Chapter

Animal Models in Orthopedic Research: The Proper Animal Model to Answer Fundamental Questions on Bone Healing Depending on Pathology and Implant Material

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Abstract

Different species vary in bone metabolism, especially in modeling and remodeling of the bone. Human-related diseases with severe outcomes on bone, such as osteoporosis or osteoarthritis, are often reflected in animal models, which cannot adequately mimic the human situation. The pre-clinical investigation of implant materials in vivo complicates the search for the ideal animal model, especially when combining pathologic bone diseases and implant material. For instance, while alterations in trabecular bone architecture are investigated in female osteoporotic rats, rodents commonly lack cortical bone remodeling or secondary osteon formation. Small ruminants are commonly used to study long bone defects or orthopedic materials, due to their comparability to humans regarding body weight, bone size, and fracture healing. Nevertheless, there are important differences between human and ruminant models: plexiform cortical bone, seasonal bone loss, and stronger trabecular bone appear in sheep compared to humans. This chapter will summarize fundamental differences in bone quality between different animal models used for orthopedic and implant material research. Thus, choosing the ideal animal model to answer the proposed research question remains the key to guarantee a solid and excellent scientific study.

Keywords: animal models, remodeling, orthopedics, pathological bone, biomaterials

1. Introduction

In vivo animal models are frequently used in orthopedic and trauma research. Due to ethical issues, researchers are focused to replace animal models by establishing novel in vitro systems. However, small tissue fragments are often used in in vitro systems thereby losing tissue's architecture at some times. Although it is possible to use in vitro organ and tissue cultures that allow preservation and

differentiation, the control of dynamic cell properties and simulation of cell interactions remain difficult. In orthopedic and trauma research, the major disadvantages involve the placing of physiological loading and the cellular and molecular orchestration compared to the in vivo system. Hence, animal models are essential not only to evaluate pathological bone but also to study tissue response, biocompatibility and mechanical properties, especially when it comes to implant materials [1].

While small animals (mice and rats) are most commonly used due to lower costs (e.g., purchase, breeding, and housing), easy handling and the feasibility to enlarge the animal number, large animals (sheep and dogs) show several advantages including bone size, body weight, and bone quality when compared to humans. Murine animal models are commonly used to evaluate pathophysiology and novel treatment strategies [2]. For example, mice are highly adaptable to pathological conditions by experimental manipulation. Moreover, molecular tools, antibodies, and the well-characterized mouse strains (including knock-out or transgenic models) make the use of these animals more advantageous [3, 4]. There has been a long debate on whether rodents are appropriate to study osteophysiology due to the lack of true skeletal maturity (e.g., lack of Haversian remodeling and closure of epiphyseal growth plate) [2]. Larger animals, such as sheep and dogs, show several advantages over small models, including their life span and extended phases of skeletally matured bone, but seasonal bone loss and plexiform cortical bone especially occur in sheep. Thus, there is no animal model that entirely fulfills all requirements making it necessary to follow a particular research question and to confirm results obtained in research on small animal models in large animals before entering the clinics.

In this chapter, we will mainly focus on four animal models including mouse, rat, sheep, and dog. Since there are several bone pathologies, which need in vivo research models, we will particularly focus on osteoporosis and osteoarthritis as one of the major bone pathologies that are also associated with implant and scaffold research. Moreover, we will provide an overview about implant research in orthopedics and trauma surgery with specialization on bioresorbable implant technology.

2. Bone biology

Animal models are usually chosen by genetic background considerations that might influence bone phenotype, thereby assessing bone properties including bone mineral density, hardness, biomechanics, and elasticity [1]. Bone quality includes several variables such as geometry, architecture, composition (e.g., collagen and matrix components), cortical porosity, turnover, and damage and bone mineral density. However, bone quantity is classified as mineral mass or bone mineral content. In general, there are two major processes involved in bone development and maintenance.

