XI. Other Forms of Injury; Section C. Fracture

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Introduction

Fractures and skeletal injury are commonly the result of blast injuries and trauma. A combined injury is defined as resulting from trauma that occurs in the setting of irradiation, such as expected after a nuclear accident, radiation dispersal device release (a “dirty bomb”), or a nuclear weapon detonation [1]. The combination of a blast injury and radiation exposure poses unique issues with regard to fracture management, in some cases because of thermal burns and exposure to other toxic substances. Enhanced-blast explosive devices increase the total energy transmitted by the explosion and cause even greater damage [2]. Those factors can interfere with the usual recommendation that surgery be accomplished within 36-48 hours of radiation exposure to mitigate the increased risk of infection [3]. Fractures can be the result of blunt force trauma or penetrating trauma to the bone, such as injury from projectiles and shrapnel, from the high pressure impact of the explosion, or secondary to a fall or building collapse. Fractures are often associated with soft tissue and skin injuries. Fractures that are associated with soft tissue wounds are termed open fractures. The presence of an open fracture significantly complicates treatment because of the increased risk of infection and soft tissue

Although most common fractures heal, surgery is often required to maximal functional outcome by restoring anatomy so that the bony segments can fuse with normal architecture. Management of soft tissue wounds and prevention of infection is paramount and often requires a multidisciplinary approach with the involvement of orthopedic, vascular, and plastic surgeons. Bone has a remarkable ability to heal. Bones are truly regenerating – without scar formation. Under microscopic analysis, healed bone is indistinguishable from normal bone. The purpose of the surgical repair of fractures is to reconstruct the anatomy of articular surfaces and restore limb alignment to optimize regeneration. Early mobilization is paramount to restore function so that mechanical loading can further promote bone accretion and maturation.

Radiation exposure can complicate bone healing. Although there is little clinical information regarding fracture healing from accidental radiation exposure, there is significant evidence that therapeutic radiation exposure during cancer treatment significantly impairs bone healing and leads to a significantly increased risk of wound breakdown and infection. The concern for combined radiation injury and blast injury has increased over the last decade, with the rise of international and domestic terrorism. Although the threat of nuclear weapon detonation is low, the possibility of an improvised explosive device, coupled with a radioactive source, is acknowledged. Additionally, as the recent nuclear accident in Fukushima illustrates, the potential for either a natural or man-made disaster at a nuclear facility exists.

Some information has come from military engagements. The increased use of improvised explosive devices (IEDs) during the conflicts in both Afghanistan and Iraq have increased our understanding of these complicated injuries. Improvised explosive devices

are typically constructed from a high explosive, wrapped in metallic objects to be used as shrapnel. The high explosive used leads to a significant concussive injury, with a huge amount of muscle and bony injury. The high energy of the explosion often drives soil and debris deep within wounds significantly contaminating the muscle and wound bed. This leads to significant infection problems, particularly fungal infection. The extremity injuries associated with improvised explosive devices, particularly those packed with high explosives, are typically devastating and often result in amputation. The military experience with IED's may not correlate well with a civilian population, as abdominal and thoracic injuries are less common in the military population due to significant advances in body armor.

While military experience has generally revolved around high explosives and high energy injuries, civilian terrorist attacks often use less powerful explosives [4]. During the Boston marathon bombing, for example, the majority of patients were injured by direct penetrating injury from shrapnel, rather than the concussive force of the blast. Those injuries are associated with less soft tissue contamination, less concussive injury, and overall less myonecrosis. Although much is known about the effects of blast injury to the musculoskeletal system and surrounding soft tissues, less is known about the effects of radiation on these injuries.

