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### Authors

Clark, Dan  
Nakamura, Mary  
Miclau, Ted  
[et al.](#)

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## Effects of Aging on Fracture Healing

Dan Clark<sup>1,2,3</sup>, Mary Nakamura<sup>4,5</sup>, Ted Miclau<sup>1,2</sup>, and Ralph Marcucio<sup>1,2</sup>

<sup>1</sup>Department of Orthopaedic Surgery, University of California at San Francisco, 513 Parnassus Ave., San Francisco, CA 94143, USA

<sup>2</sup>Orthopaedic Trauma Institute, Zuckerberg San Francisco General Hospital and Trauma Center, 2550 23rd St, Building 9, San Francisco, CA 94110, USA

<sup>3</sup>Department of Oral and Craniofacial Sciences, School of Dentistry, University of California at San Francisco, 513 Parnassus Ave., Rm. S-619A, San Francisco, CA 94143, USA

<sup>4</sup>Department of Medicine, University of California at San Francisco, San Francisco, CA 94143-0451, USA

<sup>5</sup>Department of Pathology, VA Medical Center, University of California San Francisco & Pathology Service, San Francisco, CA 94121, USA

### Abstract

**Purpose of Review**—This review summarizes research on the physiological changes that occur with aging and the resulting effects on fracture healing.

**Recent Findings**—Aging affects the inflammatory response during fracture healing through senescence of the immune response and increased systemic pro-inflammatory status. Important cells of the inflammatory response, macrophages, T cells, mesenchymal stem cells, have demonstrated intrinsic age-related changes that could impact fracture healing. Additionally, vascularization and angiogenesis are impaired in fracture healing of the elderly. Finally, osteochondral cells and their progenitors demonstrate decreased activity and quantity within the callus.

**Summary**—Age-related changes affect many of the biologic processes involved in fracture healing. However, the contributions of such changes do not fully explain the poorer healing outcomes and increased morbidity reported in elderly patients. Future research should address this gap in understanding in order to provide improved and more directed treatment options for the elderly population.

### Keywords

Fracture healing; Elderly; Senescence; Inflammatory response; Inflamm-aging

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Ralph Marcucio [Ralph.marcucio@ucsf.edu](mailto:Ralph.marcucio@ucsf.edu).

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## Introduction

The elderly population in the USA has been steadily increasing, and those aged 65 years old and older are expected to comprise 17% of the population by 2030 [1, 2]. This growing population presents their own unique health needs, and in order to meet these needs a better understanding of the physiologic changes that occur with aging is necessary. The skeletal system exhibits physiologic changes that occur with increasing age. Conditions such as osteoporosis and osteoarthritis increase with age. Additionally, many reports demonstrate a higher rate of bone fracture, and these are associated with increased morbidity and mortality [3–5]. A decline in healing potential is observed in the elderly, and this may result in increased rates of delayed healing or nonunions [6]. Delayed healing, and the resulting incapacitation, can have more severe and systemic consequences in the elderly, which poses unique challenges for the treating clinician [3, 7]. While increased age has been generally associated with a wide range of physiological changes, the mechanisms that result in decreased capacity for fracture healing are not fully understood.

An understanding of the age-related effects to fracture healing is complicated by a lack of a complete understanding of fracture healing in healthy and in young individuals. However, by analyzing the individual facets of fracture healing that we do understand we can compare differences in fracture healing between the young and old humans and animals. This review will highlight the phases of fracture healing and the respective physiologic changes that occur with age. Cellular, molecular, and genetic differences between young and old humans and animal models will be characterized to illustrate the current understanding of the effect of age on bone fracture healing. Figure 1 summarizes the effect of age on critical cells that contribute to fracture healing.

## Physiology of Bone Fracture Healing

Fracture healing proceeds through multiple phases characterized by anabolic and catabolic processes [8]. The early healing stage is characterized by a robust inflammatory response that is responsible for debriding the fracture site and contributing to the signaling milieu that will propagate the successive stages of healing, including recruitment and differentiation of skeletal tissue progenitor cells [9]. The anabolic phase follows the initial inflammatory response. Progenitor cells give rise to a soft callus with a central cartilaginous region and new bone formation at the periphery [9, 10]. The soft callus is characterized by avascular cartilage tissue that induces vascularization [11]. A hard callus begins to develop through endochondral ossification with increased mineralization and replacement of chondrocytes with osteoblast, in part, through transdifferentiation [12, 13]. Finally, remodeling of the callus occurs through catabolic processes. The callus is reduced in size and osteoblastic and osteoclastic processes alternate to reestablish the normal hematopoietic and trabecular structure, restoring bone to its pre-injured state [14].

