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A Review of Animal Models of Comorbidities in Fracture-Healing Research

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Abstract

There is clinical evidence that patient-specific comorbidities like osteoporosis, concomitant tissue injury and ischemia may strongly interfere with bone regeneration. However, underlying mechanisms are still unclear. To study these mechanisms in detail, appropriate animal models are needed. For decades, bone healing has been studied in large animals, including dogs, rabbits, pigs or sheep. However, large animal models display a limited ability to study molecular pathways and cellular functions. Therefore in recent years, mice and rats have become increasingly popular as a model organism for fracture healing research due to the availability of molecular analysis tools and transgenic models. Both large and small animals can be used to study comorbidities and risk factors, modelling the human clinical situation. However,

attention has to be paid when choosing an appropriate model due to species differences between large animals, rodents and humans. This review focuses on large and small animal models for the common comorbidities ischemic injury/reduced vascularization, osteoporosis and polytrauma and critically discusses the translational and molecular aspects of these models. Here, we review material which was presented at the workshop "Animal Models of Comorbidities in Fracture Healing Research" at the 2019 ORS Annual Meeting in Austin Texas.

Keywords: fracture healing; animal models; bone regeneration; polytrauma; osteoporosis; ischemia

1. Introduction

A variety of comorbidities and risk factors contribute to bone fracture-healing complications. For example, ischemia, impaired vascularization, osteoporosis and concomitant soft tissue injury interfere with bone regeneration. However, the cellular and molecular mechanisms are still not completely understood. There is need for reproducible animal models to mimic the clinical situation, since effective orthopaedic research is dependent on our ability to accurately model human diseases in reproducible animal models. There is usually no analogue for these conditions in wild type animals, and in these cases surgical models and molecular models (e.g. genetically engineered animals) must be developed.

Both large and small animals can be used to study comorbidities and risk factors, modelling the human situation during fracture healing. Attention must be paid to choose the appropriate model and to be aware of species differences between large animals, rodents and humans. Costs, handling and ethical aspects have to be also

considered. To study the process of fracture healing on the cellular and molecular level, genetically modified animals are desirable. Over the last two decades, the mouse became the most frequently used animal model in biomedical research due to easy handling, low husbandry costs, a short reproductive cycle, availability of transgenic mice and of specific analytic tools, including monoclonal antibodies against a broad variety of antigens to target individual molecules *in vivo* [1]. In addition, the mouse genome contents a very high number of orthologs and homologs to human genes, making the mouse a valuable model organism for bone research [2], although the bone structure and the bone remodelling process differs significantly from humans [3]. Because of the latter facts, there is also an increasing interest in non-rodent translational models due to approximate human size, similar secondary bone structure, similarities in pathophysiology and the possibility to translate knowledge from "bench-to-bedside" [4].

Here, we review material which was presented at the workshop "Animal Models of Comorbidities in Fracture Healing Research" at the 2019 ORS Annual Meeting in Austin Texas, focusing on the common comorbidities' ischemic injury, postmenopausal osteoporosis and polytrauma. There, Kurt Hankenson reported about the topic vascularization and ischemia, Anita Ignatius gave a talk on osteoporotic fracture healing models and Roman Pfeifer summarized current work about porcine polytrauma models.

2. The fracture healing process

Fracture healing is a complex process with the aim to ensure full regeneration. This process requires spatially and temporally coordinated interaction of numerous cell types and molecular mediators. In addition to biological factors, bone regeneration is

further influenced by the biomechanical environment at the fracture site. Most studies to investigate the process of fracture healing were performed using rat and mouse models. Immediately after fracture, a blood clot is formed around the bone ends through the disruption of blood vessels and incipient blood coagulation [5]. Because the oxygen supply is limited in the injured tissue area, the early fracture haematoma is characterised by low oxygen levels, decreased tissue pH due to lactate production and the accumulation of cell debris [6]. Inflammatory mediators, and, in the case of an open fracture event, pathogen-associated molecular cues recruit myeloid immune cells to the injury site. Neutrophils are the first cell type appearing in the fracture haematoma hours after injury [7]. They remove cell debris and pathogens by phagocytosis, secrete various inflammatory mediators and chemokines [8]. These pro-inflammatory factors induce the recruitment of other immune cells, including monocytes and lymphocytes [7; 9]. Neutrophils may also induce downstream processes leading to progenitor cell recruitment and neoangiogenesis [10]. Macrophages recruited by neutrophilic factors are also crucial for successful fracture repair. Their depletion resulted in complete failure of granulation tissue formation after bone injury, thereby inhibiting bone regeneration [11]. Furthermore, a switch from pro-inflammatory M1 macrophages to pro-regenerative M2 macrophages appears to be important for bone regeneration, because M2 macrophages were demonstrated to guide progenitor cell differentiation along the osteogenic lineage [12]. Moreover, cells from the adaptive immune system were also shown to influence bone healing; a complete absence of the adaptive immune system in $RAG1^{-/-}$ mice resulted in increased callus mineralisation. It was demonstrated that the lack of T lymphocytes rather than B lymphocytes predominantly causes this effect [13]. Specifically, terminally differentiated $CD8^{+}$ cytotoxic T cells were demonstrated to inhibit bone formation in the fracture callus [14]. These data strikingly demonstrate the

