[87]

Treatment of physeal injuries with MSCs has been successful in rabbit models,[64,65,87–92] as well as in pigs.[66] In addition, treatment of porcine physeal injuries with a co-MSC/chondrocyte graft yielded favorable repair tissue and prevented growth deformities.[67] However, treatment of physeal injuries in a sheep model with BM-MSCs yielded dense, fibrous repair tissue.[69] One possible explanation for this discrepancy is the chondrogenic predifferentiation of MSCs that occurred before implantation in the rabbit and porcine studies, but did not occur in the ovine study. A recent study further complicates these findings by reporting that treatment of rat physeal injuries with unstimulated BM-MSCs corrected growth arrest, while treatment with chondrogenically predifferentiated BM-MSCs did not.[62] However, treatment with either cell type decreased tether formation in the adjacent, uninjured growth plate, which could reduce growth disturbance. Overall, these studies suggest that while MSCs are a potential cell source for the treatment of physeal injuries, further work is necessary to identify the optimal MSC source and differentiation state.

In addition to the implantation of exogenous MSCs for the treatment of physeal injuries, endogenous stem cells can also participate in tissue repair. As demonstrated in Figure 3, MSCs from adjacent marrow compartments migrate into the injured growth plate, undergo osteogenesis, and form bony repair tissue.[74] However, MSCs are also capable of differentiating into a wide variety of connective tissue cells, including cartilage, and may be used to repair growth plate cartilage given the appropriate cues.[93–96]

MSCs express multiple chemokine receptors, including CXCR1, CXCR2, CCR2, and CXCR4,[97–99] and can home to these chemokines, as well as stromal cell-derived factor 1 (SDF-1), interleukin-8, platelet derived growth factors, and transforming growth factor beta (TGF-β) isoforms.[97,100–103] SDF-1 has been shown to recruit MSCs from the marrow and improve articular cartilage regeneration following injury, suggesting that SDF-1 may be a potential candidate for treatment of growth plate injuries.[104–106]

A strategic approach for physeal tissue engineering could include developing composite interpositional biomaterials that provide chemokine factors to recruit endogenous MSCs from nearby marrow compartments. Furthermore, they could provide factors that promote cartilage differentiation, encouraging endogenous MSCs to form cartilage rather than bone, leading to restoration of longitudinal growth and prevention of subsequent complications.

### *Enhancing chondrogenic potential with growth factors*

Whether the cellular based approach for physeal cartilage regeneration relies on exogenous cells or endogenous cells, the cells will need to undergo chondrogenic differentiation to form cartilage tissue successfully. Three of the most extensively studied chondrogenic factors for MSCs and other progenitor cells are insulin like growth factor-1 (IGF-1), TGF-β1 and TGF-β3. Two separate studies demonstrated that treatment of physeal injuries with IGF-I encapsulated in poly(lactic-co-glycolic) acid (PLGA) scaffolds promoted cartilage regeneration and decreased bony repair tissue compared to empty scaffolds.[63,107] Bone morphogenetic proteins (BMPs) also play a role in chondrogenesis. Differentiation of human BM-MSCs into proliferative zonal cartilage cells has been demonstrated using sequential exposure of MSCs to TGF-β3 followed by BMP-2.[108] In a sheep model, treatment of physeal injuries with locally delivered BMP-7 resulted in an overall increase in growth plate height.[109,110] These studies demonstrate the efficacy of growth factor induced reconstruction of growth plate cartilage when delivered in an appropriate manner.

### *Biomaterial-based approaches*

For cells and chondrogenic molecules to have an effect at the site of physeal injury, they must be delivered locally by a material that can serve as a temporary scaffold while new tissue forms. Materials used in the treatment of physeal

injuries have included collagen I and II,[86] fibrin glue,[87] hyaluronate-collagen-fibrin composites,[65,92,93] collagen-chitin scaffolds,[66,67,111] agarose,[61,88] chitin,[64] gelatin,[69,90] and PLGA.[63,107] The outcomes of the studies using these materials are outlined in Table 1.