Histological and molecular changes within the callus have been described during the stages of fracture healing as mentioned above and provide healing comparisons between old and young animals. Numerous studies have reported delayed fracture healing in elderly animals and have shown decreased cartilage and bone formation, delayed cartilage resorption, and

slower mineralization within the callus [15–17]. Delayed bone healing may be associated with age-related changes in the osteochondral stem cells. In general, there is an age-related decrease of stem cell quantity compounded by a decrease in proliferation and differentiation potential as demonstrated in humans and animal models [18–20]. Lopas et al. demonstrated a decrease in osteochondral stem cell proliferation associated with a significant decrease in bone and cartilage content within the fracture callus of aged mice compared to young [15].

Chondrocytes and osteoblasts arise from stem cells predominantly located in the periosteum during fracture healing [9, 21, 22]. Senescence and greater oxidative damage was associated with periosteal-derived progenitor cells from old humans compared to young [23]. Additionally, a decreased number of periosteal cells were able to be derived from the periosteum of old humans compared to young [21]. The chondrogenic potential of stem cells in the periosteum is decreased in elderly mice compared to young mice, and chondrogenic differentiation from periosteal cells is delayed in old versus young mice [22, 24]. Older animals have delayed expression of type 2 collagen (*ColII*) and delayed cartilage matrix deposition at early time points of fracture healing [24]. Similarly, osteoblast differentiation and osteocalcin expression is delayed from periosteal cells at the fracture site in elderly mice compared to young mice [24].

Stem cells contributing to fracture healing may also arise from other tissue sources that could be negatively affected by age-related changes. Cells located in the muscle appear to contribute to bone fracture healing. While muscle stem cells, known as satellite cells, may contribute only a small number of cells that comprise the skeletal tissues, they appear to regulate fracture healing possibly through signaling interactions [25]. With aging, satellite cell quantity and function decline which may negatively affect their ability to support fracture healing [26]. However, the role of other cell types that reside in the muscle is not known and worthy of investigation. The bone marrow is also a potential source of osteochondral stem cells [27, 28]. Similar to satellite cells, aging results in decreased function and quantity of bone marrow stem cells that could have a negative effect on fracture healing [29, 30].

Age also affects later stages of healing during endochondral ossification. The characteristic histological findings that describe endochondral ossification, hypertrophic chondrocytes, expression of type 10 collagen (*ColX*), and vascular invasion were all delayed in elderly mice compared to young mice [24]. Completion of endochondral ossification, characterized by complete conversion of cartilage to bone within the callus, was also delayed in elderly mice versus young mice [24].

The age-related alterations to the cellular processes that are evident during fracture healing are accompanied by changes in the regulation of critical genes involved in bone fracture healing. By using the whole genome expression analysis of fracture calluses in rats, significant differential regulation of 144 genes was found in young compared to elderly rats [31]. Functional analysis of these genes suggested they were largely involved in cell migration [31]. More specifically, bone morphogenetic protein (BMP-2) and Indian Hedgehog (IHH) expression in the fracture callus of elderly rats were decreased compared to young rats [32]. Other studies have shown comparable levels of gene expression in the early time

points of fracture healing; however, in elderly rats, expression of IHH, BMP, and TGF- $\beta$  decreased at the same rate as the young rats despite requiring almost twice as long for complete healing to be detected radiographically [33]. Thus, molecular changes occurring in cells comprising the fracture callus are evident in animals of advanced age, and these changes may contribute to the alterations in healing that are observed in older animals. However, systemic changes as a result of the normal process of aging also occur and could contribute to the delays in healing.

