Surgically-induced mouse models in the study of bone regeneration: Current models and future directions (Review)

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Abstract. Bone regeneration has been extensively studied over the past several decades. The surgically-induced mouse model is the key animal model for studying bone regeneration, of the various research strategies used. These mouse models mimic the trauma and recovery processes *in vivo* and serve as carriers for tissue engineering and gene modification to test various therapies or associated genes in bone regeneration. The present review introduces a classification of surgically induced mouse models in bone regeneration, evaluates the application and value of these models and discusses the potential development of further innovations in this field in the future.

Contents

1. Introduction

Bone regeneration has been extensively investigated during the past several decades, resulting in therapeutic progression in this field. However, critical bone defects, particularly in patients with an unfavorable healing microenvironment, remain a primary concern for surgeons (1-3). Various mouse models have been developed for the investigation of various injuries and pathological processes associated with bone regeneration, and numerous important molecular signaling pathways have been elucidated and therapies developed (1,4-6). Among all the different mouse models, surgically-induced models are prevalent in bone regeneration research (7). Regenerative medical therapies associated with bone healing employ an extensive range of various strategies that aim to repair, augment, substitute or regenerate lost tissue (4). To determine the effect of these various treatment therapies, mouse models that use surgical induction of a particular condition are frequently performed, due to their similarity to the trauma and the patient recovery process (8-10). These models are well established in combination with tissue engineering strategies, for analysis of the function of growth factors, scaffolds and stem cells (11,12). Furthermore, these mouse models may be performed in genetically modified mice, which is an important method using gene-targeting to investigate the genes involved in bone regeneration (13,14). This review briefly evaluates surgically-induced mouse models, with focus on the most important models currently used and the potential development of novel models in the future.

2. Classification and applicability

The surgically induced mouse models were divided into three different groups based on the severity of trauma and the mouse phenotypes: Simple fracture models, bone defect models and ectopic bone formation models.

Simple fracture models. Simple fracture models are used to determine the effect of various drugs and gene modifications in fracture healing. The fracture model may be further classified by anatomic location, with the fibula (15,16) and femur (17,18) among the most common sites. The fracture may be created by blunt trauma or using ophthalmic forceps (15,16). For the simple blunt fracture model, three-point bending equipment is used to create a fracture. The simple fracture model in the femur is more complex, as it requires a needle to be implanted into the intramedullary cavity via the intercondylar notch to 'fix' the fracture prior to creation. This is not required in the fibular fracture model (19-21). These models are technically simple compared with other models and are frequently used for identification of bone regeneration associated factors (22,23).

Bone defect models. Critical sized bone defects are a challenging clinical scenario for surgeons and frequently result in

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a delayed bone union or a nonunion in numerous cases (24). Therefore, surgically-induced bone defect mouse models have been extensively used for analysis of growth factors (25,26). It has previously been reported that deficiency of progranulin (PGRN), which is a downstream mediator of bone morphogenetic protein-2 (BMP-2) involved in bone healing, delayed bone healing, whereas recombinant PGRN enhanced bone regeneration. Furthermore, PGRN was required for BMP-2 induction of osteoblastogenesis and ectopic bone formation (25). When the bone defect models have been used in biomaterial research (27,28), the results indicated that osteoinduction and appropriate degradation were important in accelerating and promoting bone augmentation. This strategy appears promising as 3D temporal scaffolds for potential orthopedic applications (28). In addition, this type of model may be used in stem cell research (29-31). The findings of the experiment indicated that human muscle-derived stem cells (hMDSCs; Stem Cell Research Center, University of Pittsburgh, Pittsburgh, PA, USA) are mesenchymal stem cells of muscle origin and that BMP2 is more efficient than BMP4 in promoting the bone regenerative capacity of the hMDSCs *in vivo* (31). Local or systemic delivery of drugs may be tested using these models. Altering the genotype of the mouse involved with these models may also enable researchers to understand the molecular signaling pathways involved in fracture healing and bone regeneration. According to the size and pattern of the bone defect, these models are further divided into drill-hole or critical-size bone defect models.

Drill-hole models. Drill hole models are typically established in either the femur (32,33) or the tibia (34). To create a drill-hole, a drill is inserted into the bone while applying constant irrigation (32,34). These holes are typically created in the mid-shaft of the diaphysis of the long bone, where only cortical bone is involved. This model may be either unicortical or bicortical, in which the hole is created in either a single side or on both sides of cortical bone, respectively (25,35-37). Due to the small size of the hole, these models are predominantly used for testing the systemic delivery of medicine or to determine the effect of a specific gene modification on bone healing. These small bone defects have also been used for tissue engineering studies, in which collagen sponges are fixed in the hole position, despite the unstable location of implantation (34).