Mechanics of Bone Fractures

The extracellular matrix of bone tissue consists of an organic phase, approximately 30% by weight, and an inorganic mineral phase at 70%. The organic phase is mainly collagen type I fibers that provide elasticity and structure to the bone. Also present in small proportions in the organic phase are other structural and biologically active proteins, proteoglycans, glycoproteins, peptides, lipid materials, and adsorbed serum proteins. The inorganic portion of bone extracellular matrix is comprised of calcium phosphate mineral crystals and provides strength and rigidity to the bone. The minerals are deposited as a poorly-crystalline carbonate-apatite structure similar to, but less ordered than geologic hydroxyapatite. The small crystals constitute the resorbable reservoir of mineral ions for calcium and phosphate homeostasis, but with time, they thermodynamically become more orderly and less soluble. The amount of mineral per unit volume, termed bone mineral density contributes to the stiffness of the tissue. The combined organic and inorganic phases of bone make possible the skeleton's functions of mechanical support, locomotion by attachment of muscles, and protection of vital organs, such as the brain and heart. The load-bearing properties of bone are not static; rather, the tissue constantly undergoes resorption and formation, called remodeling, that mediate adaptation to different mechanical requirements and permit fracture healing and implant incorporation (Figure 1) [5]. It is crucial to recall that besides the mechanical function of bone, this tissue also serves a key metabolic function in contributing to calcium homeostasis by storing the mineral during times of plenty and mobilizing it when needed. Remodeling serves to prevent thermodynamic hypercrystallinization of stored mineral which would render it less soluble [6].

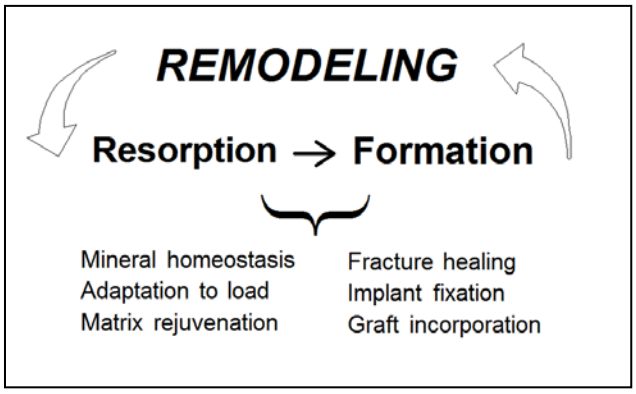


Figure 1. Bone tissue continuously undergoes remodeling in packets of focal osteoclastic bone resorption and replacement by osteoblastic bone formation. The cellular activity of remodeling allows for mobilizing the mineral reservoir for homeostasis, for alterations in architecture and mechanical properties in response to changes in loading, for matrix rejuvenation and removal of microcracks, for accurate regeneration of fractured bone, for implant fixation, and for incorporation of grafts and other osteocompatible materials.

Bone integrity can be compromised by trauma, infection, primary and metastatic tumors, and genetic disorders. Fractures occur when the load to a bone exceeds the range to which it has adapted. Fractures may be caused by a load that is greater than the ultimate strength of bone, or by repetitive application of loads of lower magnitude, as in the case of fatigue fractures. Fatigue fractures occur when low magnitude forces result in progressive accumulation of micro-damage and failure. They can occur from repetitive running or jumping and are painful and common in athletes and in military recruits undergoing basic training. Fractures may be described as low-energy fractures, high-energy as in those caused by automobile accidents, or very-high-energy as in those caused by gunshots or explosives. Fragility fractures are those that occur with low energy, such as a fall from standing height, and commonly occur to bones weakened from osteoporosis or from disuse in non-ambulatory subjects or in astronauts. Bone tumors can cause what are called pathological fractures in areas weakened by an invasive primary or metastatic lesion.

The classification of fractures according to the direction of the fracture lines includes the following: transverse (fracture runs perpendicular to the bone), oblique (fracture runs across the bone at an angle of 45 to 60 degrees), spiral (fracture has a torsional component), comminuted (fracture has more than two fragments), and impacted (fracture ends are compressed together). The direction of forces that fracture bone may be such that the resulting stresses are compressive, shear, or tensile, individually or in combination. Loads that are predominantly compressive typically cause oblique fractures, whereas torsional stresses produce spiral fractures. Tensile stresses cause flat fractures, but these are less common.

Physiology of Bone Healing

Under typical circumstances, bone tissue has a remarkable property of true regeneration following damage. This is possible because of the continuous replacement of cells and extracellular matrix in small packets of resorption and formation. Unlike post-natal skin, for example, which reacts to injury with the rapid formation of poorly organized scar tissue, bone is capable of restoring the injured parts with characteristics indistinguishable from the original material. It is commonly noted that xrays can fail to show evidence of old fracture

sites.

