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Overview of Biological Mechanisms and Applications of Three Murine Models of Bone Repair: Closed Fracture with Intramedullary Fixation, Distraction Osteogenesis, and Marrow Ablation by Reaming

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Abstract

Fractures are one of the most common large-organ, traumatic injuries in humans, and osteoporosis-related fractures are the fastest growing health care problem of aging. Elective orthopedic surgeries of the bones and joints also represent some of most common forms of elective surgeries performed. Optimal repair of skeletal tissues is necessary for successful outcomes of these many different orthopedic surgical treatments. Research focused on post-natal skeletal repair is therefore of immense clinical importance and of particular relevance in situations in which bone tissue healing is compromised due to the extent of tissue trauma or specific medical co-morbidities. Three commonly used murine surgical models of bone healing, closed fracture with intramedullary fixation, distraction osteogenesis (DO), and marrow ablation by reaming are presented. The biological aspects of these models are contrasted and the types of research questions that may be addressed with these models are presented.

Keywords

Distraction Osteogenesis; Fracture; Marrow Ablation; Murine Models; Orthopedic Surgery

Introduction

Fractures are one of the most common traumas that humans experience and are the endpoint manifestation of osteoporosis, the most common chronic disease of aging (Balogh et al., 2012). Elective orthopedic surgeries of the bones and joints also represent some of most common forms of elective surgeries performed (Praemer et al., 1999). Insufficient healing of bone fractures ranges from 10–15% for all trauma while poor healing of elective hip arthroplasties is significantly greater in diabetic patients than in non-diabetic patients (Marchant Jr. et al., 2009). Almost all of these cases of poor healing require surgical intervention at significant cost and with considerable morbidity. These cases alone represent

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about 1/3 of the cost of orthopedic treatments of bones and joints and will only increase in populations with specific co-morbidities such as diabetes, obesity, and as the population ages. The optimal repair of skeletal tissues is therefore necessary for the successful outcome of these many different orthopedic surgical treatments. Research focused on postnatal skeletal repair is therefore of immense clinical importance and of particular relevance in situations in which bone tissue healing is compromised due to the extent of tissue trauma or specific medical co-morbidities.

The repair of fractures and bone tissues as surgical treatments is unique in that bone tissues are one of the few postnatal tissues that retain a capacity for complete repair by regenerating the original structure and tissue composition. The biological processes that occur during tissue repair that occurs in response to different types of orthopedic procedures are very different and, ideally, specific surgical models should be used to assess various therapeutic modalities in the context of their surgical use. Three methodological approaches that are used to examine repair of skeletal hard tissue are presented. Specific considerations for choosing among these three approaches is reviewed here with a focus placed on the types of research questions that may be addressed with differing models (Ferguson et al., 1999; Gerstenfeld et al., 2003; Phillips, 2005).

Fracture

One of the more common models to study post natal bone formation and repair is the stabilized closed fracture due to the simplicity of the procedure (outlined in the companion article [*Copyed: reference other Gerstenfeld article 14-0161]) and that repair recapitulates limb development during embryogenesis, primarily endochondral osteogenesis. This model has been extensively used to assess the molecular mechanisms that regulate post natal skeletal tissue development, and assess the safety and efficacy of biologicals and pharmaceuticals that effect bone formation (extensively reviewed in (Ferguson et al., 1999; Gerstenfeld et al., 2003; Phillips, 2005). During fracture repair several cell types from the cortex, periosteum, bone marrow space, and surrounding soft tissue must coordinate to form a cartilaginous callus followed by the replacement of mineralized bone (Ai-Aql et al., 2008; Ferguson et al., 1999; Gerstenfeld et al., 2003). Although endochondral osteogenesis is the primary mechanism for bone repair, intramembranous osteogenesis may also be observed internal to the periosteum at both proximal and distal ends of the callus (Dimitriou et al., 2005). Four overlapping phases coupled with angiogenesis contribute to callus formation and fracture repair (Schindeler et al., 2008). The radiological, structural biological and molecular phases of fracture healing are presented in Figure 1.

Inflammatory Phase

Immediately following a trauma-induced fracture is the inflammatory response and hematoma formation (Gerstenfeld et al., 2003; Marsell and Einhorn, 2011). The inflammatory phase is crucial for the initialization of the fracture healing cascade. During the inflammatory phase, mesenchymal/progenitor cells are recruited to the site of injury and a response in the periosteum is detected within 24–48 hours post fracture. The periosteum is a bilayer membrane lining the cortical surface of bone. It is highly vascularized, provides pluripotent cells, and is required for successful bone repair (Colnot et al., 2012; Colnot et al.,

2003; Hankenson et al., 2014; Lu et al., 2005). Current literature suggests it is actually the periosteal response that provides the osteochondral progenitor cells needed to form the bridging callus (Colnot et al., 2006; Colnot, 2009). Recently a population of alpha Smooth Muscle Actin positive cells was identified which reside within the periosteum, and in response to fracture, proliferate and contribute to callus formation (Grcevic et al., 2012; Matthews et al., 2014). There are, however, other sites of origin for mesenchymal/progenitor cells that contribute to fracture repair. Mesenchymal stem cells originating from the bone marrow contribute to the inflammatory cells and osteoclast lineage (Colnot et al., 2006). Progenitor cells also reside in the endosteum, and are capable of differentiating toward the osteoblast linage contributing to bone formation via intramembranous osteogenesis (Colnot, 2009; Hankenson et al., 2014; Kumagai et al., 2008). Further research is needed to identify the originating sites for the mesenchymal/progenitor cells and its contribution to fracture repair.