In all cases, treatment with MSC- or chondrocyte-laden biomaterials produced better results than biomaterials alone. Biomaterial constructs alone often delayed or prevented bony bar formation but ultimately resulted in fibrous repair tissue and mild growth abnormalities. This suggests that a variety of biomaterials may be used to introduce cells, but that cells are ultimately key to restoring physeal cartilage. Thus, it is important to use biomaterials that can effectively deliver biofactors and/or provide an environment that encourages cells to migrate into the material and that can perhaps also direct these cells toward the chondrogenic lineage and/or maintain their chondrogenic phenotype. Longer term studies are also necessary to fully evaluate the effect of biomaterials on skeletal growth.

Physiochemical cues from the microenvironment, such as cell-ECM interactions, cell–cell interactions, and dynamic mechanical forces, influence stem cell differentiation and tissue-synthesizing capabilities.[112–117] Recent technology has allowed scientists to use biomaterials as building blocks to incorporate tissue mimetics such as ECM molecules and enzyme-sensitive cross-links for degradation. This can create a cartilage biomimetic environment to promote chondrogenic differentiation of stem cells coming into contact with the hydrogel and has led to promising *in vivo* results for cartilage repair.[118–121]

In addition to chemical cues, intrinsic biomaterial stiffness provides mechanical cues, further directing stem cell differentiation. This is especially important when the mechanical properties of the biomaterial direct cells away from the osteogenic lineage and toward the chondrogenic lineage.[122] Such cartilage-biomimetic systems warrant further investigation as potential novel interpositional materials that could lead to improved physeal tissue engineering.

A common problem with current materials used clinically, such as fat grafts, is that they do not provide sufficient mechanical support to prevent collapse of the injury site. A scaffolding construct with a load-bearing structural component could minimize force differentials observed within the surrounding physeal cartilage. Recently, groups have investigated the use of three-dimensional (3D) printing to create scaffolding structures, as well as cell-laden hydrogels for use within scaffolds.[123–125]

Biomaterial constructs that offer a cartilage promoting environment through the presentation of physiochemical cues and a load-bearing structural component may be the future of interpositional materials used after bony bar resection as they would allow for improved physeal repair and restoration of longitudinal growth. These constructs may be ideal for children that are candidates for bony bar resection, as it may be sufficient to form a cartilage-like tissue that would prevent bony bar reformation and allow the uninjured physeal tissue to continue bone elongation. However, for children that are not candidates for resection because they have more than half their physis injured, and therefore insufficient uninjured physeal tissue to continue growth, the treatment strategy would need to be more robust. In these cases, it may be necessary to recapitulate the complex zonal microarchitecture and cellular organization of the physis to restore growth.

Three-dimensional printing technology offers the opportunity to design multiple layers and heterogenous structures within a biomaterial construct to mimic the different zones of the physis with increased accuracy, which would be difficult to achieve with conventional fabrication methods.[126] Moreover, cells can be incorporated within the different layers and/or structures. Thus, combining cells, biomaterials, and 3D printing may ultimately be necessary to develop successful physeal tissue engineering approaches that can benefit all patients suffering from physeal injuries.

### *Modulating intrinsic injury pathways to prevent osteogenesis*

Although promoting cartilage repair tissue after physeal injury is of utmost importance, preventing osteogenesis and bony bar formation is also a research avenue that should be pursued. Small animal models, including the rat model of physeal injury, have elucidated critical targets for modulation in physeal injury pathophysiology. In rat growth plate injuries, vascular endothelial growth factor (VEGF) and its receptors are detected during the first 28 days postinjury.[80] Systemic delivery of bevacizumab, a humanized anti-VEGF antibody, demonstrated a reduction in osteogenic gene expression, fewer blood vessels, and decreased bony bar formation within the injury site.[71] Systemic delivery also led to a reduction in bone growth in the contralateral limb, suggesting adverse effects of systemic delivery.[71] Thus, VEGF inhibition warrants further investigation in preventing bony bar formation after physeal injury, specifically, localized antibody delivery to the injury may reduce adverse events. It is important to release the antibody during the repair process to prevent bony bar formation. However, since angiogenesis is necessary for normal bone elongation, the antibody should not remain in the area long term.[127] Local, controlled, and timed delivery of antiangiogenic factors can be achieved with drug delivery systems and warrant further investigation in the treatment of growth plate injuries.