Critical-size bone defect models. The critical bone defect model is used to simulate a greater degree of bone loss than the drill-hole model and is frequently used to study non-unions. A review of the literature revealed that two of the most frequently used methods to establish a critical bone defect include the use of either the cranial bone of the skull (38,39) or long bones of the extremities, including the femur (25,40,41) and radius. There are various differences in the methods used to establish these models. To create the cranial defect, the pericranium is removed and a trephine is used to create a circular bone defect in the skull, with meticulous care taken to avoid damaging the underlying dura mater (38,39). A drill bit is used to create the defect in the long bone defect models (25,41); however, a drill bit cannot be used to create cranial defects as the dura mater is in close proximity to the inferior aspect of the skull. In the mouse, a critical-size cranial defect is defined as a bone deficit ≥5 mm (42,43). This model has been used for the investigation of molecular signaling pathways associated with bone healing, by using knockout and overexpressing mice, and determining the effects of treatments aimed at the promotion of bone regeneration (44-46). For instance, critical-size bone defect models reveal accelerated bone formation and bone remodeling in the absence of the Toll-like receptor 4 signaling pathway. This phenotype is associated with alterations of local inflammatory cytokines and expression of osteoclastogenic factors (44). The femoral bone defect model was originally established to investigate the pathways involved in non-unions (40,47,48), and has since been used to study various treatments to promote bone healing (49). In our previous study, a 0.5 mm femoral bone defect was used to investigate bone healing. It was demonstrated that wild-type mice of the control group were able to fully heal the 0.5 mm bone defect, however PGRN knockout mice exhibited impaired bone healing (25). The mouse model was relatively complicated to create, as an intramedullary needle and a custom-made clip were implanted into the femur to fix the bone defect (Fig. 1). The use of metal devices may interfere with the bone signal when using micro computed tomography (CT; data not shown), and should be removed to minimize any of these artifacts (50). However, the removal process may result in damage to the original structure of the bone defect position.

The radial bone defect model has been extensively used for determining the effects of tissue engineering in bone repair (51-53). This is a non-union model and the bone defect will not recover spontaneously without additional treatment, which enables the use of gain-of-function studies (54). The bone defect of the radius is stable, supported by an intact ulna and scaffold carrying growth factors, to aid the implantation of cells. Furthermore, this model has previously been established in genetically modified mice to study molecular signaling pathways of fracture healing. The present study established this model in tumor necrosis factor-α receptor (TNFR)-deficient mice (Jackson Laboratory, Bar Harbor, ME, USA) to investigate the role of TNFR in the effect of recombinant PGRN protein in the promotion of bone repair (25).

Ectopic bone formation model. Ectopic bone is bone that forms in locations where bone formation does not typically occur. Several molecules have been identified to be involved in the process of ectopic bone formation. It has previously been demonstrated that ectopic bone formation may occur in PGRN knockout mice (New York University Medical Center, New York, NY, USA) (78). BMPs are extensively used to induce ectopic bone formation (55,56). Molecules and signaling pathways associated with these growth factors are investigated using models of ectopic bone formation (35,57). These models are typically either subcutaneous or intramuscular in location (25,56,58). For subcutaneous ectopic bone formation models, implants carrying genetically modified stem cells and/or growth factors are surgically implanted into a pocket beneath the skin, and bone formation is detected at indicated time points (59). Intramuscular ectopic bone formation can be established in paravertebral (51,60,61), thigh (62) or calf muscles (63). These models may be used to determine the effect of various therapies on BMP-induced bone formation, and may aid the identification of novel therapeutic strategies (25,59,64). The data from this