The inflammatory phase is a highly coordinated event where cytokines (Interleukin (IL)-1, IL-6, and Tumor Necrosis Factor (TNF) α), growth factors (Bone Morphogenetic Protein (BMP) 2, Transforming Growth Factor β (TGF-β), and Platelet-derived Growth Factor (PDGF)), and mechanical forces direct the very early stages of callus formation (Ai-Aql et al., 2008). These factors are involved with angiogenesis, recruitment of inflammatory cells, and enhancing extracelluar matrix synthesis (Ai-Aql et al., 2008; Kon et al., 2001; Marsell and Einhorn, 2011). Recent work by Dishowitz et al using a model of reduced Notch signaling showed extended periods of inflammation reduced callus size, and altered chondrogenesis and bone maturation (Dishowitz et al., 2013). Although direct and indirect effects of Notch signaling may alter the fracture healing process, this work suggests additional research is needed to elucidate the possible negative role of inflammation has on fracture repair (Colnot et al., 2006; Dishowitz et al., 2013; Hankenson et al., 2014; Xing et al., 2010).

Cartilage Formation Phase

As the inflammatory phase ends, there is an ingrowth of fibrin-rich granulation tissue at the location of the hematoma. Progenitor cells differentiate to chondrocytes producing the extra-cellular matrix (ECM) and forming a cartilaginous callus, rich in type II collagen and proteoglycans (Hankenson et al., 2014; Marsell and Einhorn, 2011; Rahn, 1982). Although the soft callus begins to form at the distal and proximal edges of the fracture and grows inward, callus formation is actually asymmetrical. Fractures of the femur produce predominately distal-forming calluses while tibia fractures produce proximal-forming calluses. It is possible that this too recapitulates long bone formation where the growth plates are located distally in the femur and proximally in the tibia (Gerstenfeld et al., 2006; Morgan et al., 2009). The soft callus stabilizes the fracture which peaks between 7–9 days post fracture (Marsell and Einhorn, 2011). It should be noted that mechanical forces due to the stability of fractures also influence callus size. Relatively large calluses are produced with less mechanically stable fractures; however there is a threshold at which no callus will form if the fracture is too unstable (Ai-Aql et al., 2008; Colnot et al., 2012; Hankenson et al., 2014).

Cartilage Resorption and Primary Bone Formation Phase

Several events must then occur while the callus is transitioning between a soft cartilaginous tissue and a rigid calcified bone tissue (primary bone formation). Chondrocytes proliferate, mature, and become hypertrophic resulting in increased synthesis of type X collagen and matrix mineralization. Increased expression of TNF-α during this phase, initiates apoptosis of the hypertrophic chondrocytes. However, overexpression of TNF-α can also lead to deficiency in bone healing due to premature cartilage removal, demonstrating a highly coordinated transition for primary bone formation. There is also increased expression of Macrophage Colony Stimulating Factor (M-CSF), Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), and Osteoprotegerin (OPG), which recruits and regulates differentiation of osteoclasts (Barnes et al., 1999; Gerstenfeld et al., 2003; Hankenson et al., 2014; Kayal et al., 2007; Lehmann et al., 2005; Marsell and Einhorn, 2011). These multinucleated cells may contribute to the resorption of the mineralized cartilage, however, these cells by themselves are not solely responsible for the removal of the soft callus even though they are present at four-fold greater numbers during the resorption phase of the mineralized cartilage than seen in during the resorption of the hard callus (Gerstenfeld et al., 2006).

More recent data suggest that matrix metalloproteinases (MMPs) expressed by multiple cell types, including osteoblasts, hypertrophic chondrocytes, osteoclasts and vascular endothelial cells, are key in driving the resorption of the soft callus (Behonick et al., 2007; Galis et al., 2002; Kosaki et al., 2007). Currently more than 20 MMPs comprise a family of proteinases that modify the extracellular matrix in multiple processes (McDonald et al., 2013). Of these members, MMP-9, MMP-14, and MMP-13 are involved with endochondral ossification and bone formation (Ortega et al., 2010). Inhibition of MMPs impaired the endochondral union and knockout of MMP-9 or MMP-13 in mice altered fracture repair shown by the increased size of the cartilage callus, non-union/delayed union, and decreased vascular invasion in the hyptrophic cartilage callus (Behonick et al., 2007; Colnot et al., 2003; Kosaki et al., 2007). Further, the use of bisphosphonates which inhibit osteoclast activity (zoledronic acid) or numbers (clodronate) do not result in altered cartilage resorption. It is the remodeling phase that is inhibited with bisphosphonates (McDonald et al., 2008). As the mineralized cartilage is being resorbed, blood vessels and osteoblasts infiltrate the area. The bone-forming cells, osteoblasts, use the soft callus as a template to deposit woven bone replacing the resorbed mineralized cartilage (Schindeler et al., 2008). As the hard callus replaces the cartilaginous soft callus, it starts at the edges and move inward. A thin outer cortical shell forms with an inner-supporting trabecular structure connecting the outer shell with the cortex (Gerstenfeld et al., 2006; Morgan et al., 2009). The hard callus formation peaks around day 14 post fracture (Marsell and Einhorn, 2011).

Second Resorptive Phase

A second resorptive phase follows the primary bone formation. Whereas MMPs are crucial for the resorption of the soft callus and osteoclast function appears to be of lesser importance, osteoclasts are needed for the second resorptive phase. Numerous studies have shown that inhibiting osteoclasts with either bisphosphonates or anti-resortpive agent (Denosumab) that blocks osteoclast differentiation results in altered bone resorption,

resulting in larger calluses within increased mineralized tissues (Gerstenfeld et al., 2009) and strength (Gerstenfeld et al., 2009; McDonald et al., 2008). Bone remodeling occurs with osteoclasts resorbing woven bone followed by the replacement of lamellar bone formation by osteoblasts. During this process the original cortical and trabecular bone is restored and the medullary cavity is re-established. As a result the biomechanical properties return (Ai-Aql et al., 2008; Schindeler et al., 2008). There is increased expression of TNF-α and IL-1, continued BMP2 expression and decreased expression of other members of the TGF-β family. RANKL and M-CSF expression begin to decrease (Ai-Aql et al., 2008; Gerstenfeld et al., 2003; Hankenson et al., 2014; Marsell and Einhorn, 2011; Mountziaris and Mikos, 2008). This is the longest phase for bone repair (Hankenson et al., 2014; Marsell and Einhorn, 2011).

