

# Physiological Challenges of Bone Repair

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**Summary:** Bone healing after fracture occurs in a well-organized manner and involves a multitude of cell types, inflammatory cytokines, growth factors, prostaglandins, and certain vitamins. Some of the means by which alterations in these essential components affect bone repair are understood, whereas others still need to be delineated. Based on clinical experience and basic science research, certain clinical conditions have become associated with delays in bone repair after fracture. These conditions include chronic inflammation, diabetes, hypovitaminosis, aging, and polytrauma. This brief report reviews some of the ways by which these conditions have been shown to negatively influence bone repair.

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## INTRODUCTION

Bone healing after fracture occurs in a well-organized manner. Bone repair occurs in 1 of the 2 ways as a direct result of the local mechanical environment. Bone repair can occur through direct bone healing (primary bone healing or intramembranous bone healing) or indirect bone healing (secondary bone healing or endochondral bone healing) or a combination of both. Regardless of the means by which bone repair occurs, the process is influenced by a multitude of factors, some understood and others still to be delineated. Bone repair begins after hematoma develops as a result of the fracture and injury to

the surrounding soft tissues. Hematoma formation is generally followed by an inflammatory response. It is during this phase that macrophages and degranulating platelets infiltrate the fracture site and release inflammatory cytokines, including platelet-derived growth factors (PDGF), transforming growth factor  $\beta$  (TGF- $\beta$ ), interleukin 1 and 6 (IL-1 and IL-6), prostaglandin E<sub>2</sub>, and tumor necrosis factor  $\alpha$ . In this early phase, periosteal pre-osteoblasts and local osteoblasts begin to form new bone. Mesenchymal cells and fibroblasts proliferate and are associated with the expression of fibroblast growth factors. Mesenchymal and osteoprogenitor cells are associated with the expression of bone morphogenetic proteins (BMPs) and the TGF- $\beta$  family of proteins. As the hematoma matures, a collagenous matrix develops in which new blood vessels can be found. These new blood vessels provide a conduit for progenitor cells and growth factors for mesenchymal cell differentiation. These cells ultimately stimulate the development of cartilage, which is later converted to bone through endochondral ossification. This early cartilage can be identified by the expression of type 1 and 2 collagen. Local chondrocytes further proliferate and hypertrophy and express factors that stimulate ossification of the cartilage matrix. Conversion of this hypertrophic cartilage to bone involves terminal differentiation of the chondrocytes, cartilage calcification, and woven bone formation. This conversion of cartilage to immature woven bone is associated with the release of multiple factors, including BMPs, TGF- $\beta$ , Insulin-like Growth Factor's, osteocalcin, and collagen types 1, 5, and 11. As the bone is formed, the chondrocytes die off as a result of apoptosis and additional vascular ingrowth occurs. Over time, the newly formed woven bone is remodeled through osteoblast and osteoclast activity into mature lamellar bone.

Numerous physiological and environmental factors can negatively influence the process of bone repair after fracture. These factors include chronic inflammation, diabetes mellitus, hypovitaminosis, aging, and polytrauma. This brief report will review some of the ways by which these factors have been shown to negatively influence bone repair.

## CHRONIC INFLAMMATION

Although inflammation plays an important early role in bone repair, prolonged inflammation as a result of chronic inflammatory diseases, polytrauma, or infection can actually retard this process. Over the past several years, the concept of chronic inflammation challenging the process of bone repair has been investigated. For example, the leakage of lipopolysaccharide (LPS) endotoxins from the gut of severely traumatized individuals has been shown to cause bone resorption.

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Reikeras et al demonstrated that local and intraperitoneal administration of LPS impaired osteotomy/fracture repair in rats. LPS seemed to delay fracture healing by inducing hypertrophic and immature callus formation by promoting proinflammatory activity of macrophages.<sup>1</sup> Bastian et al outlined the effects of systemic and local inflammation on neutrophilia and neutrophil priming and their potential effects on fracture healing. Based on available data, they proposed a hypothetical model whereby neutrophilia and neutrophil priming during systemic inflammation lead to an increased influx of neutrophils into a fracture hematoma. This increased influx alters local concentrations of growth and differentiation factors and thereby disturbs the downstream processes of bone repair (Fig. 1).<sup>2</sup>

Lindsey et al,<sup>3</sup> working with an open femur fracture model, showed that the inflammatory cytokine IL-12 could be used to immunomodulate the environment and actually aid bone formation. Roszer published a comprehensive review of inflammation as it relates to fracture healing, particularly in the setting of diabetes. His report outlined the conditions, such as diabetes, that are responsible for inducing local or systemic inflammation and are likely to delay bone repair. The mechanism for this delay appears to be related to the excessive inflammatory mediators shortening the lives of chondrocytes and osteoblasts while prolonging the lives of osteoclasts.<sup>4</sup>