The mechanical conditions at a fracture site determine the pattern of repair. Direct, or primary bone healing refers to a situation in which there is only a small fracture gap and little or no motion across the gap. This can occur naturally with so-called hairline fractures. It can be achieved surgically by stabilizing the fracture fragments and placing them in close contact with plates and screws or intramedullary nails so that remodeling can cross the fracture line and unite the bone with neo-vascularization and neo-osteogenesis. Hematoma formation in the gap helps to guide regeneration of the missing tissue. The second type of bone healing by indirect or secondary repair involves multiple processes (Table 1), as initial

Table 1. Stages of Fracture Healing by Secondary Intention
~ Hematoma Formation
~ Inflammation
~ Revascularization
~ Cartilage Formation
~ Cartilage Calcification
~ Woven Bone Formation
~ Bone Remodeling

local inflammation stimulates new bone to form from the outer layer of periosteum and the inner endosteum. Depending upon the amount of vascularity and micromotion, there can be an initial mixture of cartilage and woven bone formation. That rapidly laid down tissue is called the hard callus and can reduce mobility of fragments to a great extent. Meanwhile, necrosis of fracture fragments stimulates revascularization and signals osteoclasts to invade the location and remove the damaged cells and matrix. Remaining hematoma within large or mobile spaces becomes replaced with granulation tissue and in avascular regions differentiates to cartilage as a temporary bridge. With orderly vascularization, the tissue undergoes endochondral ossification (replacement of cartilage by marrow and bone tissue) and maturation of the bony architecture. The rate and extent of repair are also influenced by the subtle balance of immobilization and pro-osteogenic deformation. Thus, there seems to be an optimal window of interfragmentary motion needed for normal healing. Sequential cascades of inflammatory mediators and growth factors orchestrate the orderly regeneration of bone. Studies with knock-out mice revealed the roles of specific factors to initiate and to conclude each phase of healing [7]. Delayed or non-union can result from an inappropriate mechanical environment, co-morbidities such as diabetes and vascular disease, drugs such as corticosteroids, infection, and poor vascularity secondary to smoking. It is the goal of surgery to convert secondary to primary healing by reducing micromotion and the gap between bony segments. Infants and children heal fractures more rapidly than do adults, but there is little evidence that aging *per se* affects the rate or extent of fracture healing.

Animal Models of Bone Wound Healing with Radiation Exposure (Combined Injury)

Bone Defect Models. There are two major categories of experimental osseous lesions that are used as research models; it is important to understand the distinction because of the differences in physiology, significance, and relevance (Table 2). The most common one is the surgical creation of a large osseous defect that exceeds the capacity of the bone to

regenerate spontaneously. One example is an wide intersegmental gap or osteotomy in a long tubular bone, such as the femur, requiring fixation with plates and screws, an external device, or an intramedullary nail to hold the segments in place. The general rule is that a gap length 1.5 to 2 times the diameter of the bone should not heal completely. There is always the possibility that excess blood can pool in a void and provide a stable clot for bone formation. Other examples use large drilled holes. Key principles for defect models include controlling mobility and preventing hematoma stability. It is the hematoma that initiates fracture healing; when the gap is too large for hematoma stability, there is no or minimal healing and the defect endures. Large empty defects fill with fibrous connective tissue. Defect models are useful to test natural and synthetic bone-substitute implant materials and composites against the "gold standard" of fresh autogenous bone grafting, which when properly done achieves complete incorporation, remodeling, and restoration of the defect. Rats, rabbits, dogs, sheep, and goats are used for such evaluations [8]. Complicating infections are rare, particularly with rodent models, but rodents do not have Haversian type of bone and remodeling but are used to investigate the initial phases of bone formation. Dogs, sheep, and goats have bone turnover rates that are much higher than in humans. Thus, non-human primates are the best choice, but are costly, with limited availability [9].