Role of Angiogenesis during Fracture Repair

Fracture repair is dependent on angiogenesis, the formation of new blood vessels from pre-existing vessels, and blood supply. When either angiogenesis is inhibited or ischemia is induced, the fracture healing process is hindered (Hausman et al., 2001; Keramaris et al., 2008; Lu et al., 2007). Although angiogenesis may be involved during the inflammatory phase, the central role of angiogenesis appears to be involved with the endochondral process for the transition from a soft, cartilaginous callus to a hard, mineralized tissue (Ai-Aql et al., 2008; Lehmann et al., 2005).

Regulating angiogenesis are two key pathways: Vascular Endothelial Growth Factor (VEGF) -dependent and angiopoietin-dependent pathways. Angiopoietin-1 and -2 are vascular morphogenetic proteins. Angiopoietin-1 is expressed early during inflammation and cartilage phases suggesting a possibility of an initial in growth of vessels (Ai-Aql et al., 2008; Gerstenfeld et al., 2003; Lehmann et al., 2005; Suri et al., 1996). However, the current literature suggests that the VEGF-dependent pathway is the key regulator of angiogenesis during fracture repair. The addition of VEGF promotes fracture healing. And it is this pathway that couples angiogenesis with cartilage resorption and the primary bone formation phase (Ai-Aql et al., 2008; Gerber et al., 1999; Street et al., 2002). Members of the VEGF family, VEGF-A, VEGF-C and VEGF-D are expressed during the end of cartilage mineralization by both the hypertrophic chondrocytes and the emerging osteoblasts (Ai-Aql et al., 2008; Yeh and Lee, 1999). However, the bioavailability and removal of the hypertrophic cartilage allowing for vessel invasion is heavily dependent upon MMPs for endochondral bone formation found in fracture healing and the growth plate (Behonick et al., 2007; Colnot et al., 2003; Holmbeck et al., 1999; McDonald et al., 2013; Vu et al., 1998). Osteoclasts and smooth muscle cells express MMP-9 while osteoblasts and hypertrophic chondrocytes express MMP-13. It is possible that other cell types express MMPs, including MMP-9 and MMP-13 (Behonick et al., 2007; Colnot et al., 2003; Kosaki et al., 2007; McDonald et al., 2013; Ortega et al., 2010). The knockout of MMP-9 not only resulted in altered fracture healing, but reduced vascular invasion of the hypertrophic cartilage. The PECAM1 positive endothelial cells stayed at the periphery of the fracture callus while in the wild type the PECAM1 positive cells infiltrated the callus (Colnot et al., 2003). Similar angiogenesis results were seen with the knockout of MMP-13 (Behonick et

al., 2007; Kosaki et al., 2007). The release of VEGF regulates both angiogenesis and recruitment of osteoclasts.

Distraction Osteogenesis

Distraction osteogenesis (DO) is a bone regenerative process where an osteotomy is created followed by a gradual separation of the two pieces of bone resulting in the formation of a gap and increased bone length, this is also known as “distraction” (Fig. 2). This distraction yields formation of new bone within the gap, forming a bridge between the two ends of the bone. The newly formed bone is thought to be a response to the longitudinal mechanical strain applied to the callus during healing (Ai-Aql et al., 2008; Codivilla, 1905; Ilizarov, 1989). This technique is useful to study bone regeneration due to the three mechanisms for bone formation. During the early stages of DO, endochondral ossification occurs, where cartilage formation is limited to the periosteum, not within the distraction gap. It is thought that the distraction environment may suppress cartilage development while periosteal disruption is not influenced by these forces (Jazrawi et al., 1998). Chondroid bone forms with a gradual transition to bone, called transchondroid ossification. Lastly, the predominant process of ossification is intramembranous bone formation, the direct formation of bone without the intermediate of cartilage. It is observed in the later stages of DO (Choi et al., 2002; Yasui et al., 1997). In all there are three phases of DO which are defined by the distraction process.

Latency

The first phase of DO is termed latency and consists of the initial trauma response. It is very similar to the early phase of fracture repair. Hematoma forms at the osteotomy site. Inflammation occurs and there is increased expression of pro-inflammatory cytokines, IL-1 and IL-6. By the end of latency, IL-1 and IL-6 return to baseline (Ai-Aql et al., 2008; Cho et al., 2007). Mesenchymal stem cells are recruited to the site. The contribution of precursor cells localized in the periosteum to the newly formed bone is not known for DO, although they are key for fracture repair (Abou-Khalil and Colnot, 2014; Ai-Aql et al., 2008; Colnot et al., 2006). There is increased expression of BMP2 and BMP4 during early latency and these contribute to the differentiation of precursor cells into chondrogenic and osteogenic cells. Cartilage formation begins at the periosteum. Compared to the early expression of BMP2 and BMP4, a peak for BMP6 expression is detected later at the end of latency [*Author: the following phrase seems to be missing a word or two. Please edit to clarify.] and early phase of active distraction reflecting the ossification mode transitioning from endochondral to intramembranous. The primary inflammatory process is completed by the time of active distraction (Farhadieh et al., 2004; Lammens et al., 1998; Li et al., 1998; Liu et al., 1999; Rauch et al., 2000; Sato et al., 1999).