### DIABETES

There are thought to be over 21 million individuals in the United States with diabetes mellitus, and an estimated 600,000 new cases are diagnosed each year.<sup>5</sup> Not only does this pose a huge medical and financial burden on society, the negative influence that diabetes has on fracture healing further increases the impact that diabetes has on a person's quality of life after injury. Activated platelets within a fracture hematoma release growth factors important for the initiation of bone repair. These factors include PDGF, TGF-β, and vascular endothelial growth factor (VEGF). Tyndall et al studied the expression of PDGF in the fracture callus of spontaneously diabetic BB Wistar rats. Immunohistochemistry, reverse transcription, and polymerase

chain reaction demonstrated decreased localization of PDGF and decreased PDGF messenger RNA (mRNA) in the early fracture callus. This reduced concentration of essential growth factors is believed to play a role in delayed bone repair in diabetics.<sup>6</sup> Interestingly, patients with fracture nonunions due to reasons other than diabetes have been shown to have decreased levels of PDGF and VEGF at the bone repair site as well. Modulating the presence of these early growth factors or providing exogenous growth factors to fracture sites can potentially reverse the negative effects of decreased presence of growth factors in diabetic fracture callus.

### HYPOVITAMINOSIS

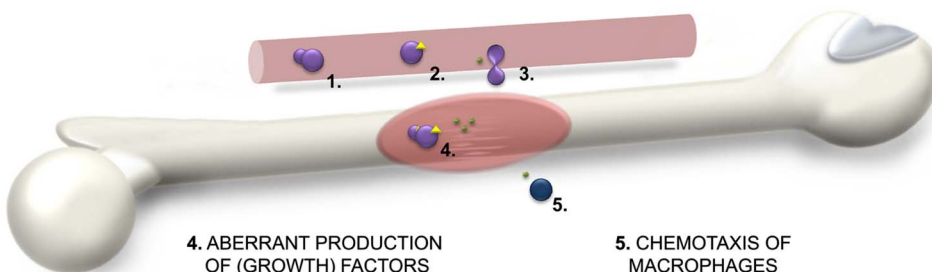
Hypovitaminosis, particularly abnormal low vitamin D levels, has been associated with delay in bone repair. The usual vitamin D pathway to activation includes 7-dehydrocholesterol in the skin, conversion to 25 (OH) vitamin D in the liver, and then the formation of the active form of vitamin D, 1,25 (OH) 2 vitamin D in the kidney, and locally in the bone. Vitamin D metabolites have been shown to be critical for fracture healing.<sup>7</sup> Low vitamin D levels (25 (OH) vitamin D <32 ng/mL) have been associated with secondary hypoparathyroidism as well. Bogunovic et al<sup>8</sup> found that 60%–70% of patients with trauma had low vitamin D levels, whereas Brinker et al<sup>9</sup> found that 68% of patients with established nonunions had low vitamin D levels. From these and other studies, it appears that low vitamin D levels are associated with impaired bone repair. Not only have low vitamin D levels been associated with the development of nonunions, Adami et al<sup>10</sup> showed that individuals with vitamin D levels of <20 ng/mL had a 1.77 increased risk of fracture compared with individuals with normal vitamin D levels.

There are several potential causes for the development of vitamin D deficiency. These include decreased exposure to sunlight, inadequate vitamin D intake, malabsorption of vitamin D in the gut possibly as a result of prior bariatric surgery, and certain other conditions and medications (particularly antiepileptics). Oily fish, including wild salmon, are known to contain considerable amounts of 25(OH) vitamin D,

### HYPOTHESIS

#### SYSTEMIC INFLAMMATION IMPAIRS FRACTURE HEALING BY INAPPROPRIATE HOMING OF PRIMED NEUTROPHILS TOWARDS THE FRACTURE HEMATOMA

- 1. NEUTROPHILIA
- 2. SYSTEMIC NEUTROPHIL PRIMING
- 3. INCREASED HOMING OF PRIMED NEUTROPHILS



**FIGURE 1.** Hypothetical model of the early inflammatory phase of fracture healing during systemic inflammation. Neutrophilia and neutrophil priming during systemic inflammation lead to an increased influx of neutrophils into the fracture hematoma, which alters the local concentrations of growth and differentiation factors and thereby disturbs downstream processes of bone repair (with permission from Bastian et al<sup>2</sup>).

and certain vegetables and other animal proteins are also good sources of vitamin D.

Treatment of patients known to have low levels of vitamin D (<32 ng/mL) should begin with the addition of dietary supplementation and a search for and correction of the cause for the low vitamin D levels. Recommendations for correction of serum vitamin D levels include consumption of 2000 units of vitamin D per day combined with 1500 mg of calcium per day.

Low vitamin C levels can also influence bone repair by causing an impairment of collagen synthesis, a process extremely important in bone repair and ultimate bone strength. A recent Framingham study showed that patients who consumed 250–400 mg of vitamin C each day had a decreased fracture rate compared with those who did not routinely take vitamin C supplements.<sup>11</sup>

In general, hypovitaminosis, particularly regarding vitamins D and C, has a negative effect on bone repair and causes an increase in the risk of fracture. Treatment of these deficiencies begins with vitamin supplementation and the identification and correction of the cause for these deficiencies.

