Published in final edited form as:

N Engl J Med. 2015 October 29; 373(18): 1776–1778. doi:10.1056/NEJMcibr1510090.

Fibrinolysis as a Target to Enhance Fracture Healing

Regis J. O’Keefe, M.D., Ph.D.

Department of Orthopedic Surgery, Washington University School of Medicine, St. Louis

The skeleton is among the most commonly injured body tissues. Every year more than 16 million fractures of long bones occur in the United States. Fortunately, bone has excellent regenerative potential, and most fractures heal efficiently over a period of 6 to 12 weeks, with restoration of skeletal integrity and locomotion. However, the rate of delayed or incomplete healing of common fractures, such as fracture of the femoral shaft, ranges from 0 to 15%.¹ Failure to unite the separated fragments of a fracture into a single, stable segment of bone results in pain, weakness, and reduced mobility. These complications are more common in elderly patients, in whom the failure of a fracture to completely heal often results in serious compromise of overall health status.

Although fibrosis and scar formation are components of normal regeneration in most tissues, the healing of bone requires de novo regeneration of tissue. Bone regeneration during the healing of fractures occurs in a manner that is analogous to the process that occurs during embryonic development and involves two interrelated mechanisms — intramembranous ossification and endochondral ossification (Fig. 1).²

In intramembranous ossification, mesenchymal progenitor cells in the environs of the injury differentiate into osteoblasts, which then directly form bone. In endochondral ossification, mesenchymal progenitor cells instead differentiate into chondrocytes, initially forming a cartilage intermediate that is replaced as bone is regenerated. In this process, the chondrocytes must undergo a maturation process that involves the formation of a calcified cartilaginous template that serves as a scaffold for primary bone formation. Terminally differentiated chondrocytes undergo apoptosis and secrete factors (e.g., vascular endothelial growth factor [VEGF]) that stimulate the formation of vascular channels that invade the calcified cartilage. Primary bone is formed when pericytes — mesenchymal progenitor cells associated with the vasculature — differentiate into osteoblasts, which subsequently form bone on the calcified cartilage template and eventually replace the cartilage tissue.

Intramembranous and endochondral ossification occur simultaneously and are temporally and spatially organized within the fracture callus. Intramembranous ossification occurs early along the bone surface in the peripheral regions proximal and distal to the injury site. In contrast, endochondral ossification occurs centrally, overlying the fracture site.² Fractures heal when new bone crosses the fracture gap and unites the previously separated bone fragments into a continuous segment of bone. Given the importance of skeletal integrity to vertebrate survival, this initial healing process occurs rapidly, and the bone that is initially

formed is disorganized. Over time, the fracture is remodeled as the disorganized bone matrix is replaced with a more organized, biomechanically superior bone matrix. On completion of healing, the shape and form of the new bone is similar to that of the original bone.

Fracture healing depends on the recruitment, proliferation, accumulation, and subsequent differentiation of mesenchymal progenitor cells at the site of the fracture.³ The disruption of any component in the complex series of exquisitely regulated cellular, molecular, and tissue-related events can lead to impaired fracture healing. In a series of genetic experiments involving mice, Yuasa et al. recently found that fibrinolysis is a required step in the normal healing of a femur fracture.⁴ Like other injuries, fracture results in hemorrhage and the initiation of the clotting cascade, followed by the deposition of a fibrin matrix. A long-held hypothesis has suggested that the fibrin clot, or "fracture hematoma," stimulates the local inflammatory response and is necessary for the recruitment of mesenchymal progenitor cells and the initiation of fracture healing. The results reported by Yuasa et al. do not support this hypothesis. In their study, normal fracture healing occurred in mice incapable of making fibrinogen.