Table 2. Comparison of Experimental Bone Defects and Wounds

	Bone Defects	**Bone Wounds**
Characteristic	Non-healing void	Healing injury
Examples	Segmental defects, large intraosseous lesions	Experimental fractures, small intraosseous lesions
Mechanism	Too large or mobile to heal	Spontaneous osteogenesis
Purpose	To test bone grafts, bone-substitute implants, tissue engineered constructs for osteoaugmentation	To define healing process, to test materials for osteocompatibility
Utility	To examine influence of age, diseases, knockdown genes, irradiation, treatments	To examine influence of age, diseases, knockdown genes, irradiation, treatments

It is advisable to include empty defects and fresh autogenous grafts as negative and positive controls, respectively. Model validations and timecourse studies are needed to identify the mechanism(s) by which a test material results in bone formation in the defect. We distinguish three mechanisms: osteogenesis, osteoconduction, and osteoinduction [10]. Osteogenesis refers to the bone formation process that occurs when viable osteoblasts and/or their progenitors are transplanted from one part of the body to another; a fresh graft, marrow, or cell-based products are examples of osteogenesis. Osteoconduction refers to the material providing a compatible scaffold that supports the ingrowth of new bone from the margins of the defect; this is reminiscent of the passive osteoconductive role of a hematoma. Osteoinduction is rather different; it refers to the property of a material to induce non-bone-forming cells, such as found in connective tissue, to change phenotype and produce bone, usually through the endochondral pathway. A material or factor can be established as osteoinductive by its ability to induce host tissue ossification when implanted in a subcutaneous site. Demineralized bone is an example of an osteoinductive material. A test material can elicit a combination of these processes and composite materials can be

designed to optimize multiple mechanisms [5].

To monitor the activity and fate of a test bone substitute material, investigators use quantitative radiographic, histomorphometric, and mechanical analyses. The comparatively flat parietal bone on the skull has advantages for its surgical ease and reproducibility, lack of need for fixation because it is intraosseous, radiographic and histologic simplicity, and experience with inexpensive rodents. Although some cranial models entail drilling a wound that spans both parietal bones, thus traversing the midline suture, the caveat is that perforation of the dura with bleeding happens and wastes animals. A safer, more reproducible approach entails a defect within each parietal bone [11]. Not only does this avoid risks associated with the midline suture and provides two defects per animal, it also precludes hematoma formation and avoids the regenerative contribution of local activated sutural cells. The disadvantage of the calvarial defect is that it is not a weight-bearing model. The rat mandibular ramus is a good site for defects because it offers an environment subject to compressive and shearing forces [12].

Most published studies with defect models to evaluate grafts or bone-substitute implants use healthy, growing animals. Those findings may have little relevance for intended use in aged, diseased, or chronically medicated patients. There is some information with aged, diabetic, or other disease models. For example, a comparison of the effects of recipient age showed that a BMP-2 device healed calvarial defects by 4 weeks in young mice; in old mice healing was delayed and incomplete [13]. Defect repair with bone graft was reported to be superior in control rats than in streptozotocin-induced diabetic rats [14]. Ovariectomy results in reduced bone formation with bone grafts in the mandibles of aged rats [15]. Strains of rodents with immune disorders are often used to evaluate cellular or acellular tissue matrices from other species to avoid rejection. Connectivity of findings from experiments in athymic test animals to euthymic animals or patients is very weak and, in fact, can result in mis-information because they do not reveal inflammatory reactions to the implant.

Bone Wound Models. Investigations of fracture healing require a standard method to make a reproducible fracture and to quantitatively monitor healing. There are many fracture models that attempt to mimic traumatic fractures of long bones with associated soft tissue trauma and inflammation. Controlled osteotomies lack the acute soft tissue trauma, but are more reproducible [16]. As with defects, most of the literature concerns normal, healthy animals for research on effects of agents to stimulate healing, such as prostaglandin PGE2 [17] and of inhibitory drugs, such as indomethacin [18].

Considered a variation of fracture healing, Distraction Osteogenesis (DO) is a technique for expanding bone volume in oral and orthopedic surgery by exploiting bone's innate capacity for osteogenesis in response to tension forces applied across an osteotomy by an external distraction device. Models in the minipig [19] and rat [20] mandibles have been validated, the latter being used to show nicotine inhibition of neovascularization and osteogenesis in the distraction gap.