2.1 Modeling

Bone modeling in general describes bone formation without prior osteoclastic resorption (uncoupled bone formation). This is the case during initial bone growth due to embryogenesis, as well as due to sequences in bone fracture healing and pathological bone situations, including inflammation or bone tumors. Bone modeling results in bone microstructures which are referred to as primary and woven bone. Histologically, primary bone can be separated into three types of structurally different bone tissues: primary lamellar bone, plexiform or laminar bone, and primary osteons. Depending on the vertebrate species, the state of development,

but also the site of the skeleton, fulfills different kinds of functions. Primary bone is usually build up fast and gets remodeled to secondary bone during maturation. Woven bone, on the other hand, is a repair tissue, which builds the callus during fracture healing. There is no osseous or cartilage template (anlage) needed to build up woven bone. This kind of bone tissue shows a higher degree of mineralization and more porosity then secondary bone, but exhibits less mechanical qualities, as the embedded collagen-fibers are more or less disorganized. Typically, it also gets remodeled to secondary bone during bone maturation, with a few exceptions (e.g. alveolar bone and sutures of the cranium) [5, 6].

2.2 Remodeling

Bone is permanently rebuilt throughout the body to assure bone mineral homeostasis, to regenerate microfractures, or to adapt the bone to new load. Bone-degrading osteoclasts and bone-forming osteoblasts work together in a highly concerted procedure. The balance between bone resorption and formation is crucial for physiological bone metabolism. If the balance in between resorption and formation is disturbed, this can result in diverse disease patterns. In osteoporosis, for example, more bone is resorbed than is subsequently build; conversely, in osteopetrosis, more bone is formed than was previously degraded. Bone remodeling takes place within microscopical construction sites, the Basic Multicellular Units (BMUs). A BMU includes those osteoclasts, osteoblasts, and osteocytes involved in a particular remodeling event. BMUs in average are about 1–2 mm long, with a diameter between 0.2 and 0.4 mm. Cortical bone remodeling results in a secondary osteon (Havers' System), which in the center includes a neuro-vascular channel to provide the bone with nutrients and signals. Trabecular remodeling takes place in the spongy parts of the bone and results in so called avascular hemi-osteons. Trabecular bone is provided with nutrition by blood vessels from the medullary cavity [6–8].

3. How to choose the right animal model depending on bone pathology

In translational research, in vivo animal models are an important tool and have to be chosen carefully, when studying pathophysiology of diseases, implant materials or treatment options. To investigate diseases, there are several approaches including xenograft and genetically engineered models as well as inbred strains. However, results obtained from in vivo animal studies differ in their translatability to the clinical condition [9]. Generally, there are several other factors which have to be taken into account when choosing the animal model, for example, length of the experiment, costs for food and housing, experiment type and primary outcome measures.

Small animal models, especially mice and rats, exhibit several advantages including easy handling, lower costs, and quick experimentation, due to their short life span and enhanced metabolism. While small animals serve as ideal models to examine pathophysiology and pathogenesis as well as new treatment options, large animals, such as sheep and dogs are also often used to study long-term diseases processes and treatment options. Therefore, researchers suggest to additionally confirm treatment options' efficiency in large animal models before clinical use [10].

3.1 Rodents

Rodents are well-established in vivo models preferably used in translational research of different disciplines as well as in bioactivity and feasibility studies due

to their well-defined genetics, biology, and immunology. Accordingly, the reproducibility is quite high. Due to their limited life span, rodents are favorably used for age-related bone metabolic and regenerative studies [11, 12]. Bone biology strongly depends on gender and age. However, there are also several differences within animal strains and after genetic manipulations. For example, fracture healing was strongly enhanced in C57BL/6 mice compared to C3H and DBA/2 [13]. Bone modeling (growth and reshaping) of the skeleton occurs throughout rodent's life cycle, and the epiphyseal growth plate still remains open throughout adulthood. Trabecular bone content is limited in rodents, and Haversian remodeling does not occur, whereas cancellous remodeling is established in rodents [14].