Active Distraction

Tensile forces are applied to the callus at a specific rate and rhythm in order to stretch the callus during active distraction. The mechanical force increases BMP2 and BMP4 expression, contributing to bone formation. The early phase of active distraction shows a high ratio of RANKL/OPG resulting in resorption of cartilage that formed during latency

and endochondral bone formation begins (Ai-Aql et al., 2008; Li et al., 1998; Rauch et al., 2000; Sato et al., 1999). During this phase several zones are created with certain cell types also contributing to the bone regeneration process. The ‘fibrous interzone’ (FIZ) is a central fibrous area with collagen fibers running parallel to the bone. It is enriched with chondrocyte-like cells, fibroblasts, and oval cells (Aronson, 1994; Sato et al., 1998; Vauhkonen et al., 1990). Oval cells are morphologically intermediates between fibroblasts and chondrocytes that express IL-6 once distraction begins. It is suggested that the IL-6 stress response contributes to the intramembranous osteogenesis by enhancing differentiation of cells committed to the osteoblast linage (Ai-Aql et al., 2008; Cho et al., 2007). Along the collagen bundles within the FIZ, osteoid (non-mineralized bone) is deposited by osteoblasts. Mineralization occurs forming the zone of ‘microcolumn formation’ (MCF). An area of highly proliferating cells between FIZ and MCF is called the ‘primary matrix’ or ‘mineralization front’ (PMF) (Aronson et al., 1990). Once the desired length is achieved, distraction ends and consolidation begins.

Consolidation

The period after active distraction, consolidation, is when mineralization and remodeling occurs. The bone columns formed during active distraction begin to interconnect. The expression of BMP2 and BMP4 decrease to undetectable levels, though this may take up to two weeks (Marukawa et al., 2006; Yazawa et al., 2003). Exogenous BMP2 shortens treatment time by accelerating bone formation during consolidation (Yonezawa et al., 2006). This suggests that BMPs may have a role in differentiation of cells needed to complete bone regeneration during the early consolidation. Osteoclasts are recruited and remodeling occurs. This process will result in the newly formed bone to be fully integrated with the femoral structure including the cortical bone and restored medullary canal.

Angiogenesis

Similar to fracture, DO also requires angiogenesis and increased blood flow. Inhibition of angiogenesis during DO can result in a fibrous non-union (Aronson, 1994; Carvalho et al., 2004; Fang et al., 2005; Jacobsen et al., 2008). New vessel formation may begin during active distraction, however, vessel volume maximal increase occurs during consolidation, similar to bone volume increases during consolidation, suggesting a link between angiogenesis and bone formation (Matsubara et al., 2012; Morgan et al., 2012). A comparison between the induction of angiogenesis that occurs during distraction compared to fracture is seen in Figure 2C. While there is a considerable increase in the number of new vessels in the muscle tissues and within bone after osteotomy, as compared to controls, the application of mechanical stimulus of distraction produces a much greater angiogenic response, seen in comparing the distracted sample to the osteotomy alone. It is unclear, however, if the increased angiogenesis in the muscle is in response to supporting bone formation or if it is also related to the effects of distraction on increasing muscle mass (Nishisho et al., 2012).

Regulating angiogenesis during DO is thought to be VEGF-A and neuropilin, an alternative receptor for VEGF. These proteins are expressed many times more than other VEGF and receptors during DO (Carvalho et al., 2004). Blocking VEGF receptors with antibodies

results in decreased angiogenesis and significant decrease in bone formation (Jacobsen et al., 2008). In addition, VEGF-A expression is localized to maturing osteoblasts at the PMF and to osteoclasts in the MCF zone, directing angiogenesis in this region of the distraction gap (Choi et al., 2002). Although VEGF-D does peak at the end of latency and early phase of active distraction, it decreases at later stages when angiogenesis is most active (Carvalho et al., 2004). Other angiogenic factors expressed during DO are angiopoietin-1 and -2. Angiopoientin-2 is antagonistic to angiopoietin-1, however, the co-expression of angiopoietin-2 and VEGF-A may stimulate new vessel formation and enhance the plasticity of existent larger vessels (Pacicca et al., 2003).

Interestingly, there is also emerging evidence suggesting a link between angiogenesis and BMPs. Endothelial and smooth muscle cells express BMP2 during bone regeneration (Matsubara et al., 2012). In return, BMPs initiate the expression of VEGF and associated receptors (Deckers et al., 2002; Yeh and Lee, 1999). However, a conditional knockout of BMP2 in endothelial cells does not inhibit bone growth during embryogenesis and post-natally (McBride et al., 2014). Although there is strong evidence that BMPs and VEGF members may interact during angiogenesis and bone formation, the exact relationship between these different morphogenetic signals has not been fully resolved.

Marrow Ablation

Of the three surgical models described here, marrow ablation by surgical reaming is the simplest to perform, requires the least instrumentation and is the most rapid regenerative process, being completed in 21 days. This procedure was first developed in rats to primarily study coupled remodeling (Suva et al., 1993), with our laboratory subsequently adapting the procedure to mice (Gerstenfeld et al., 2001). [*Author: Figure 3 here?] The procedure may be carried out in either lower extremity long bones, however, in the femur, reaming should be executed from the distal condyle and used to ablate the distal metaphyseal trabecular bone, while in the tibia the reaming is performed through the proximal condyle and ablates the proximal metaphyseal bone. In either bone, the reaming is specifically directed to ablate the trabecular bone under the primary growth plate surface from which the bone grows in length since it is believed that the stem cells that give rise to the stromal element are found in niches under these growth plates (Farnum et al., 2003; Kuhn et al., 1996; Pritchett, 1992).