significant effect of the inflammatory phase after bone fracture on later callus development and bone regeneration.

In the intermediate phase of endochondral fracture healing, also called indirect bone healing, the biomechanical and biochemical environment near the fracture gap with high tissue strains and low oxygen saturation promotes chondrogenesis of mesenchymal progenitor cells and periosteal progenitor cells. Cartilage tissue is formed at both sides of the fracture gap and grows towards the fracture line to ensure an initial stability of the fracture gap. In contrast, low tissue strains and higher oxygen levels promote direct bone formation at the rims of the fracture callus [15]. During callus maturation, chondrocytes become hypertrophic and start to mineralise their surrounding matrix. Some chondrocytes undergo apoptosis to allow the recruitment of osteoprogenitor cells to the empty lacunae, whereas others initiate transdifferentiation into osteoblasts to promote bone formation in the fracture callus [16-18]. Therefore, the percentage of cartilage and bone in the intermediate fracture callus provides important information about the progress of fracture healing. After achieving a high degree of stability at the injury site through bony bridging of the fractured cortices, haematopoietic progenitor cells of the monocytic lineage differentiate into osteoclasts and start to resorb the external fracture callus. Remodelling continues until the original bone structure and contour is restored [19].

3. Comorbidities influencing fracture healing

3.1. Ischemic injury/vascularization

A crucial part of successful fracture healing consists of maintaining an adequate blood supply (vascularization) and promoting development of new blood vessels

(angiogenesis) at the fracture site. Indeed, inadequate blood supply is a well-recognized risk factor for delayed and non-union healing in patients [reviewed in [20]], about 46% of fracture patients with vascular injuries experience healing problems. Vascular injury frequently accompanies bone injury and can result in ischemia at the site of injury. This in-turn causes a lack of oxygen (hypoxia) and nutrients to reach tissues, while also failing to clear CO_2 and other waste metabolites [21]. In addition to physical vessel injury, comorbidities might be associated with decreased vascularity; such as diabetes, ageing, and smoking that influence healing due to decreased blood flow to the fracture site, therefore hampering bone regeneration [22; 23]. Studying animal models with poor vascularization associated with bone healing is essential to develop new therapeutics to treat clinical patients.

Mouse models have been used to examine the effect of surgically-induced ischemia on fracture healing. Femoral artery ligation and transection and removal followed by tibial fracture is the established approach. Lu et al. investigated non-stabilized and stabilized tibia fracture healing in 10-14 week old male mice with induced ischemia by femoral artery resection [24]. Post-injury analysis from non-stabilized fractures showed a significant decrease in blood flow to the fracture site and delayed perfusion. Callus size, cell proliferation, bone and cartilage matrix production decreased considerably during non-stabilized fracture healing due to ischemic injury. The authors determined that ischemic conditions caused a decrease in blood supply and delayed union in non-stabilized fracture models. A study performed by Miedel et al. 2013 used wildtype (WT) and Thrombospondin-2 (TSP2)-null mice with ischemic tibial fractures in order to investigate whether the absence of the vascularization inhibitor TSP2 would enhance ischemic fracture healing [25]. Results found that TSP2-null mice had improved vascular perfusion, increased callus angiogenesis, and

accelerated endochondral ossification compared to WT mice. TSP2-null mice also exhibited an increase in cell proliferation (20%) and decrease in apoptosis (15%) when compared to WT mice. Additionally, non-unions did not develop post-fracture; the authors assume this could be due to stabilizing the fractures with an intramedullary pin, allowing for further mechanical stability. It was concluded that during ischemic fracture healing, eliminating TSP2 resulted in increased vascularization and improved bone regeneration.