3.2 Large animals

Within processes which are related to body size or metabolic characteristics, like biomechanics (e.g., fracture fixation) or bone healing efficiency, respectively, the animal model (size and anatomy) should be as close to the human situation as possible [15, 16]. Martini et al. discussed the utilization of animal models in the field of orthopedic research from 1970 to 2001. Within the first decade (1990–2001), they reported a relative increase of sheep from ~6 to 8–9%, when compared with the two decades before. However, in parallel to the increase of sheep models, the relative amount of dogs used in orthopedic research decreased for about the same percentages (due to, for example, easier handling, ethical reasons) [17].

To the best of our knowledge, no deeper literature recherché was performed since then. Nevertheless, we expect a further increase of sheep being used as an animal model for orthopedics and traumatology.

3.2.1 Sheep

The cortical fraction of mature long bones in sheep is reported to exhibit a mixture of primary and secondary bone tissue. Plexiform bone appears close to the periosteum, while Haversian tissue occurs close to the endost, with a mixture of both in the mesosteal zone. Young animals up to 3–4 years in contrast exclusively show plexiform bone throughout whole sections of femora. For sheep, significantly higher bone densities have been observed compared to human bone. For example, the trabecular bone density of sheep femora is about 1.5–2-times higher than the density of that in humans. These values, however, are strongly related to the bone site where they have been measured and might not be predictive for the trabecular bone density of other bone locations, such as vertebrae [18]. Even though there are clear differences in bone microstructure, studies reported that sheep exhibit similar bone remodeling and turnover when compared to the human situation [19]. Sheep might be also an alternative model for studying osteoporosis. However, as there are differences in endocrinology and the gastrointestinal tract, it has been suggested to investigate the influence of these parameters on seasonal factors, hormones or low bone turnover during long days [20].

3.2.2 Dog

Bone composition, density, and quality were investigated in different species including chicken, cow, pig, dog, and sheep. On basis of the weight of ash, the content of hydroxyproline, extractable protein, and IGF-1, canine bone showed the greatest comparability to human bone. When it comes to bone density, dog and pig were suggested to closely mimic human bone. However, it was concluded that the canine model seems to represent the human situation the best [21]. Kimmel et al.

stated that there are similarities in trabecular bone; however, the bone turnover might be more difficult to match between human and the canine model, as even the same bone types from different sites of the same animal show high variability in turnover [22].

In comparison with the typical secondary osteonal microstructure in human cortical bone, Wang et al. reported that canine cortical bone rather consists of a secondary osteonal core, which is flanked to both sides (periosteal and endosteal) by plexiform bone. Plexiform or laminar bone is found predominantly in large and fast growing animals, but not in humans after embryogenesis [23].

3.3 Osteoporosis

3.3.1 Clinical significance

The advancing prevalence of post-menopausal osteoporosis is associated with increasing age of the population. Osteoporosis is characterized by weakening of the bone mass and density consecutively increasing the risk of bone fractures. In 2010, 3.5 million incident fragility fractures (fractures under osteoporotic conditions) were recorded in the European Union, which also increases the economic burden associated with high healthcare costs [24]. The strong increase in age is closely associated with the increase to suffer not only from a single fracture but also from multiple fractures at an advanced age. Worldwide, 1 in 3 women and 1 in 5 men over 50 will experience osteoporotic fractures [25, 26]. A quarter of those with hip fractures never walk again or even die [27].

Since the 1940s, when Fuller Albright demonstrated that estrogen can reverse negative calcium balance in post-menopausal women, there is a remarkable advance concerning osteoporotic drugs. However, concerns have been raised when it comes to anti-resorptive drugs, such as bisphosphonates, especially about rare side effects [28]. Therefore, researchers also focus on enhancing patient's acceptance and compliance with anti-resorptive drugs and in parallel evolving novel drugs without long-term side and prolonged anabolic effects.