Phases of Marrow Ablation

The process in characterized by three phases: an initial inflammatory phase in which the marrow space is filled with a blood clot which lasts about three to four days. This is followed by a period of rapid marrow stromal stem cells recruitment in which the reamed space completely fills with newly formed trabecular bone and fibrous tissue. Interestingly, during this early period, the reamed space in which the new bone is formed is devoid of any observable hematopoietic tissues. Subsequently, beginning somewhere between 7 and 10 days post-ablation, resorption of trabecular bone is initiated and a prolonged period of coupled remodeling occurs during which time the hematopoietic tissues are reestablished in the marrow space (Suva et al., 1993). It is interesting to note the expression of multiple stem cell markers that are potentially associated with marrow stromal stem cells that give rise to trabecular bones show two peaks of expression during this process. One is related to the

initial phase after injury within the first seven days. The second is the period in which coupled remodeling is taking place, although the expression of these markers is much lower during this second period of coupled remodeling (Marsell et al., 2014; Wigner et al., 2013).

Differences between the Three Surgical Models

Fracture repair, DO, and marrow ablation all induce bone formation that is initiated by an inflammatory response followed by recruitment and differentiation of precursor cells in order to stabilize and/or restore the bone to its original shape and biomechanical properties. Although these are similar, there are distinct differences including the mechanism for ossification, timing, and signaling that promote bone repair or regeneration. While selecting the appropriate mouse model to examine a hypothesis, these disparities need to be considered. A key difference is whether the model drives bone formation predominantly through an endochondral or an intramembranous ossification process. Both during fracture repair and DO, endochondral and intramembranous ossification occurs. However, fracture repair predominantly relies on endochondral ossification while DO and marrow ablation regenerates bone primarily through intramembranous ossification (Ai-Aql et al., 2008; Hankenson et al., 2014; Kuroda et al., 2005; Marsell and Einhorn, 2011; Suva et al., 1993; Wise et al., 2010). It should be noted that closed fractures are usually stabilized with an intramedullary pin which may impede repair from within the endosteum while the DO is externally fixed with different stability. The sources of contributing stem cells to these processes also have not been fully defined. In this context marrow ablation only produces trabecular bone and is completely endosteal whilst DO will regenerate both cortical and trabecular elements in the region of the regenerative tissues in the distraction gap. When comparing marrow ablation to DO, one must also take into consideration if periosteal tissue contributes stem cells to the regenerative process. In marrow ablation the only source of stem cells is from those within the marrow space or those that are potentially recruited from the circulation (Marsell et al., 2014).

During the early phases of bone regeneration there is formation of a hematoma or blood clot and inflammation. The pro-inflammatory cytokines IL-1 and IL-6 are both induced shortly after the induction of fracture or DO (Ai-Aql et al., 2008; Cho et al., 2007; Kon et al., 2001), whilst IL-1β is increased during marrow ablation (Kuroda et al., 2005). The increases in IL-1 and 6 soon decrease below basal levels during endochondral ossification and only returns to basal levels during remodeling phase for fracture repair. A second peak for IL-6 expression occurs with mechanical strain during the active distraction phase of DO. It has been suggested that IL-6 may have opposing effects on bone regeneration depending on the mouse model. This is demonstrated by the anabolic effect (DO) and catabolic effect (fracture repair) of IL-6 (Ai-Aql et al., 2008; Cho et al., 2007). Its expression is decreased during marrow ablation, only reaching basal level at day 5 and peaking at day 14, well past the osteogenic phase. Also present during fracture repair and marrow ablation is TNFα (Kuroda et al., 2005). Its expression is involved with the transition of cartilage to bone in fracture repair. During marrow ablation it peaks at day 5 with a larger peak at day 14. The expression level of TNFα is much lower during DO; this may be due to less cartilage that needs to be resorbed and the osteotomy injury is not as profound as with the fracture (Cho et al., 2007; Kon et al., 2001; Kuroda et al., 2005).

The timing of events during fracture healing, DO, and marrow ablation are also different. This is demonstrated by the difference in timing and extent of intramembranous and endochondral bone formation and when osteoclast mediated resorption and coupled remodeling is initiated. These differences are exemplified by comparing mRNA expression profiles for representative genes that are associated with these processes (Figure 4). The cartilage response during fracture repair is quick and robust, producing a large volume of tissue outside of the bone occurring within the first week after fracture and peaking shortly after (Ai-Aql et al., 2008). This strong cartilage response is not observed during DO or marrow ablation. Only minimal cartilage is produced during the early phase of DO. Further, this small amount of cartilage is resorbed quickly once distraction begins. Instead of large amounts of cartilage to stabilize the bone defect, during DO large amounts of osteoid is produced within the distraction gap. The synthesis of osteoid in the distraction occurs approximately during the time that the callus in fracture repair is calcified and primary bone formation occurs. Cartilage, however, is not detected histologically, although some gene expression for cartilage is detected, with marrow ablation, (Kuroda et al., 2005; Okuda et al., 2007; Wise et al., 2010). The lack of cartilage formation is most likely due to the population of precursor cells that reside within the endosteum. These cells are restricted to the osteogenic linage, while the periosteum, which contributes to both fracture repair and DO, consists of precursor cells capable to differentiate to both chondrocytes and osteoblasts (Colnot, 2009).