## Inflammation and Fracture Healing

The initial phase of fracture healing is characterized by a robust inflammatory response. Secretion of pro-inflammatory cytokines at this time is necessary to initiate the healing process and achieve adequate healing [34]. Temporal control of the inflammatory response is crucial for proper bone fracture healing. After the initial pro-inflammatory phase, inflammation must be resolved in order to allow the anabolic processes to begin and continue the subsequent healing phases. However, changes in the inflammatory system occur with age, and chronic inflammation and/or a decreased ability to resolve inflammatory processes could negatively affect bone fracture healing.

## Inflamm-Aging and Immunosenescence

An excessive or prolonged inflammatory phase can have detrimental effects on fracture healing [35, 36]. In animal models, prolonged inflammation results in delayed chondrogenesis and smaller callus size [37]. Recently, it was shown that elevated systemic levels of a pro-inflammatory cytokine, TNF $\alpha$ , in induced diabetic mice negatively affected angiogenesis during fracture healing [38]. In humans, an elevated inflammatory status is related to certain systemic conditions, including diabetes, smoking, and increased age. These conditions are all associated with poorer fracture healing outcomes, but the mechanisms remain incompletely understood. The term “inflamm-aging” has been used to describe a chronic increased pro-inflammatory status in the elderly [39]. Elderly people are found to have higher levels of circulating proinflammatory cytokines, even in healthy individuals. It appears that the increased pro-inflammatory status in the elderly predisposes them to the range of systemic diseases including osteoporosis, Alzheimer’s disease, Type II diabetes, atherosclerosis, and Parkinson’s disease [40–42]. Currently, it is unclear what drives this increased inflammation. Inflamm-aging has been suggested to be the result of a defect in the proper resolution of the normal inflammatory response, or the result of an unknown chronic mechanism that signals and prolongs the inflammatory response [43, 44]. As the inflammatory response is a critical step in proper fracture healing, any disruption of the inflammatory response could negatively affect fracture healing.

Inflamm-aging may also be a result of age-related changes to the immune response. Aging of the adaptive immune response has been described as immunosenescence [45]. Immunosenescence describes a loss of immune function that is associated with a predisposition to infection and disease in the elderly [45]. Increased age is associated with changes in T and B cell production and maturation. T cell production and maturation is negatively affected by age-related changes to the bone marrow hematopoietic compartment

and to the thymus [46, 47]. With increasing age, the source of T cell progenitors, the hematopoietic compartment, decreases in size and is associated with a decrease in T cell progenitor quantity and proliferation potential [47]. The T cell progenitors migrate to the thymus for further differentiation and maturation. A decrease in thymus function and T cell output is seen with increasing age as involution and atrophic changes are present [46]. The lack of a sufficient quantity of T cells produced by the thymus negatively affects the ability to mount an effective immune response. Additionally, the lack of T cells limits the availability of regulatory T cells that are present to resolve the mounted immune and inflammatory response [46, 48]. Immune cell trafficking has also been shown to be of importance in fracture healing studies showing impaired fracture healing in CCR2 deficient mice [49]. CCR2 is a chemokine receptor for the ligands Ccl2, Ccl7, and Ccl12 that is expressed on monocytes, myeloid-derived cells, a subset of T cells, and mesenchymal stem cells. Recent studies in a muscle regeneration model have suggested that CCR2 deficiency in young mice results in an inflamm-aging environment similar to changes seen with aging [50]. In this way, it has been proposed that the elevated pro-inflammatory status of inflamm-aging may be a response to age-related defects of the immune response. Figure 2 demonstrates a conceptual model of the effects of inflammatory response dysregulation on the subsequent stages of fracture healing.