In addition to VEGF, other molecular pathways have been implicated in bony bar formation, including those related to Wnt/β-catenin signaling and BMP signaling.[72] During late inflammatory and early fibrogenic phases, the Wnt/β-catenin pathway is a key regulator of the osteogenic differentiation of endogenous MSCs at the injury site.[72,128] Inhibiting the Wnt/β-catenin pathway after physeal injury in rats by an orally administered inhibitor led to decreased expression of Wnt target genes, decreased osteogenesis and bony bar formation, and also led to increased cartilage tissue in the repair area.[128] This suggests that the Wnt/β-catenin signaling pathway is another potential target for preventing bone formation after physeal injury, and this can also be achieved through localized biomaterial-based drug delivery systems.

Modulating pathways involved in ossification could also be achieved using short interfering RNA (siRNA), where key genes responsible for osteogenesis can be silenced, potentially inhibiting bony bar formation. Localized and sustained siRNA delivery has been shown to be possible through biomaterial-based delivery systems.[129–131] siRNA molecules have been successfully encapsulated within degradable

Table 1. Brief Overview of Biomaterials Used to Treat Growth Plate Injuries

| *Reference* | *Model* | *Cell type* | *Biomaterial* | *Cell loading* | *Growth factor* | *Implantation method* | *Histology and overall results* |
|---|---|---|---|---|---|---|---|
| Foster *et al.*[86] | Sheep, prox. tib. | Lamb growth plate chondrocytes | — | — | — | Implanted, moldable | Growth plate-like repair tissue. No BB, AD, GA |
| | | | Collagen 1 | Seeded | — | Implanted, moldable | BB formation. Immune reaction |
| | | | Collagen 2 | Seeded | — | Implanted, moldable | Fibrous and collagenous repair tissue. Prevented BB, but immune reaction |
| | | Untreated | — | — | — | — | BB formation. |
| Hui *et al.*[87] | Rabbit, prox. tib. | BM-MSCs | Fibrin | Mixed w cells | — | Injected | Growth plate-like repair tissue. No AD |
| | | Peri-MSCs | Fibrin | Mixed w cells | — | Injected | Growth plate-like repair tissue. No AD |
| | | AT-MSCs | Fibrin | Mixed w cells | — | Injected | Hyaline-like cartilage. Significant AD |
| | | — | Fibrin | — | — | Injected | BB formation. Significant AD. |
| Gál *et al.*[91], Plánka *et al.*[92] | Rabbit, prox. tib. | BM-MSCs | Hyaluronin/ collagen/ fibrin | Seeded | — | Implanted, rigid | Hyaline-like cartilage. Prevented AD, GA |
| | | — | Untreated | — | — | — | BB formation. Significant AD |
| Planka *et al.*[66]; Planka *et al.*[67] | Pig, distal femur | BM-MSCs | Col1/chitosan | Seeded | — | Implanted, rigid | Hyaline-like cartilage. Prevented AD, GA |
| | | — | Col1/chitosan | — | — | Implanted, rigid | Fibrous repair tissue. Mild AD, GA |
| | | — | Untreated | — | — | — | BB formation. AD and GA |
| Chen *et al.*[88] | Rabbit, prox. tib. | Peri-MSCs | Agarose | Seeded | — | Implanted, moldable | Growth plate-like repair tissue. No BB, AD, GA |
| | | — | Agarose | — | — | Implanted, moldable | BB formation. AD and GA |
| Li *et al.*[64] | Rabbit, prox. tib. | Peri-MSCs | Chitin fiber | Seeded | — | Implanted, rigid | Growth plate-like repair tissue. No BB, AD, GA |
| | | — | Chitin fiber | — | — | Implanted, rigid | Fibrous and collagenous repair tissue. Prevented AD and GA |
| McCarty *et al.*[69] | Sheep, prox. tib. | BM-MSCs | Gelfoam® | Seeded | TGFβ1 | Implanted, rigid | Dense fibrous repair tissue. Prevented progression of BB |
| | | — | Gelfoam | — | TGFβ1 | Implanted, rigid | Dense fibrous repair tissue. Prevented progression of BB |
| Ahn *et al.*[90] | Rabbit, prox. tib. | BM-MSCs | Gelatin 5% | Seeded | — | Implanted, rigid | BB formation. Reduced AD |
| | | BM-MSCs | Gelatin 10% | Seeded | — | Implanted, rigid | BB formation. More reduced AD |
| | | BM-MSCs | Gelfoam | Seeded and cultured 1wk | TGFβ3 | Implanted, rigid | Growth plate-like repair tissue. No AD |
| | | — | Gelfoam | — | — | Implanted, rigid | BB formation. Significant AD |
| Sundararaj *et al.*[107] | Rabbit, prox. tib. | — | PLGA | — | IGF-1 | Implanted, rigid | Hyaline-like cartilage |
| | | — | PLGA | — | — | Implanted, rigid | Delayed BB formation |
| | | — | Untreated | — | — | — | BB formation |
| Clark *et al.*[63] | Rabbit, prox. tib. | — | PLGA | — | — | Implanted, rigid | BB formation. GA and AD |
| | | — | PLGA | — | IGF-1 | Implanted, rigid | Increased chondrogenic repair tissue. Mild AD |
| | | BM-MSCs | PLGA | Seeded | IGF-1 | Implanted, rigid | Increased chondrogenic repair tissue. Mild AD |