Diabetes is a metabolic disorder; it is associated with increased fracture risk, affects bone formation, hinders fracture healing, and is also linked to decreased angiogenesis [22; 26]. Rodent models of diabetes are prevalent and thus have permitted a thorough characterization of bone healing with metabolic dysfunction. For example, a study by Brown et al. 2014 observed the healing of tibia fractures in mice with diet-induced diabetes [27]. C57Bl/6 mice were provided with a control lean diet or a high-fat diet. Results indicated that high-fat diet mice presented a delay in fracture healing, increase in callus adiposity and weak biomechanical properties under high-fat conditions. microCT showed a decrease in callus vascular volume and a considerable decrease in fracture callus bone volume post-fracture in high-fat diet mice compared to the control models. Investigators have also used streptozocin treatment to induce diabetes in mice and similarly demonstrate poor fracture healing [28]. Diabetic fracture models have also been studied in rats. Follak et al. 2004 investigated the histomorphometry and histology of bone formation and remodeling during fracture healing in 100 spontaneously diabetic BB/O(ttawa)K(arlsburg) rats [29]. The rats were placed into groups based on their blood glucose levels; well-compensated or poorly-compensated metabolic state. Results found that spontaneously diabetic rats in poorly-compensated metabolic states had a complete delay in early fracture healing, showed severe

mineralization and delayed cellular differentiation in comparison to spontaneously diabetic rats in well-compensated metabolic state. The authors suggested that strict blood glucose control, with insulin and a resulting well-compensated metabolic state may avert issues observed in diabetic animal models. Streptozocin has also been used to induce diabetes in rats, and development of diabetes is similarly associated with poor fracture healing outcome [30]. When choosing an appropriate animal model for diabetes, the research aim of the study has to be taken into account, for example if diabetes type I or II should be studied.

Physiological changes associated with ageing have a great effect on vascularization and angiogenesis during fracture healing [31]. Lu et al. 2005 observed juvenile (4 weeks), middle-aged (6 months) and elderly (18 months) mice, with non-stabilized tibia fractures, and compared cellular, molecular and histological progression of fracture repair [32]. Results indicated decreased bone formation, impaired bone remodeling, delayed angiogenic invasion of cartilage, prolonged endochondral ossification and delays in cell differentiation associated with aging. Middle-aged mice had considerably larger calluses compared to elderly mice, suggesting a delay in callus formation and growth in the older mice models. Additionally, elderly mice displayed a delay in cartilage resorption in comparison to the younger mice models. It was noted that while the fractures in elderly mice healed with time, it was evident that the healing capabilities of animals continues to diminish as they age. Further study by Lu et al. 2008 examined the effect age has on vascularization during tibial fracture repair in juvenile (4 weeks), middle-aged (6 months), and elderly (18 months) mice [33]. The authors investigated and compared vascularization of fracture calluses, and expression of HIF-1α, VEGF, MMP-9 and MMP-13 between different age groups. Results indicated that juvenile mice showed increased surface density of blood vessels

and were found to have a more robust angiogenic response during fracture healing compared to adult mice. On the other hand, elderly mice had decreased angiogenic response in comparison to middle-aged mice. Although HIF-1α protein and VEGF transcripts were detected in fracture calluses for all age groups, there was earlier expression in juvenile mice. Furthermore, juvenile mice displayed robust MMP-9 and MMP-13 expression 7 days post-fracture, while the other two age groups presented delayed expressions. All these data were acquired using a model of non-stabilized tibia fracture, therefore a potential influence of fracture fixation is not taken into account.

Larger animal models of impaired vascularization besides instable fracture fixation techniques, which might also lead to delayed vascularization, and particularly surgically-induced-ischemic models have not been reported so far. While surgical ischemia is feasible in rodents without resulting in significant morbidity, this is less tenable in larger animals. In rabbits, exposure to nicotine is a model that results in reduced vascularization and impaired bone healing has been demonstrated in a mandibular defect models [34; 35].