Fibrin is degraded by plasminogen. To determine whether the catabolism of fibrin is necessary for normal fracture repair, Yuasa et al. generated mice that do not produce plasminogen. In wild-type mice, fibrin was catabolized and completely absent in calcified cartilage at the initiation of vascular invasion, primary bone formation, and tissue remodeling. However, in the mice without plasminogen, residual fibrin remained within the cartilage matrix. In these mice, the callus size was normal and hypertrophic chondrocytes secreted VEGF, but the investigators did not observe any vascular invasion into the calcified cartilage.⁴ Vessels were abundant in the intramembranous bone component of the fracture, but no vessels extended past the junction between the intramembranous bone callus and the calcified cartilage callus (Fig. 2). In the absence of vascular growth, the primary formation of bone on the surface of the calcified cartilage template and subsequent remodeling were impaired. Thus, whereas in wild-type mice the fracture site had healed and was remodeled within 42 days, in the mice lacking plasminogen, a large cartilage callus and a nonunited fracture site remained. To determine whether these outcomes were caused by events other than the impairment of fibrin catabolism (plasmin has biologic effects that are independent from its catabolism of fibrin), the authors knocked out the expression of fibrin in the plasminogen-deficient mice. When these mice were then treated with antifibrinolytic small interfering RNA, they had reduced levels of fibrin in the cartilage matrix and improved fracture healing⁴ (Fig. 2).

Whereas genetic experiments clearly show that the formation of a fibrin clot at the site of a fracture is not necessary for normal healing, Yuasa et al. have established that the removal of fibrin is essential to the growth of vascular tissue and its penetration of the cartilage callus. Fibrin-containing cartilage matrix fails to undergo primary bone formation and remodeling and impairs fracture healing.

Because alterations in fibrinolysis and fracture healing are both associated with advanced age, various environmental exposures, and many chronic diseases, these findings should stimulate further studies.⁵ However, important questions must be answered before these

insights can be translated into therapies. Is residual fibrin a component of fracture-callus tissue that has failed to heal? Is fibrinolysis in a fracture callus compromised by cigarette smoking or by obesity, diabetes, and other chronic inflammatory disease states? In patients who have such diseases, could interventions that enhance fibrinolysis promote fracture healing? The answers to these questions could lead to new therapies that will improve bone regeneration in patients at risk for compromised fracture healing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

I thank Georgia Stobbs-Cucchi for her help with an earlier version of the figures.

References

1. Taitsman LA, Lynch JR, Agel J, Barei DP, Nork SE. Risk factors for femoral nonunion after femoral shaft fracture. *J Trauma*. 2009; 67:1389–92. [PubMed: 19704386]
2. Grimes R, Jepsen KJ, Fitch JL, Einhorn TA, Gerstenfeld LC. The transcriptome of fracture healing defines mechanisms of coordination of skeletal and vascular development during endochondral bone formation. *J Bone Miner Res*. 2011; 26:2597–609. [PubMed: 21826735]
3. Worthley DL, Churchill M, Compton JT, et al. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. *Cell*. 2015; 160:269–84. [PubMed: 25594183]
4. Yuasa M, Mignemi NA, Nyman JS, et al. Fibrinolysis is essential for fracture repair and prevention of heterotopic ossification. *J Clin Invest*. 2015; 125:3117–31. [PubMed: 26214526]
5. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. *Cardiovasc Ther*. 2010; 28(5):e72–e91. [PubMed: 20626406]

Figure 1. Intramembranous and Endochondral Ossification during Fracture Healing

Along the surface of the bone at sites flanking the fracture, periosteal mesenchymal progenitor cells differentiate into osteoblasts and directly form bone. In the central region of the fracture, mesenchymal progenitor cells accumulate and initially differentiate into chondrocytes. As the population of chondrocytes matures, vascular endothelial growth factor (VEGF) and other angiogenic factors are expressed, and the matrix is calcified and invaded by vascular channels. Vessel-derived mesenchymal progenitor cells subsequently differentiate into osteoblasts and form bone on the cartilage template. Functional healing

occurs when calcified callus tissue bridges the fracture site and unites the bone fragments. The healing process is completed when osteoclast-mediated remodeling occurs, replacing the immature bone, and bone morphology returns to a normal state.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Impaired Fracture Healing in Plasminogen-Deficient Mice

In plasminogen-deficient mice, fibrin remains in the cartilage callus (Panel A). Although vascular channels extend into the intramembranous bone, reaching the junction of the calcified cartilage callus, the vessels fail to invade the tissue of the callus. The resulting absence of primary bone formation on the cartilage callus template impairs subsequent healing. In contrast, in plasminogen-deficient mice whose capacity to express fibrin has been knocked out, reduced levels of fibrin are found in the cartilage callus. Vascular channels are able to invade the callus, primary bone forms on the cartilage template, and fracture healing proceeds to completion.