## AGING

Experienced clinicians have the impression that bone repair occurs faster in younger patients compared with older patients. Investigations into why this occurs have approached the differences in the rate of fracture healing in animals of different ages from a variety of perspectives. Age-related changes in tissues and cells have been identified, and these changes appear to contribute to the observed delay in bone repair with aging.

Meyer et al found that on average, young rats (6 weeks old) healed a closed femur fracture considerably quicker than older rats. On average, normal biomechanics of the fractured femurs in young rats was restored within 4 weeks, whereas in older rats, normal biomechanical strength was not recovered for approximately 26 weeks. The investigators showed that levels of mRNA gene expression, in general, followed the same pattern in both groups, but significantly, lower levels of mRNA for Indian Hedgehog (ihh) and BMP-2 were detected in the fracture calluses of the older rats. Indian Hedgehog is thought to be involved in chondrogenesis and bone repair, whereas BMP-2 stimulates bone induction through cartilaginous remodeling. The reduction in BMP-2 may partially account for the slower onset of soft callous formation in the older animals.<sup>12</sup>

In a rabbit model, O'Driscoll et al used an organ-culture model to investigate the relationship between chondrogenic potential of the periosteum and aging. Periosteum was harvested from the tibiae of 82 rabbits, aged 2 weeks to 2 years. After culturing, the explants were analyzed using histomorphometry, collagen typing, wet weight measurement, H-thymidine and S-sulfate uptake, autoradiography, and proliferating cell nuclear antigen immunostaining. The 3 most significant findings were that chondrogenesis declined significantly with age (6-month-old rabbit explants formed 50% less cartilage than younger rabbit specimens) as did the thickness of the

periosteum (87%) and the total number of cells within the cambium layer of the periosteum.<sup>13</sup>

Understanding that mesenchymal stem cells (MSCs) residing in bone marrow are the progenitors for osteoblasts and other cell types, D'Ippolito et al hypothesized "that a decrease in the number of osteoblasts and the age-related reduction in bone formation and in the mechanical properties in humans reflect decreased osteoprogenitor generation during aging." Using bone marrow cells from postmortem thoracolumbar (T1–L5) vertebral bodies of 41 donors (25 men/16 women), they found that the number of MSCs with osteogenic potential decreases with age and hypothesized that this reduced osteogenic potential could be related to age-related bone loss (osteoporosis). It stands to reason that this diminished number of MSCs and cells with osteogenic potential that occur with aging could also negatively influence bone repair in older patients.<sup>14</sup>

Gruber et al summarized the experimental evidence on age-related changes at the cellular and molecular level. This study found that delays in cell differentiation and angiogenic invasion of cartilage lead to a prolongation of endochondral ossification, delayed onset of periosteal reaction, decreased overall bone formation, and impaired bone remodeling with aging. Restoration of biomechanical characteristics after fracture was also shown to occur within 4 weeks in 6-week-old rats, within 10 weeks in 26-week-old rats, and >6 months in 1-year-old rats.<sup>15</sup>

Age-related changes at the cellular level were further delineated by Syed and Hoey<sup>16</sup> and Almeida et al.<sup>17</sup> These studies showed that the mesenchymal progenitor cells had a decreased responsiveness to signaling molecules, a decrease in the actual number and division capacity, decreased blood vessel formation, lower osteoinductive activity, and a decrease in the local and systemic levels of signaling molecules with aging.

## POLYTRAUMA

Normal bone repair is dependent on a complex process that involves the interaction between inflammatory mediators, cytokines, and a variety of growth factors. The inflammatory response is one of the initiating factors for bone healing. Polytrauma often results in injury to the body's soft tissues, solid organs, appendicular and axial skeleton, and these injuries stimulate a substantial inflammatory response. It is believed that as a result of this response to injury, particularly the increase in circulating inflammatory mediators, bone repair and generalized healing is delayed. When infection occurs, the interference with bone and soft tissue healing is prolonged further. Some of the mediators, which have a direct effect on fracture healing after trauma, include TNF- $\alpha$ , leukotrienes, VEGF, TGF- $\beta$ , and PDGF.<sup>18</sup> Gerstenfeld et al<sup>19</sup> demonstrated impaired fracture healing in the absence of TNF- $\alpha$  signaling and explored the role of TNF- $\alpha$  in endochondral cartilage resorption. They found that TNF- $\alpha$  played an important regulatory role in postnatal endochondral bone repair. Wixted et al studied leukotrienes, potent inflammatory mediators, and demonstrated enhancement of the bone repair in the presence of leukotriene antagonists. This enhanced bone repair took place in the setting of increased chondrocyte proliferation and early bone formation.<sup>20</sup> Sibylle

et al found enhanced VEGF in polytrauma and burn patients with uncomplicated healing. Decreased VEGF levels were more commonly found in patients with sepsis, adult respiratory distress syndrome, and multiple organ failure.<sup>21</sup>

### SUMMARY

Normal bone repair depends on a complex pathway that includes many different types of circulating molecules. Disturbances in the regulation, presence, or timing of these molecules can lead to alterations in bone repair. Physiological changes associated with inflammation, diabetes, hypovitaminosis, aging, and polytrauma to delay bone repair are also experienced.

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