Altered temporal and spatial development of angiogenesis is observed between the three surgical mouse models. Although VEGF signaling is involved with both fracture and DO, the mechanism may not be similar. There is greater vascularization with DO compared to fracture and marrow ablation (Aronson et al., 1990; Matsubara et al., 2012; Morgan et al., 2012; Raines et al., 2011). However there is greater expression of VEGF with fracture repairs compared to DO. Between 7 and 14 days post injury angiogenesis peaks during fracture repair. At this time cartilage is transitioning to bone by hypertrophic chondrocytes expressing VEGF and resorption of the soft callus. Chondrocyte signaling on the other hand is not thought to regulate angiogenesis during DO or marrow ablation. Active distraction initiates angiogenesis and the volume of vessels peaks during consolidation (Matsubara et al., 2012; Morgan et al., 2012). Both the medullary space of the bone and surrounding muscle see an increase in vessel volume during DO (Morgan et al., 2012). However, vessel formation is mostly restricted externally to the fracture repair callus (Ai-Aql et al., 2008; Claes et al., 2002). Neovascularization can be seen as early as day 3–7 during marrow ablation. There is induction of VEGF expression which increases at day 7 and remains increased through at least day 14 when vessel volume also peaks (Kuroda et al., 2005; Raines et al., 2011).

Conclusions

This article presents a review of the biological processes that occur during the progression of bone healing and regeneration in three commonly used murine orthopedic surgical models. Basic radiological histological and molecular features of these three models are discussed and the primary similarities and differences in the biological processes of these different surgical models are summarized.

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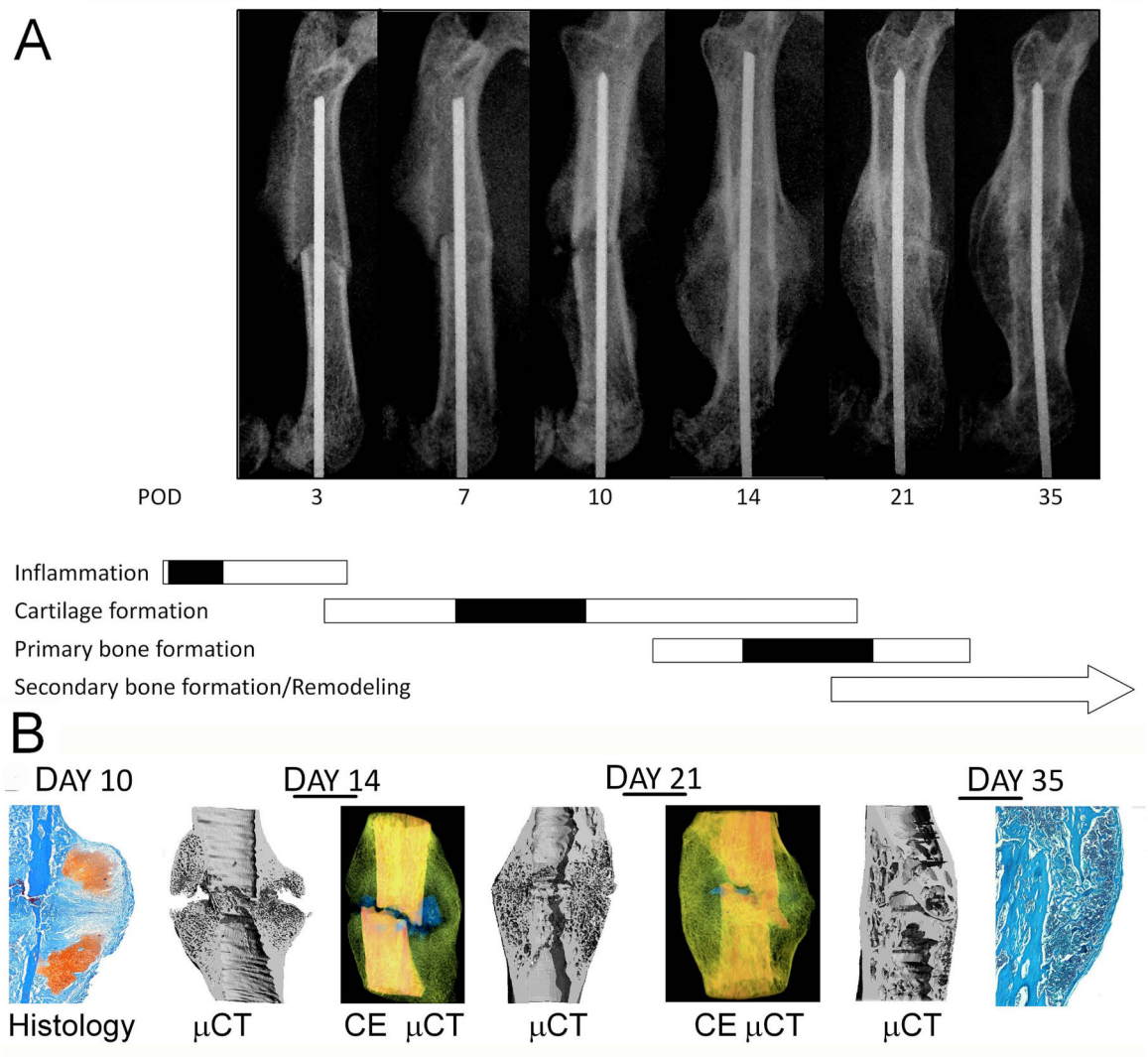

Figure 1.

Progression of Fracture Healing. All bone repair studies that are depicted in the figures were carried out in C57/BJ mice at 8 to 12 weeks. A) Radiographic progression of trauma induced simple transverse fracture healing fixed with an intramedullary pin. Post-operative days (POD) after trauma are denoted in the figure. The stages of the fracture healing are indicated in the figure with the black segment of each bar indicating the medium period when that stage of healing is maximal. B) The progression of changing tissue and material compositions across the time course of femur fracture healing. Transverse histological sections from day 10 and day 35 fracture calluses were stained with Safranin O and fast green. Cartilage is stained red and other tissues are shades of blue. Transverse cross-sections of day 14 and day 21 specimens are μCT reconstructions showing the distribution of tissue mineral, and translucent μCT reconstructions are composite rendering of consecutive reconstructions using contrast enhancement agent [CE] (Hayward et al., 2013) to distinguish cartilage from mineralized tissues. Images are pseudo-colored: yellow, original cortical bone; blue, cartilage; and green, new bone.