The age-related effects on inflammation during fracture healing can be investigated independently of other age related changes to the organism by using a chimeric animal model. Xing et al. used such a chimeric model of lethal irradiation followed by bone marrow transplant [51]. By irradiating aged mice and transplanting the bone marrow of young mice to those animals, investigators found that the osteochondral stem cells were derived from the aged host and the inflammatory cells were derived from the young donor in a fracture model. In this manner, the older mice receiving young bone marrow had larger calluses and more bone in the early healing time points, more rapid callus remodeling at later stages of healing, and a rejuvenation of the inflammatory response compared to older mice receiving age-matched bone marrow transplants [51]. A similar study by Baht et al. utilized a parabiosis and a bone marrow transplant model of old and young mice in a fracture healing study [52]. Fractures in old mice healed significantly better with shared circulation from a young mouse or with bone marrow transplants from young mice compared to aged-matched old mouse donors. No cells from the young donor circulation or bone marrow differentiated into skeletal cells in the callus suggesting a component of the circulation or bone marrow could revert osteochondral stem cells of old mice to a more youthful phenotype. They further found that  $\beta$ -catenin was differentially expressed in old and young mice during fracture healing. Shared circulation with young mice resulted in decreased levels of  $\beta$ -catenin and was associated with improved fracture healing. Decreasing  $\beta$ -catenin activity in old mice resulted in similar improvements in healing as sharing circulation with young mice. The two studies described above suggest a crucial component regulating fracture healing of the hematopoietic environment that is affected by age-related changes.

## Cellular Regulation of Inflammation

An understanding of the intrinsic age-related changes to cells involved in the inflammatory response may explain, in part, the poorer healing potential in the elderly. Cells of the innate

and adaptive immune system local to the fracture site assist in regulating the inflammatory response. Macrophages are powerful regulators of inflammation. In the early inflammatory stage of healing, macrophages are classically activated and have an M1 phenotype [53, 54]. M1 macrophages are proinflammatory and release cytokines IL-1, IL-6, TNF- $\alpha$ , and iNOS to elicit and propagate the inflammatory response [53, 54]. As inflammation is down-regulated in the later phases of fracture healing, the macrophages acquire an alternatively activated M2 state. M2 macrophages express anti-inflammatory cytokines, such as IL-10 and promote healing through secretion of growth factors TGF $\beta$ , VEGF, and PDGF [53, 55]. Temporal control of the polarization of M1 and M2 macrophages is important to regulate inflammation during the healing process.

Intrinsic age-related changes to macrophages may perturb the inflammatory response in the elderly and may have negative consequences for fracture healing. Aged macrophages were found to be less responsive to granulocyte macrophage colony-stimulating factor (GM-CSF) that resulted in decreased proliferation compared to young macrophages [56]. Additionally, elevated serum levels of chitotriosidase, a marker for chronically activated macrophages, was found in elderly humans compared to young controls [57]. The negative effects of age-related changes to macrophages are further supported in animal models of healing. Cutaneous wounds heal slower in aged mice and can be rescued with transplants of macrophages from young mice [58]. Further, aged macrophages appear to be detrimental to healing. In blocking macrophage recruitment to the fracture site, elderly mice appear to exhibit better fracture healing compared to elderly mice that had normal macrophage activity [59]. Conversely, in the same experiment, blocking macrophage recruitment to the fracture site in young mice had a negative effect on fracture healing [59].

Macrophages are known to contribute to bone healing. A tissue resident population of macrophages, termed “osteomacs,” has recently been described [60]. Osteomacs regulate osteoblast function and promote fracture healing; blocking their activity has deleterious effects on bone healing [60, 61]. While it appears clear that circulating macrophages that are recruited to the site of bone injury have age-related changes that hinder fracture healing, whether age-related changes occur in osteomacs is not known.

Cells of the adaptive immune system also contribute to fracture healing [62]. T and B cells are present within the early callus during the inflammatory phase [63]. Regulatory B cells negatively regulating inflammation through expression of IL-10 and downregulating pro-inflammatory cytokine expression in T cells within the callus [64]. B cells from patients with poor fracture healing outcomes had decreased expression of IL-10 compared to patients that healed normally [64]. As healing continues, cartilage formation within the callus is associated with a local increase in Treg cells [65]. At later time points of healing, T and B cells are readily present at sites of mineralization and in direct contact with osteoblasts and osteoclasts [63]. Models of fracture healing using mice with genetic modifications have further elucidated the role of T and B cells. Deficient T and B cell function in Rag1<sup>-/-</sup> mice demonstrated impaired fracture healing which was associated with decreased IL-17F expression [66]. IL-17F was shown to promote osteoblast maturation in vivo [66]. Additionally, dysregulated T cell recruitment and activation within the bone in a mouse model of lupus was associated with increased bone turnover and decreased bone fraction

within the fracture callus [65]. As discussed above, T and B cell quantity and function are negatively affected by age. Due to the involvement of T and B cells in fracture healing, such age-related changes could have a negative effect on fracture healing.