While there is clarity in the association of poor-vascularization with reduced bone healing in the previously discussed animal models, in most cases these compromised vascular models have not been used to study novel therapeutic interventions to promote bone healing. On the other hand, methodologies to enhance bone healing through activation of vascularization have been attempted in a variety of non-compromised bone injury models over the past two decades. As an example, VEGF delivery (to enhance blood vessels) was first shown to enhance healing of bone fracture 17 years ago [36]. It is notable that approaches to enhance vascularity are now at

the forefront of tissue engineering strategies to repair large bone defects, as it is assumed that poor vascularization may be one factor associated with poor healing of large bone defects. Particularly co-delivery of angiogenic and osteogenic factors is highly topical [37; 38]. As this field moves forward it will be essential to examine potential bone regeneration therapeutics in vascular compromised models. As an example, Donneys et al. have used deferoxamine, which stabilizes HIF1α and promotes angiogenesis, to enhance bone healing in irradiated rat mandibular defects [39].

In conclusion, most data about the influence of impaired vascularization on fracture healing were gained using rodent models. Specific models are available mimicking different patient-specific comorbidities like diabetes, ischemia or enhanced age. The clinical relevance of these models has to be proven in future studies comparing data gained from animals and human patients, since there are significant differences between the used models and the clinical situation, for example the use of artery ligation instead of a severe blood loss model.

3.2. Osteoporosis

Osteoporosis is one of the emerging health problems of aging societies in developed countries. It is estimated that 200 million people worldwide suffer from osteoporosis and the incidence is still growing [40]. Currently, one out of three women and one out of five men aged over 50 years will experience an osteoporotic fracture during their life [41]. Osteoporotic fractures are commonly located in bone regions with a high proportion of trabecular bone including the vertebral bodies, the distal radius and the proximal femur, because bone loss starts in these metabolically active bone regions [42]. The treatment and healing of osteoporotic fractures is often associated with

orthopaedic complications. These can result from implant anchorage problems in the fragile bone and/or from the disturbed healing capacity of the bone tissue itself [42-46]. There is need for suitable animal models to test newly developed implants and biomaterials on the one hand and on the other hand to better understand the poor bone healing capacity and delayed fracture healing on a cellular and molecular level [2]. Generally, animal models to investigate the osteoporotic fracture healing process should reflect the clinical scenario of bone loss, e.g. postmenopausal, senile or secondary osteoporosis. Furthermore, the animal model should allow the generation of metaphyseal fractures.

The best established murine model for osteoporosis is the induction of postmenopausal osteoporosis by ovariectomy (OVX) in female mice. Following OVX, the animals display a very rapid loss of trabecular bone, resulting in an osteopenic phenotype [47]. The mechanism behind that is a shift in bone remodelling towards bone resorption due to the lack of ovary-derived estrogen, mimicking the human situation of high bone turnover in the early postmenopause [48]. Limitations of this animal model are that the total BMD loss is less than in human patients [49], that bone loss after OVX varies greatly between different mouse strains [47] and that OVX does not completely mimic the kinetic and the underlying cause of natural menopause. During bone healing, OVX mice display delayed fracture bridging, callus maturation, disturbed immune response and reduced angiogenesis [46; 50] [43; 51; 52] as well as diminished mechano-responsiveness [53; 54] similar to osteoporotic patients [44; 50; 55], demonstrating the translational relevance of this animal model. One drawback is that most fracture healing studies using OVX mice are conducted in a diaphyseal fracture setup [56]. However, there are some models available trying to target the metaphyseal region of the mouse bone, which is challenging due to the small skeletal size [57].

In large animals like sheep, the similar size of the bones compared to humans is a clear advantage for testing of orthopaedic implants or biomaterials. Furthermore, the bone structure of sheep is comparable to human bone with the presence of a Harversian remodelling system. In contrast to humans, the cortical bone of young sheep is still plexiform, but number of secondary osteons increase with aging [58]. The skeletal turnover kinetics is closer to the human situation as in small animals, although most sheep strains do not display age-related bone loss [59]. Importantly, sheep have a much higher bone mineral density then humans. The BMD at the lumbar spine was shown to be more than twice as high as in humans [60], which might negatively affect translation of research findings into clinical practice especially regarding implant testing. Another disadvantage is the different gastrointestinal system of sheep as they are ruminants. Therefore, these animals are not suitable for studying the effects of orally administered drugs on bone and fracture healing. In sheep, bone loss can be induced by OVX [61], calcium and/or vitamin-D deficient diet [62] or administration of glucocorticoids [62; 63]. However, OVX and deficient diet do only induce minor bone loss and glucocorticoids have severe side effects including increased susceptibility to infection [64]. Therefore, a new sheep model of centrally induced bone loss was developed by surgical disconnection of the hypothalamus and the pituitary gland (HPD) [65; 66]. The hypothalamus is a regulating centre in the brain which is, among others, also important for maintaining homeostasis. It closely links the nervous system and the endocrine system via the pituitary gland. The hypothalamus secrets certain hormones and neuropeptides, and these in turn stimulate or inhibit the secretion of pituitary hormones, such as growth hormone, gonadotropin and thyroid stimulating hormone, which then regulate peripheral endocrine organs. Thus, the surgical disconnection of this axis leads to an insufficiency of the pituitary and the peripheral