AD, angular deformity; AT-MSCs, adipose tissue mesenchymal stem cells; BB, bone bridge; BM-MSCs, bone marrow mesenchymal stem cells; Col1, collagen type I; GA, growth arrest; IGF-1, insulin-like growth factor 1; Peri-MSCs, periosteal mesenchymal stem cells; PLGA, poly(lactic-co-glycolic acid); prox. tib, proximal tibia; TGFβ1, transforming growth factor beta 1.

hydrogels demonstrating sustained release of siRNA molecules. Furthermore, successful integration of siRNA molecules into nanoparticle delivery systems and biodegradable solid polymers show promise for localized gene silencing.[131,132] Localized, targeted, gene silencing through siRNA molecules may have a role in modulating intrinsic growth plate pathogenesis and warrant further investigation for correcting and preventing bony bar formation.

MicroRNA (miR) has recently become another target that can be modulated to affect tissue repair. Inhibiting miR-222 expression *in vitro* improved osteogenic differentiation of human MSCs as demonstrated by increased expression of *Runx2*, *COL1A1*, and *BGLAP*.[133] These results translated into improved fracture healing in a rat fracture model when miR-222 inhibitor was administered.[133] Since upregulation or inhibition of various miRNA expression patterns has the potential to promote or inhibit osteogenic and chondrogenic differentiation of MSCs,[134–136] localized and controlled delivery of miRNA modulators to physeal injury sites may prove important to prohibiting the initial formation of a bony bar and promote physeal cartilage regeneration. Successes in identifying and manipulating key genes in fracture healing using miRNA offer insight into the possibilities of modulating pathways involved in physeal pathogenesis. These successes warrant additional investigation in elucidating and subsequently modulating patterns of miRNA expression in physeal injury.

## Conclusion

Physeal injury remains a significant cause of morbidity among the pediatric population. The most significant complication of physeal injury is bony bar formation, either leading to angular limb deformities or complete growth arrest. Current management, surgical or otherwise, has significant limitations and may result in further morbidity in the form of additional surgeries, further development of growth arrest, or progression of angular limb deformities. As such, there is a critical need to develop new treatment strategies for physeal injury that not only prevent bony bar formation but also lead to regeneration of healthy growth plate cartilage, thus restoring normal bone elongation.

Methods under investigation include modulating intrinsic injury pathways to prevent osteogenesis, as well as recruiting or adding stem cells for regenerating damaged physeal cartilage. Additional methods include modulating cellular microenvironments of the injury site and creating interpositional materials with the structural support necessary to prevent collapse of the resection site. Development of an interpositional material with structural support has immediate translational potential as current materials used after bony bar resection, such as fat grafts, lead to poor outcomes. Further developing these interpositional materials using biomaterials that promote cartilage tissue will also be of great benefit to treat children undergoing bony bar resection.

In severe cases, where children are currently not candidates for bony bar resection due to a large physeal injury, it will likely be necessary to engineer a construct that mimics the complex zonal structural and cellular organization of the physis to ensure bone elongation. This will be more challenging, as proper signals will need to be available to the cells to progress from a resting state chondrocyte to a mineralizing cell. It may also be necessary to incorporate exogenous cells, which will increase the regulatory oversight needed to reach clinical trials. However, advances in cell-instructive biomaterials, delivery of cell signaling molecules, and 3D printing technology will allow progress to be made in the development of complex structures, ultimately allowing their clinical translation. Together, these will have important implications in preventing significant morbidity in the pediatric population affected by physeal injuries.

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No competing financial interests exist.

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