Mesenchymal stem cells are also involved in the inflammatory response and act as powerful immunomodulators at the site of injury to control excessive inflammation and promote repair [67–69]. Suppression of inflammation mediated by MSCs occurs, in part, through signaling and interactions with local inflammatory cells. MSCs have been shown to interact with T cells, B cells, NK cells, and dendritic cells through signaling that limits proliferation of inflammatory cells or promotes secretion of anti-inflammatory molecules [70]. Additionally, research has suggested that MSC and macrophages interact to promote alternatively activated macrophages in the downregulation of inflammation and promotion of healing [71]. The immunomodulatory properties of MSCs have been utilized clinically and have been reported beneficial in the treatment of graft versus host disease, Crohn's disease, renal failure, and heart failure [72–75].

Intrinsic age-related changes to MSCs could explain the impaired healing in older humans and animals as a result of dysregulated inflammatory response. The quantity of MSCs isolated from bone marrow is decreased with age [30]. Additionally, there are increased markers for oxidative damage of MSCs from elderly human samples [58•]. These age-related changes to MSCs corroborate findings in experimental wound healing studies that show a benefit of therapeutic administration of MSCs from young animals but no benefit in healing with aged MSCs [58•].

## Vasculature and Fracture Healing

Successful bone fracture healing requires adequate vascularization of the tissue [76]. The contribution of the vasculature to fracture healing includes providing blood supply for delivery of nutrients and cells, providing the endothelial cells that express angiogenic and osteogenic signaling molecules locally, and providing the source of oxygen to the healing callus [9, 77, 78]. However, the complete contribution of the vasculature to fracture healing is not fully understood.

With increasing age, perturbations in bone fracture healing are associated with age-related dysfunction to the bone vascular system and its ability to regenerate in healing. Generally, the vascular perfusion of the skeleton decreases with age [79]. Elderly rats display significantly higher ossifications and decreased patency of bone marrow blood vessels compared to younger rats [80]. The decrease in vascularization at the time of fracture may delay angiogenesis during the fracture healing. The fracture callus at early healing time points in young mice have a higher surface density of blood vessels compared to the elderly mice [77]. The increase in vascular density was associated with an early detection of vascular endothelial growth factor (VEGF) and hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) in young mice but not elderly [77]. Additionally, increased *Mmp9* and *Mmp13* transcripts were detected throughout early and late stage healing in young compared to old mice [77]. Differential expression of angiogenic factors, including VEGF, HIF-1 $\alpha$ , and *Mmps*, has been well demonstrated in young animals versus old during bone fracture healing [81–83].



## Conclusions

Increasing age has been shown to negatively affect the cellular and molecular processes throughout the different stages of bone fracture healing. Inflammatory regulation, cellular differentiation, and signaling cascades are all affected, in part, by age-related changes. Our current understanding of these age related changes explains, only partially, the decreased healing potential and increased complications observed during fracture healing in elderly patients. A more complete understanding is necessary to allow for therapeutics to target the specific age-related deficiencies and provide better care for the increasing geriatric population.