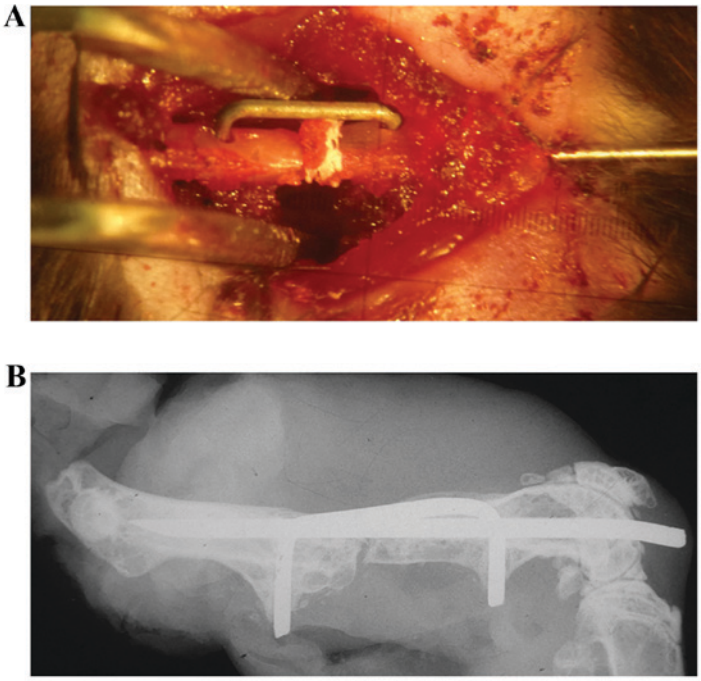

Figure 1. Establishing a femoral bone defect model. (A) Intramedullary needle and custom-made clip were implanted into the femur to fix the bone defect. (B) Post-operative X-ray analysis.

type of model demonstrates that a focused approach to develop targeted differentiation protocols in adult progenitor cells may be achieved via the identification and subsequent stimulation of genes, proteins and signaling pathways associated with calcium phosphate mediated osteoinduction (64).

3. Advantages and limitations

Mouse models have numerous advantages compared with larger animal models, and are used for a broad range of applications (Table I) (65). Mice are docile, tolerate the surgical procedures and are able to ambulate with the implanted limb within a short time following surgery (66). Additionally, genetic alterations are easily created in mice and therefore certain genes can be targeted for knockout or overexpression. This allows the investigation of the effect of drug therapies on bone regeneration and the identification of the underlying molecular mechanisms involved. Furthermore, various mouse models have been well established in the literature, and researchers may select an appropriate model based on the aim of the experiment.

However, surgically-induced mouse models have limitations. In numerous cases, genetic modification results in a defect during development, which may involve bone growth (67,68). This may subsequently interfere with bone healing, and therefore artificially alter the results of the experiment. In these cases, inducible genetically modified mice may be used to eliminate any effect on bone development (69).

4. Discussion, conclusion and perspective

The mouse is currently the most commonly used animal model in basic research (Table I) (70). The ease of maintenance, relative low cost and abundance of pre-established mouse models provide advantages compared with other species (65). The ability to use mouse models in an effective manner in order to gather valuable scientific information is the responsibility of the researcher. Researchers should select appropriate models according to the aim of their project. Fig. 2 presents a proposed outline for the various regenerative modalities of fracture healing in surgically induced mouse models. Various cells, particularly osteoblasts (71,72) and osteoclasts (73), participate in the bone regeneration process, and induce bone formation and remodeling. In simple bone regeneration models, periosteum and intramembranous ossification is important in the regeneration process (74,75). In the bone defect model, the indicated cells accumulate towards the location of the bone defect. The use of scaffolds (76) and exogenous growth factors (77) may further promote the targeted accumulation and function of endogenous and implanted cells. The surgically-induced mouse model is the environment in which all of these interactions occur. Further studies are required to determine the potential long-term effects of such treatments on bone repair using various fracture models (73). Numerous discoveries using mouse model of bone regeneration have already been clinically tested and translated into clinical applications. For instance, BMP-2 and -7 were initially investigated using a surgically-induced mouse model of bone regeneration and are now available for clinical use to promote bone regeneration and healing (77).

The use of surgically-induced mouse models of bone regeneration have the potential to be improved. Firstly, more efficient devices may be developed for fixation of these models. Fixation devices that are used near the surgical site should be free of degrading particles to result in a more purified microenvironment for bone regeneration. Novel devices are required for more convenient fixation and less damage to the surrounding soft tissue, so that the blood supply to the area of healing is protected. Imaging modalities used for these

Table I. Comparative list of various models and associated information.