A bone wound model is an experimentally created osseous lesion that heals spontaneously. The wound location and species should be validated to show that healing follows the processes that occur in fracture healing. We developed a healing unicortical tibial wound in rats, first for the purpose to monitor osteocompatibility of implant materials [21]. As expected, implantation of demineralized bone powder was compatible with wound healing. Some proposed bone-substitute materials were found to evoke local inflammatory reactions when placed near marrow; excessive inflammation inhibited bone formation [22]. We concluded that a healing wound model adds information about materials to that obtained from a non-healing defect model. Subsequently, we adapted the tibial wound model for C57BL/6NHsd female mice [23]. Histopathological evaluation indicated that tibial wounds healed spontaneously with complete bridging with new bone by 35 days. Because this wound model was intraosseous and without mobility to the void, there was no cartilage phase. There was rapid filling of the intramedullary marrow space with robust temporary bone. Osteoclast-mediated resorption removed that bone while the cortical void was regenerated. In contrast, immunocompromised NOD/SCID mice showed little evidence of cortical healing, with poor revascularization, persistence of residual hematoma, and weak fibrosis in the medullary canal [24]. This shows the importance of inflammation for osteogenesis. We described other genotype differences in wound healing [24]. A study of the effect of age on wound healing compared 5-month-old SAMR1 with SAMP6 mice, the latter known for normal skeletal development but with premature osteopenia beginning at 4 months of age. There was significantly slower wound healing in SAMP6 than in control SAMR1 mice, at all timepoints. Such genotype differences are important to consider whether one is designing studies to delay or to accelerate wound healing.

Effects of Irradiation on Repair of Bone Injury. A mouse study of mortality due to total body irradiation (TBI) combined with exposure to blast pressure in a detonation chamber showed that most of the deaths in high blast groups occurred on the day of exposure [25]. There was little evidence of increased ensuing mortality except in the group with highest radiation does (600 r) and highest blast pressure (7.1 kilogram-force per square centimeter). Skeletal effects in that model were not examined by those researchers. A 1959 report of whole-body irradiation to young growing rats with experimental femoral fractures showed the expected dose-dependent mortality over 400 r. Lower doses produced a delay in fracture healing for 3 weeks, with gradual recovery [26].

TBI adversely affects static parameters of bone status. Mice exposed to TBI showed profound abnormalities in trabecular architecture at 110 days post-irradiation, including markers of osteopenia [27]. A higher dose of TBI in young and older mice resulted in early bone and bone marrow loss, but recovery in older mice was significantly diminished [28]. TBI resulted in bone loss and reduced the pool of MSCs [29] and increased osteoclast number and markers of bone resorption [30]. Extent of bone loss was shown to be different for different mouse genotypes [31]. In a study with B6D2F1 mice, skeletal tissue loss was exacerbated by TBI plus skin wound injury, compared with TBI alone [32]. This shows long-term, exacerbated bone effects when TBI was combine with skin wound trauma.

Models of combined bone injury with TBI universally show detrimental effects on healing parameters. A study of femoral fractures with fixation in retired breeder rats showed atrophic non-union in the TBI group [33]. Some studies use bone injury models with irradiation restricted to the site of injury in order to remove the influence of systemic effects. Local irradiation to legs of rats with closed femoral fractures showed a 4-week delay in recovery of biomechanical properties [34]. A follow-on study by those investigators with irradiation at intervals after the fracture demonstrated that there was a window of inhibition [35]. Because fracture healing was diminished less when irradiation was delayed for 10 days post-fracture compared with 3 days, it can be concluded that events in the first few days following injury are crucial to healing and are vulnerable to irradiation. The model of rat mandibular DO was used to show that irradiation of the distraction region produced voids and fibrosis rather than bone fill and bridging [36].

Another approach using titanium conduction chambers in rabbit tibial metaphyses that allow for quantification of bone ingrowth showed a dose-dependent decrease in bone formation, with partial recovery over time after irradiation [37, 38].

We used the mouse tibial wound method to assess effects of irradiation on bone healing [23]. With C57BL/6KNsd mice, there was a dose-dependent delay in tibial wound healing with a single dose of 10, 20, or 30 Gy delivered to the leg; local doses of 40 and 50 Gy produced severe soft tissue damage and skin ulceration that precluded further analyses. Because of the baseline differences in rates of wound healing for different mouse genotypes, there were also genotype differences in the magnitude of inhibition with 20 Gy irradiation to the leg [24]. Serial histological analyses showed evidence of partial recovery and late osteogenesis in C57BL/6NHsd, SAMR1, and SAMP6 mice, but not in NOD/SCID mice. The effects of irradiation on bone repair have also used beagles [39] and rats [40].