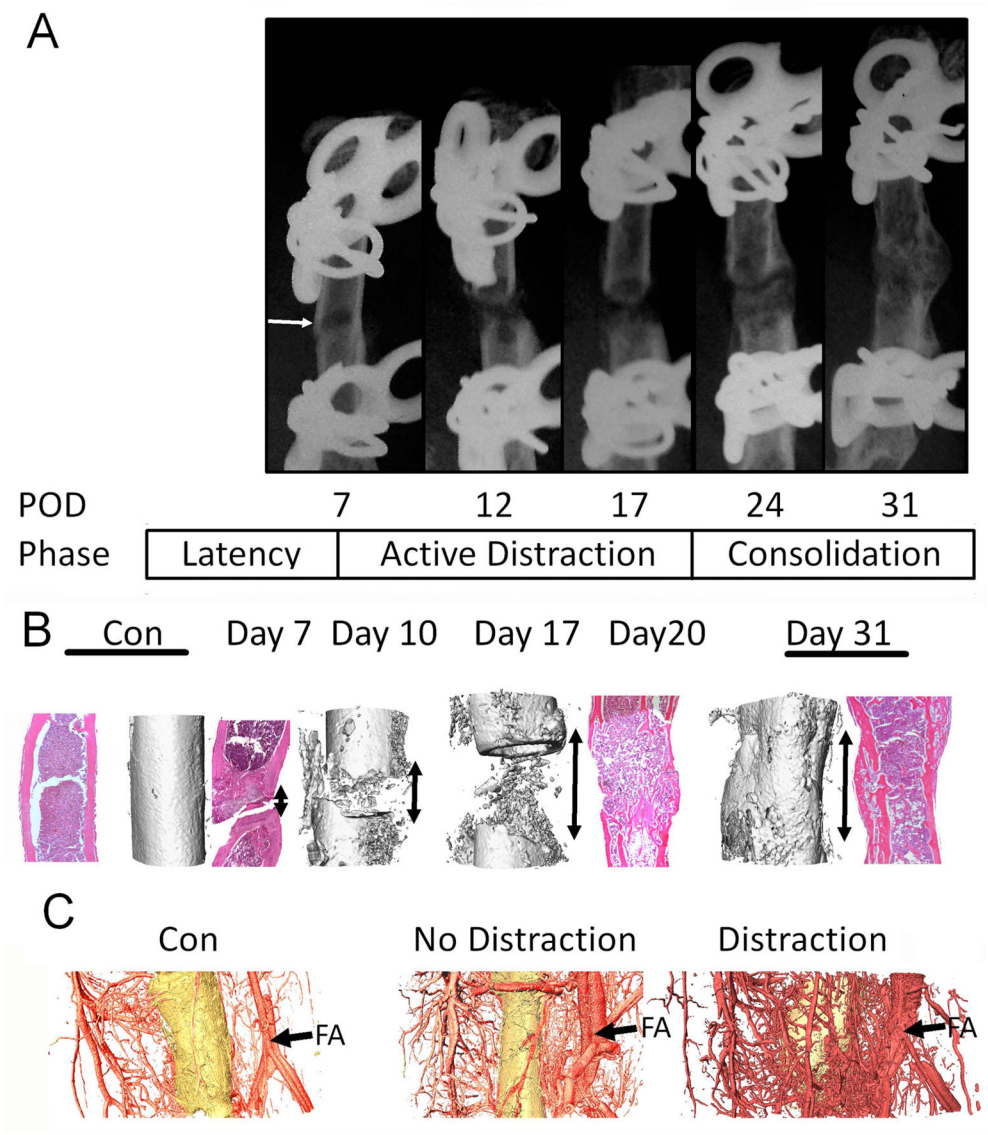

Figure 2.

Progression of Bone Formation During Distraction Osteogenesis. All bone repair studies that are depicted in the figures were carried out in C57/BJ background mice at 8 to 12 weeks. Radiographic progression and the stages of bone formation induced by distraction osteogenesis are shown. Post-operative days (POD) after trauma are denoted in the figure. Arrow depicts osteotomy. B) The progression of changing tissue and material compositions across the time course of distraction osteogenesis. Days after surgery at which transverse histological sections and μCT were obtained from are denoted in the figure. Sections were stained with hematoxylin and eosin. Arrows denote the size of the gap. C) Comparisons of angiogenic response elicited by distraction osteogenesis. The vascular tissue beds within the muscle and bone compartments of the upper mouse leg of control (no surgery), osteotomy and osteotomy followed by distraction are depicted.