First author, year	Model	Main classification	Implement	Application	Refs.
Cheng, 2010; Kayal, 2010; Holstein, 2010; Holstein, 2009; O'Neill, 2012; Einhorn, 1995; Kellum, 2009; Wigner, 2012 Gerstenfield, 2003	Simple fracture models	Fibular fracture models, femoral fracture models	Blunt trauma, ophthalmic forceps	Identify the bone regeneration associated factors	(15,16,17,18,19,20,21,22,23)
Haddock, 2013; Zhao, 2013; Ben-David, 2013; Annibali, 2013; Yang, 2013; Zhang, 2013; Fricain, 2013; Gao, 2013; Tanaka, 2010; Katae, 2009; Behr, 2010; Tang, 2009; He, 2011; Jawad, 2013; Meszaros, 2012; Behr, 2012; Liu, 2013; Manassero, 2013; Krebsbach, 1998; Lee, 2001; Wang, 2012; Levi, 2012; Lo, 2012; Garcia, 208; Zwingenburger, 2013; Lin, 2012; Holstein, 2011; Kimelman-Bleich, 2009; Moutsatsos, 2001; Kimelmen-Bleich, 2011; Tai, 2008	Bone defect models	Drill-hole models, critical-size bone defect models	Drill, trephine	Analyze the growth factors, biomaterials and stem cells	(24,25,26,27,28,29,30,31,32, 33,34,35,36,37,38,39,40,41 42,43,44,45,46,47,48,49,50, 51,52,53,54)
Zhao, 2013; Tang, 2009; Bergeron, 2012; Kamiya, 2012; Chen, 2010; Wagner-Ecker, 2013; Frescaline, 2013; Sheyn, 2008; Medica, 2010; Shimaro, 2011; Eckmans, 2013; Zhao, 2015	Ectopic bone, formation models	Subcutaneous bone formation models, intramuscular bone formation models	BMPs molecules	Identify molecules and signaling pathways associated with growth factors	(25,35,55,56,57,58,59,61,62, 63,64,78)

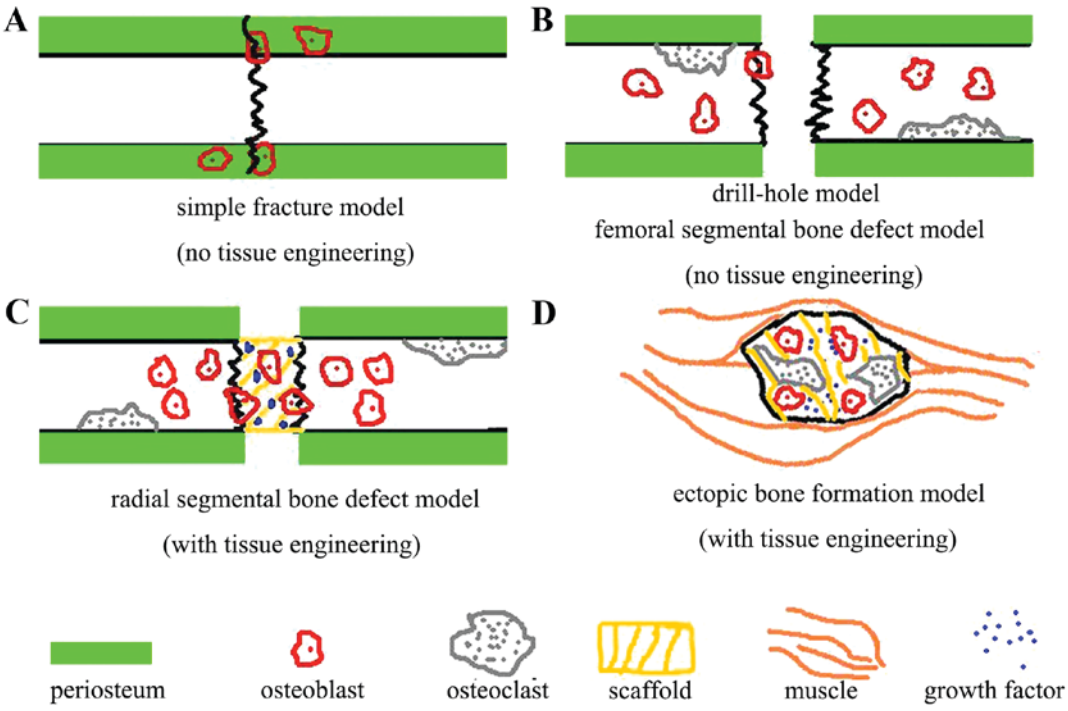

Figure 2. Proposed outline for the different regenerative modalities of fracture healing in various surgically-induced mouse models. (A) Simple fracture model. (B) Drill hole model. (C) Radial segmental bone defect model. (D) Ectopic bone formation model. Various cells, particularly osteoblasts and osteoclasts, participate in the bone regeneration process and induce bone formation and remodeling. In simple bone regeneration models, periosteum and intramembranous ossification are important in the regeneration process. In the bone defect model, the indicated cells accumulate towards the location of the bone defect. The use of scaffolds and exogenous growth factors may further promote the targeted accumulation and function of endogenous and implanted cells.

small areas of bone regeneration also require improvement, including micro CT and magnetic resonance imaging. Finally, inducible transgenic mice should be used more frequently in the establishment of these models, as this would eliminate any alterations in bone formation that occur during development.

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