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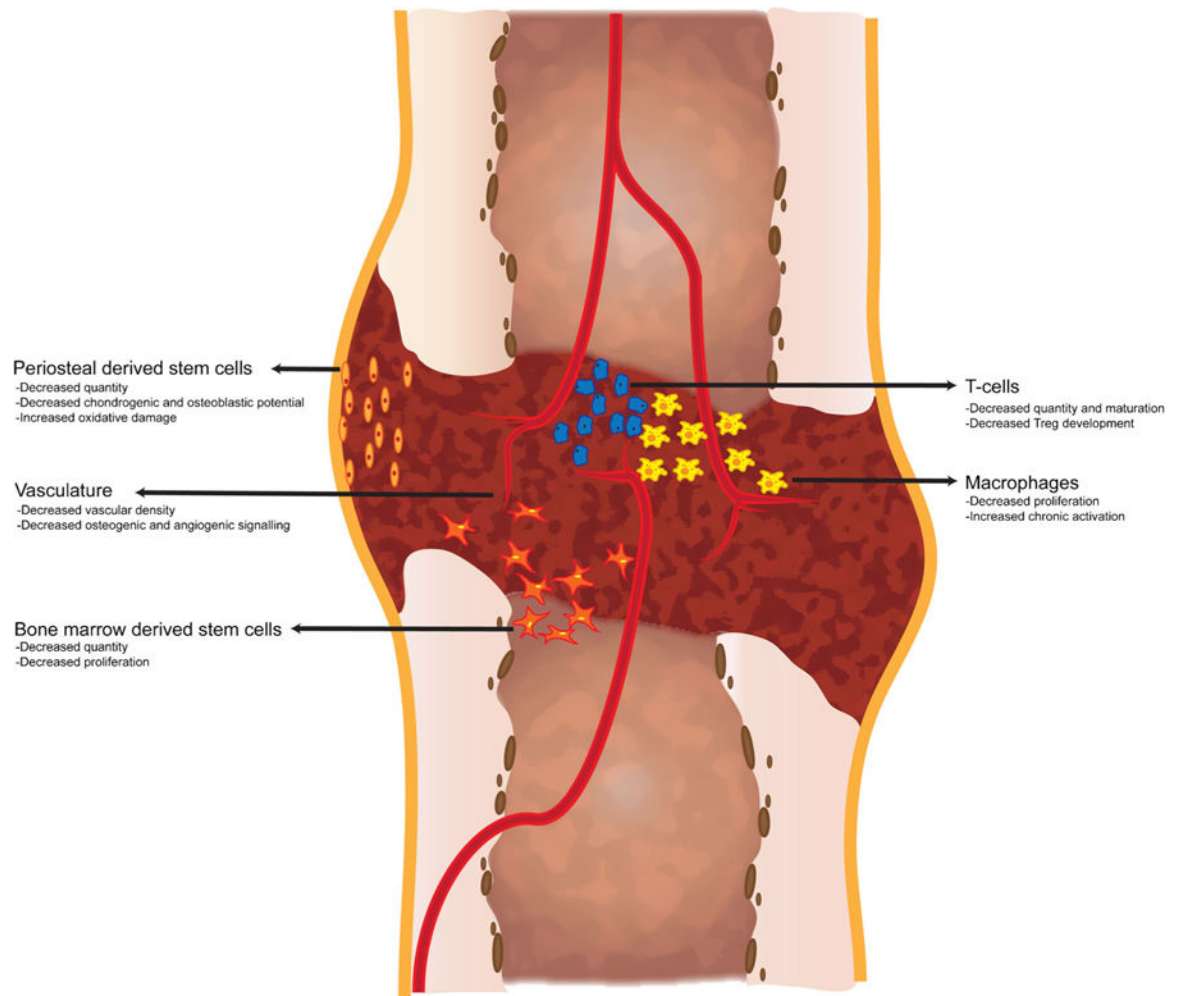
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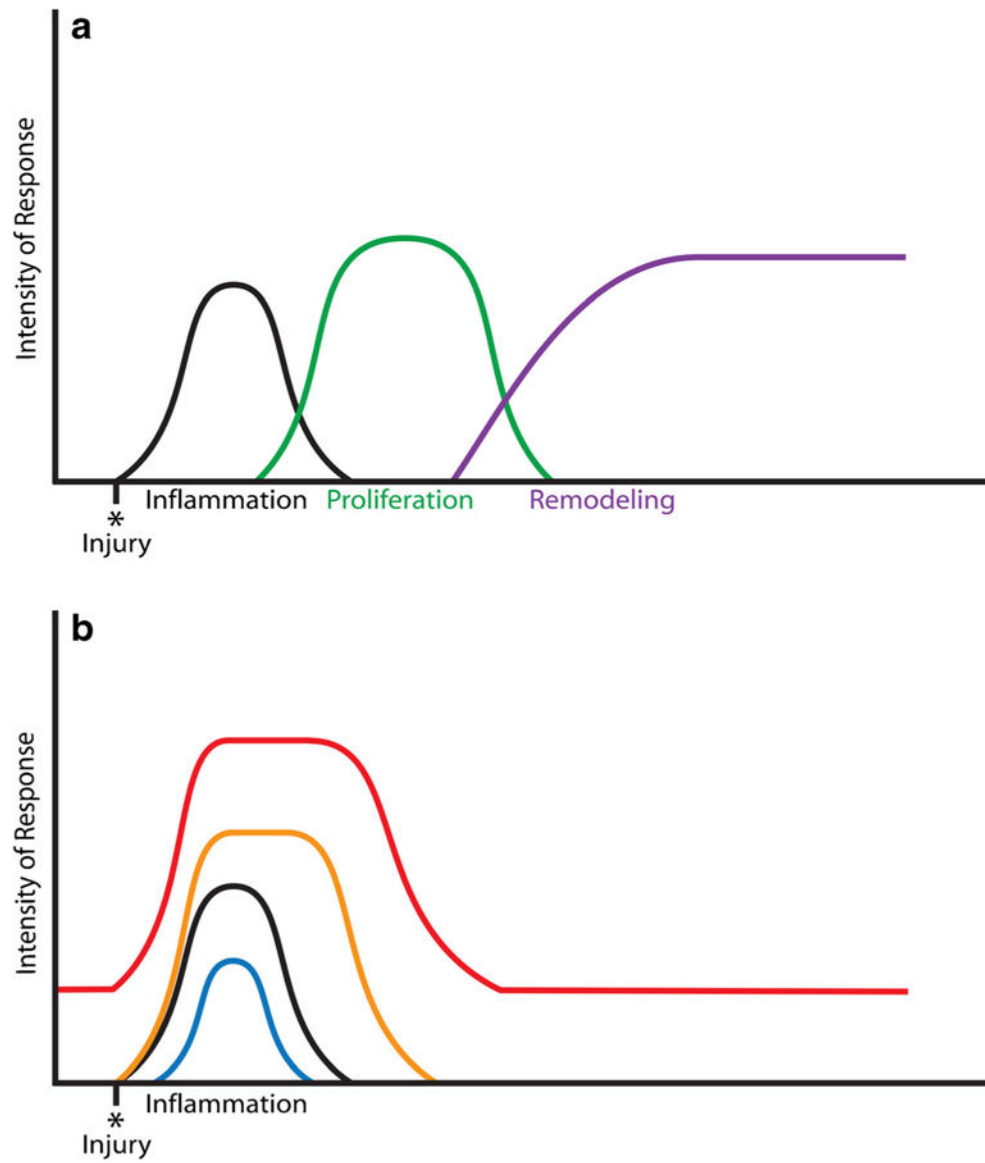
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**Fig. 1.**