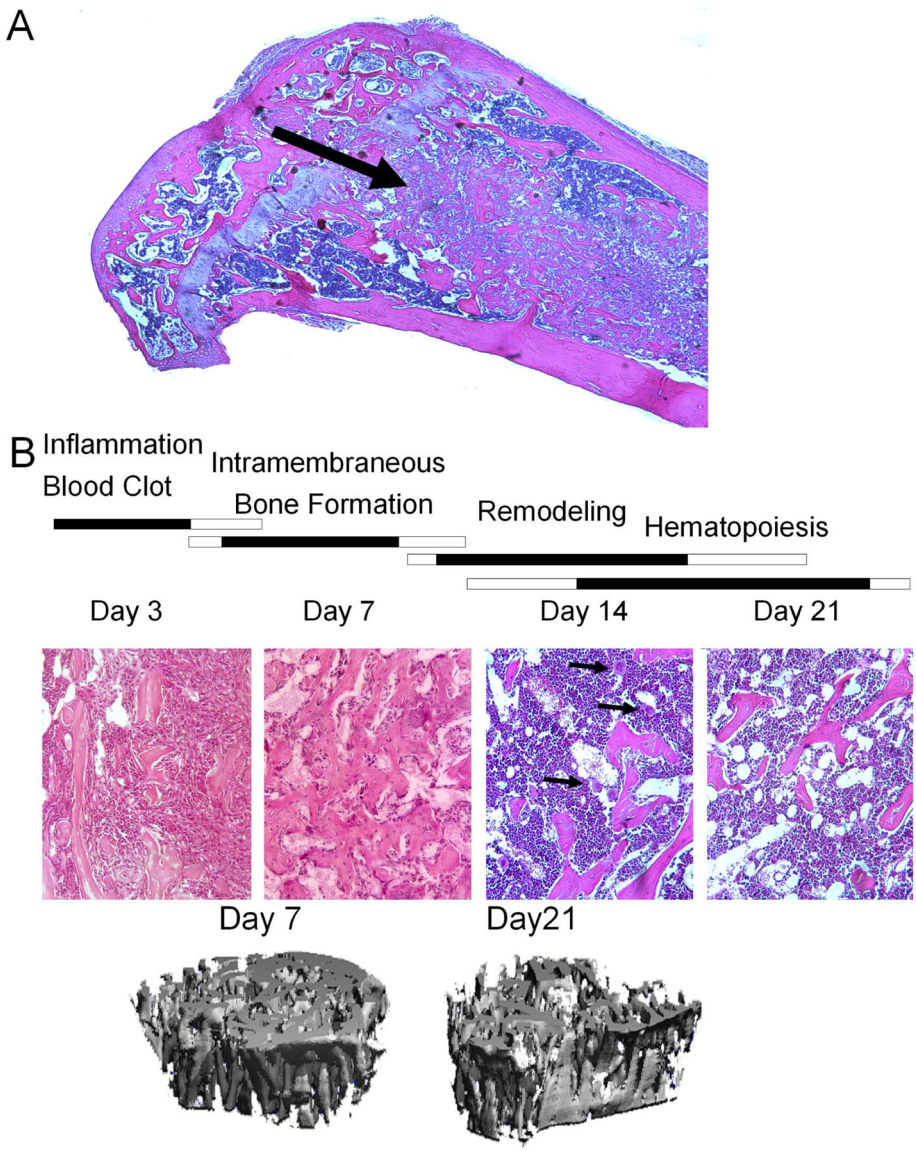

Figure 3.

Progression of Bone Formation After Marrow Ablation by Reaming. A) Gross appearance of transverse section of the tibia at seven days after reaming. Large arrow denotes the entry point of the syringe needles that were used to ream the bone. Histological image depicts the extensive trabecular bone formation in repose to the reaming. B) Series of higher magnification images of the stages of intramedullary bone formation which peaks at day 7 POD followed by resorption seen with the extensive formation of multi- nucleated osteoclast (denoted with arrows) at day 14 POD. At day 21 POD the marrow space is returning to its pre reaming configuration however many more adipocytes are now seen in the marrow. C) The structural progression of changing material compositions across the time course of marrow ablation as characterized by μCT POD days 7 and 21.

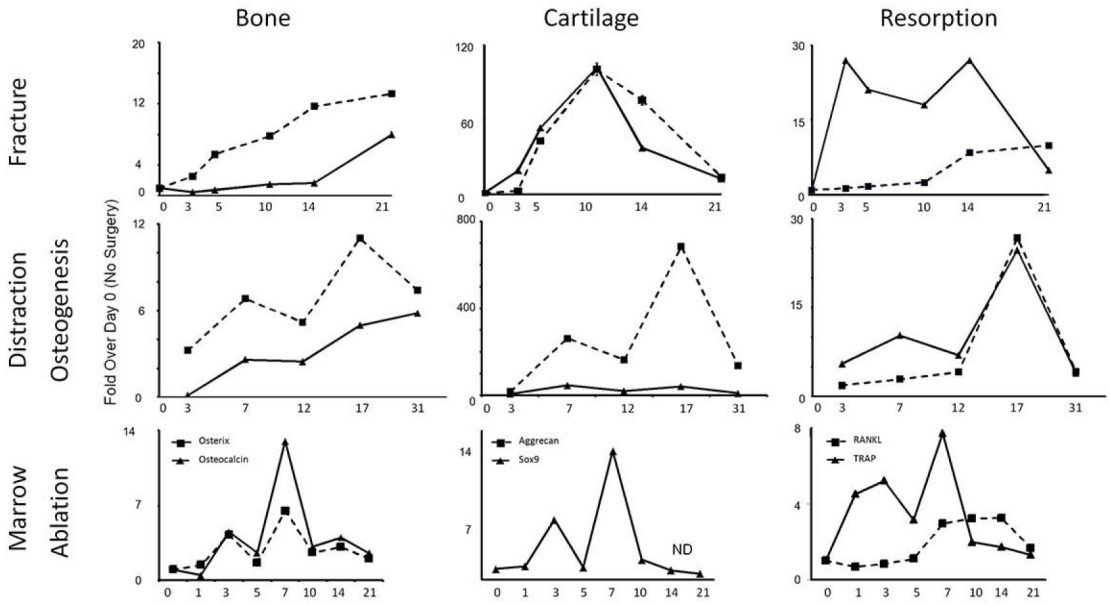

Figure 4. Comparison of Representative Gene Expression during the three different Surgical Models

Two representative genes are shown for each biological process, bone (Osterix and Osteocalcin), cartilage (Sox9 and Aggrecan), and resorption (TRAP and RANKL) during fracture, DO, and marrow ablation. No detectible (ND) expression was observed for Aggrecan during marrow ablation.