endocrine organs. HPD sheep displayed a 30% reduction in trabecular bone mass after 12 months and 60% after 24 months, whereas the bone formation rate was reduced by 70%, mimicking human low-turnover osteoporosis. Cortical bone loss was 25% after 12 months and sustained over a period of 24 months after HPD [65]. Furthermore, HPD sheep displayed significantly delayed metaphyseal fracture healing, indicated by an increased amount of soft tissue and cartilage, but less bone in the fracture area [67]. This demonstrated the relevance of this large animal model for osteoporotic fracture healing research, although the HPD procedure clearly lead to an artificially generated type of osteoporosis.

In conclusion, there is no ideal animal model to mimic all the aspects of osteoporotic fracture healing. When choosing the right animal model, the initial research question and the specific pros and cons of each model have to be considered.

3.3. Polytrauma

Clinical studies indicate that fracture healing is severely disturbed in patients with multiple trauma [68], however, the pathomechanisms are still poorly understood. To investigate the underlying mechanisms, rat and mouse models have been developed in recent years, which combine a fracture with an additional injury, such as a blunt chest trauma [69] or a soft tissue injury [70]. Further polytrauma models often include bone fractures but rarely analyze the broken bone tissue to unravel the pathomechanims behind poor fracture healing. Polytrauma is still a leading cause of death in patients below 40 years of age [71]. Severe traumatic insults are associated with a pronounced immunologic response (genomic storm [72]) and tissue damage affecting the coagulation system and increasing susceptibility for infections leading to multiple organ dysfunction or even failure. Several small and large animal models of polytrauma

have been used to investigate the pathophysiology and mechanisms of multiple trauma and therapeutic interventions. Rodents, especially genetically manipulated, allow analysis of trauma associated molecular mechanisms [73]. However, non-rodent translational models have the advantages of the approximate human size, similarities in pathophysiology and anatomy, high-developed central and peripheral nervous system and the high possibility to translate knowledge to the clinics [4]. Especially for multiple and polytrauma research questions, porcine models have been shown useful since they allow clinically applied surgical emergency interventions, demonstrate cardiovascular and hemodynamic responses and provide multiple samples large sample size per animal.

The "TREAT-Network" (**Tr**anslational Larg**e** **A**nimal Research Ne**t**work) has been founded with a main aim to study pathophysiological changes associated with multiple trauma, surgical treatment strategies and emergency interventions [74]. This network introduced a standardized thoracic trauma in combination with controlled hemorrhage, standardized laparotomy including liver laceration and additional extremity trauma [75]. This large animal model is reproducible and clinically relevant traumatic insult with controlled hemorrhage. Using this model, the role of induced hypothermia (34°C) for 3 hours has been studied and revealed no relevant effects of therapeutic hypothermia on hemodynamics and coagulation system [76; 77]. However, hypothermic animals showed reduced hepatic inflammatory response [78]. Hepatic cytokine expression and liver damage were significantly increased and animals with normothermia compared with hypothermic group. In a further analysis, the observation period after traumatic insult has been extended to 48 hours. Again, hypothermia (33°C) was induced for 12 hours with a subsequent rewarming period of 10 hours [79]. This study revealed that prolonged hypothermia was associated with a

significant elevation of systemic and local HBGB-1 (High-Mobility-Group-Protein B1) levels and Interleukin (IL)-6 levels [79]. Authors discussed a possible effect of hypothermia and re-warming on prolonged immunologic imbalance after trauma.