Another reproducible model follows alveolar bone healing histologically in tooth extraction sockets in rats. The socket fills with capillary-rich granulation tissue within 2-3 days post-extraction, followed by osteoblastic bone formation in the socket and also outside the socket in mandibular periosteum especially if growing rats are used. Socket healing is delayed in animals irradiated immediately after extraction and even if irradiated 2 weeks prior to extraction [41]. The rat molar extraction model was used in a short-term (5 days) study to compare the effects of local and whole-body irradiation (8 Gy) on healing of the bony socket [42]. Whole-body irradiation was profoundly damaging to bone formation in the socket, but had little effect on the periosteal bone formation. This suggests that periosteal cells may be more differentiated and less radiosensitive than the progenitor cells needed for bone socket healing.

Possible Countermeasures

Skeletal effects of TBI have been mitigated by different approaches. Tibial axial compression is a method to apply anabolic mechanical stimulus to a mouse limb. The anabolic effect on net accrual of cancellous and cortical bone mass was not diminished by

TBI; that observation suggested that mechanical stimulation may be an effective way to improve bones exposed to ionizing radiation [43]. Limb unloading models have also been used, in which one hindlimb is suspended from the top of the cage. The extent of TBI-induced bone loss was similar in control and hindlimb-unloaded mice, but only the irradiated specimens showed oxidative damage. Irradiation-induced bone loss was mitigated with administration of an anti-oxidant, alpha-lipoic acid, in that study with C57/Bl6 mice [44]. In a study with Wistar rats, hindlimb suspension caused greater bone loss than did TBI, but the group exposed to both treatments showed even greater bone loss [45].

Acute effects of TBI on normal tissues vary with dose, but are most prominent in tissues with rapidly proliferating cells, such as in the skin or alimentary tract [46]. Some common mechanisms can be described. For example, DNA damage can result in cell death, apoptosis, senescence, or recovery by DNA repair enzymes. Activation of tissue-specific cellular signaling pathways usually manifest as inflammation, vascular injury, oxidative stress, and initiation of fibrosis. Thus, normal wound healing can be retarded because of the importance of cell proliferation and neo-angiogenesis in the repair process. Most of the classical agents used to prevent radiation damage have been tested as radioprotectants, i.e., administered prior to exposure to therapeutic radiation and include corticosteroids, non-steroidal anti-inflammatory drugs, antioxidant drugs and natural products [47]. Amifostine was shown to be an effective radioprotectant, administered 45 minutes prior to irradiation of the rat mandible, and prevented the deleterious effect of irradiation on distraction osteogenesis [36]. More research is needed to test such agents following combined injury, but some progress has been made. In a rat model of mandibular fracture, a combination of the radioprotectant amifostine and the angiogenic stimulus of desferoxamine improved all metrics of healing of irradiated fractures [48]. With the murine model of tibial wound healing to test radioprotection and mitigation, we reported normal bone healing with either amifostine or a novel gramicidin S-conjugated nitroxide (JP4-039) when administrated before or after irradiation [23]. Similar results were found in tibial wounds in SAMP6 mice treated with JP4-039 at the time of irradiation [24].

Studies with irradiated cultured cells may be useful to discover agents to restore functionality. *In vitro* irradiation of cultured cells, such as human Mesenchymal Stem Cells (MSCs), results in stress-related senescence, as exemplified by dose-dependent decreased proliferation, increased doubling time, increased senescence-associated β-galactosidase (SA-β-gal) activity, and activation of p53 and p21 [49]. Each of those effects of irradiation are exactly those that are seen in comparisons of MSCs from older vs. younger subjects [50]. This concordance, most familiar for cultured fibroblasts [51], suggests that *in vitro* irradiation may also serve as a model for stem cell senescence. This is further supported by analysis of the senescence-associated secretory phenotype in irradiated and otherwise stressed conditions [52].