The effect of age on the cellular contribution to fracture healing. Stem cells and immune cells involved in fracture healing demonstrate age-related changes that may negatively affect fracture healing. Osteochondral stem cells arise from the periosteum and bone marrow and demonstrate decreased quantity, increased oxidative damage, and decreased osteoblastic and chondrogenic differentiation potential with age. T-cells contribute to fracture healing and production and maturation of T cells is negatively affected by age-related changes to the bone marrow hematopoietic compartment and to the thymus. Macrophages are important regulators of inflammation during fracture healing. Aged macrophages demonstrate decreased proliferation and increased activation that may contribute to the poorer healing outcomes associated with aged macrophages compared to young. Finally, adequate vascularization is required for successful fracture healing. Aged animals demonstrate decreased vascular density within the callus which is associated with decreased levels of key angiogenic factors required for healing



**Fig. 2.** The effects of inflammatory response perturbation on the stages of fracture healing. **a** Fracture healing follows three general stages of inflammation, proliferation, and remodeling. The initial inflammatory response is tightly regulated and crucial in initiating the subsequent stages of healing. **b** Systemic conditions, including increased age, have an effect on inflammation and may result in differential inflammatory responses during fracture healing. Senescence of the inflammatory response results in a decreased and limited inflammatory response (blue curve) that may result in inadequate activation of the proceeding healing stages. An exaggerated and sustained response (orange and red curves) can result from inadequate resolution of the response and may negatively affect the proceeding stages. An increased basal level of inflammation (red curve) is proposed to occur with inflamm-aging and would have possible negative effects throughout the healing process