Long-term observation in such clinically relevant models allow the analysis of remote organ injury and their dynamics. Animals were observed for 72 hours under ICU conditions after induction of above-mentioned polytrauma. The investigation of cardiac function after thoracic trauma and hemorrhagic shock has shown that multiple trauma leads to an early cardiac dysfunction, which was associated with cardiac cell damage, local inflammation with in cardiac tissue, disturbed cytoskeletal and gab junction architecture [80]. With a new diagnostic tool: Electric Impedance Tomography (EIT) respiratory function and severity of thoracic trauma was assessed [81]. The method revealed significant differences between the traumatized and healthy lungs. The EIT radiation-free measurements for bedside diagnostics identified significant reduced ventilation on injured lung with a compensatory increase of contralateral uninjured lung. In addition, the role of biomarkers of thoracic trauma (IL-8 and Leukotriene B4 (LTB4)) were studied during these long-term observational experiments [82]. Previously, clinical studies have shown that LTB4 and IL-8 may indicate patients at risk for pulmonary complications [83]. Similar patterns were observed in a standardized polytrauma model. Animals with severe thoracic trauma demonstrated local and systemic elevated levels of LTB4.

A special focus of “TREAT-Network” was the analysis of local inflammation associated with an extremity fracture in a translational porcine model [84], since local inflammation appears to play an important role in fracture healing [79; 85; 86]. However, how the composition of cytokines and mediators affects the fracture healing and

repairing process is still not known. Moreover, other measurements confirmed that the duration and severity of hemorrhagic shock has an influence on systemic biomarkers (HMGB-1 (High-Mobility-Group-Protein B1) and HSP-70 (heat shock proteins-70)) levels. Those markers are known to predict outcomes after polytrauma [79; 87; 88]. Severe hemorrhagic shock has not only effect on organs, such as lung, liver, kidney, etc., but also on peripheral musculature, peripheral soft-tissues and bone [89]. After deep hypoperfusion, relevant alterations of local microcirculation appear and persist up to 72 hours after trauma. Even after resuscitation animals appear to develop a hyperemia in peripheral soft tissues for at least 72 hours [89]. Whether these changes have an effect on fracture healing, systemic or local inflammation need to be shown in future studies. Surgical strategies may also be studied using a porcine trauma model. After induction of a femoral fracture, animals were subjected to femoral nailing or external fixation and soft-tissue microcirculation was measured repeatedly over a period of 72 hours. These measurements have demonstrated a significant increase in local blood microcirculation in animals treated with intramedullary reaming and nailing in comparison to external fixation. These alterations might be of importance for fracture healing and local and systemic inflammation as well [90].

Several clinically relevant questions in fracture healing research can be addressed in the future using the above-mentioned animal models. In hemodynamic instability, in presence of acidosis or coagulopathy, patients are initially subjected to a, so-called, damage control procedure with a temporally stabilization of fractures with external fixation or casts [91]. In addition, surgical debridement and decontamination is performed. During the clinical course, after patient physiology is restored, factures are definitely treated. However, it is not fully clear how the systemic and local inflammatory response in polytrauma affect the fracture healing. Moreover, the time

point of definitive surgery and reconstruction may be crucial and need to be studied in the future research projects.

Taken all these studies and evaluations together, porcine polytrauma models can provide a high level of clinical relevance, when they are properly designed and mimicking the clinical situation. On the other hand, rodent models are useful to unravel molecular pathway underlying disturbed regeneration after polytrauma.

4. Conclusions

Both clinical and experimental data support the hypothesis that comorbidities like reduced vascularization/ischemia, osteoporosis and concomitant soft tissue trauma have negative effects on bone fracture healing. Experimental data were gained from animal models for fracture repair.

Regarding impaired vascularization and ischemia, most studies used surgical, genetically modified or metabolic mouse or rat models. Larger animal models of impaired vascularization, particularly surgically-induced-ischemic models have not been reported so far due to significant morbidity. Therefore, small animal models seem to be favorable to study the influence of impaired vascularization and ischemia on fracture healing, however studies testing novel therapeutic interventions to promote bone healing in such cases are lacking so far.

To study osteoporotic fracture healing, there are both well-established large and small animal models available. When choosing the right animal model, the initial research question and the specific pros and cons of each model have to be considered. Mouse models are especially useful to unravel molecular mechanisms in osteoporotic fracture healing but have limitations to study metaphyseal bone healing. Sheep models allow

to study metaphyseal bone healing but induction of osteoporosis requires complicated experimental procedures, keeping also the animal welfare in mind. A combination of large and small models might be needed to transfer findings from basic research to clinical application. This might be the case not only to study osteoporotic fracture healing, but for fracture healing research in general.

In the case of polytrauma, porcine large animal models can provide a significant level of clinical and translational relevance, since there have been significant efforts to design these models and experiments to closely mimic the human situation. Further investigations will show if findings from these models can be transferred into clinical practice. Also, further development and refinement of animal models might increase the translational relevance of these models in the future.

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