DNA damage is a characteristic effect of certain levels of ionizing radiation, with activation of a network of damage response pathways to mediate recovery [53]. Zoledronate is a potent nitrogen-containing bisphosphonate used clinically for osteoporosis and other bone-

resorption disorders. Because of its mechanism as an inhibitor of prenylation of many GTPases, it was tested as a radioprotectant with pediatric MSCs [54]. Many beneficial effects of zoledronate were described first for non-irradiated expanded MSCs, including extension of proliferation and differentiation potentials, reduced levels of SA-β-gal, p21, p16, and DNA damage. Those benefits were also described for cells treated with zoledronate prior to irradiation, with evidence of inhibition of mTOR signaling mediating the drug's known action. Efficacy *in vivo* was demonstrated with 3-days of zoledronate pretreatment resulting in lower number of DNA damage foci and higher numbers of bone mesenchymal progenitors in marrow isolated 24 hours after irradiation of C57Bl/6 mice.

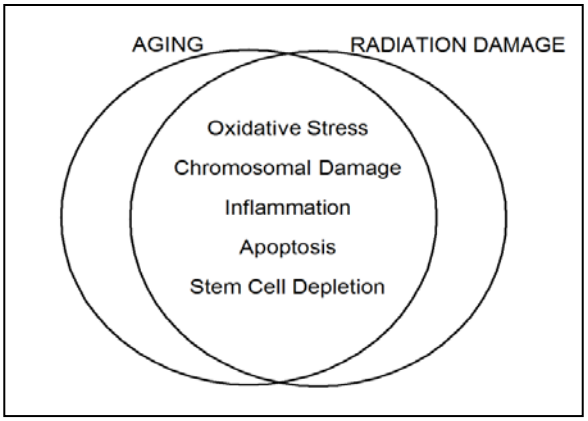
Mouse MSCs deficient in the Fanconi anemia group protein FANCD2 were shown to be more radiosensitive to ionizing radiation, taken as evidence of the importance of double strand DNA break repair [55]. Furthermore, systemic administration of JP4-039 ameliorated radiosensitivity *in vitro* of *FancD2*$^{-/-}$ fibroblast cell lines [56].

A murine osteoblast-like cell line, OCT-1 was used to show dose-dependent irradiation-induced DNA-damage and G2 arrest with some preliminary evidence of increased mineralization [57], but the osteogenic effect may be considered a low-dose and time-sensitive effect as suggested by others [58].

Future Directions

There is insufficient understanding of the pathophysiology of combined injuries. More knowledge is needed in order to identify those injuries that need prompt attention and to develop optimum therapies. Well-characterized animal models are needed to help define the impact of all the variables involved. Nuclear accidents and wartime incidents show general principles, but are not documented well enough to establish protocols. It is unclear whether trauma in the face of irradiation is affected only by the progressive symptoms of irradiation, or whether the combination represents unique mechanisms of injury potentially sensitive to distinct therapeutic approaches. The critical symptoms of radiation sickness develop over weeks after exposure. That is why skin injuries, for example, sustained at the time of irradiation can show early evidence of healing, but relapses occur after one to two weeks [59]. Progressive thrombocytopenia causes later hemorrhages in wound sites and granulocytopenia reduces resistance against wound infection. Increased probability of infection and risk of hemorrhages in the weeks following exposure indicate that life-saving surgical procedures should be performed immediately.

Fig. 2



Many of the cellular effects of irradiation are the hallmarks of aging (Figure 2), including the molecular and cellular underpinnings of skeletal aging [60]. Genetic mechanisms include DNA damage; oxidative stress damage includes accumulation of reactive oxygen species (ROS) and p53; "inflammaging"

involves activation of cytokines, NF-κB, and fibrosis. Exploration of features common to combined injury research and aging research may advance solutions to both endeavors.

Identifying shared and unique mechanisms of impaired wound healing in the setting of whole-body irradiation requires animal models with potential to identify targets for therapeutic intervention. Evidence from existing animal models suggest that it is unlikely that prevention of acute radiation sickness alone will mitigate damage to wound healing. It will be important to define the impact of delay and duration of treatments and possible synergy with different classes of agents. Agents that target ROS, osteoblast progenitors, damaged endothelium, apoptosis, and necrosis may be optimized together or in series.

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