3.3.2 Osteoporosis-related outcome on bone

Osteoporosis is a skeletal disorder that is generally subdivided into primary and secondary osteoporosis, latter describing osteoporosis as a secondary outcome to chronic diseases such as Cushing's syndrome. In contrast, primary osteoporosis involves type 1 post-menopausal and type 2 senile osteoporosis. Post-menopausal osteoporosis is a multifactorial disease characterized by weakening of the trabecular and cortical bone structure (**Figure 1**). During osteoporosis, loss of bone results in decreased total mineralization, leading to reduced tensile bone strength and increased risk of fracture. During bone fracture healing, mechanical and biological factors are negatively affected by osteoporosis [29]. Under healthy conditions, however, cellular and molecular events are carefully orchestrated, thereby producing a template for regeneration and remodeling of the fracture site, followed by bone function restoration, resulting in successful fracture healing [30]. Under osteoporotic conditions, reduced numbers and/or reduced activity of osteogenic cells including mesenchymal stem cells and osteoblasts, while osteoclast activity increases. An imbalance of anabolic and catabolic local factors has also been linked to osteoporosis [31]. Osteoporotic bone fractures are also associated with an impaired bone cell proliferation rate, reduced mechanical stress, and inhibited reactivity to local and systemic stimuli. Impaired vascularization has been observed under osteoporotic conditions [29]. However, spontaneously elevated

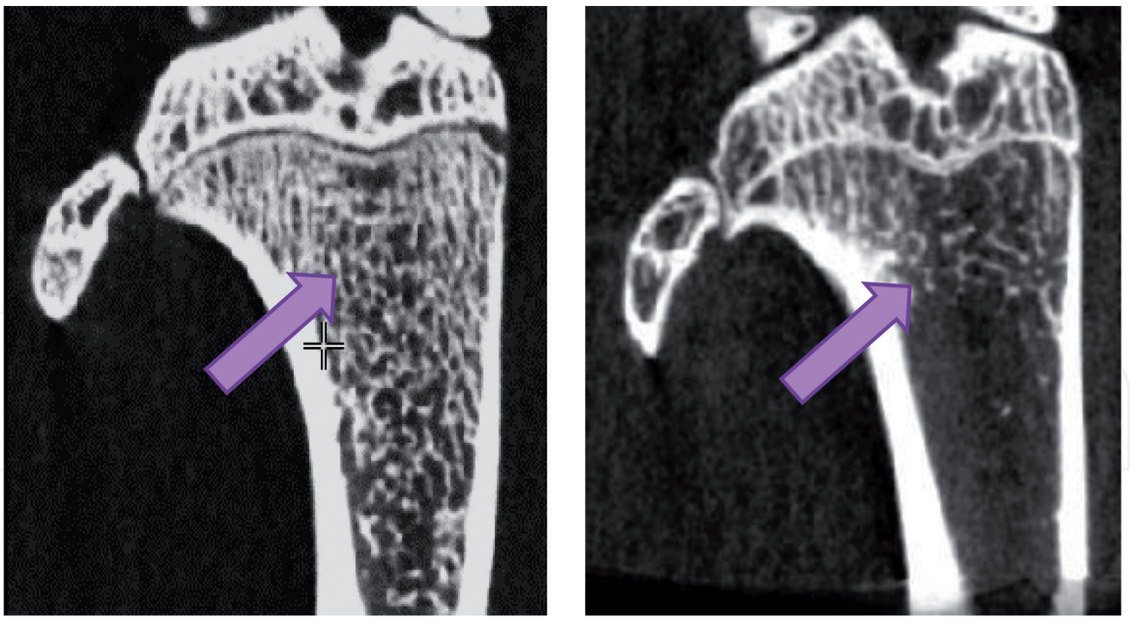

Figure 1.
Osteoporosis leads to reduced trabecular bone structure after ovariectomy in female Sprague Dawley rats. Micro-computed tomography pictures of the left proximal tibia are presented 4 weeks (left) and 8 weeks (right) after ovariectomy (unpublished data).

pro-inflammatory cytokine expression such as TNF-α, IL-6, and IL-1 and decreased bone forming factors (IGF-1 and TGF-β) are associated with osteoporosis [31].

3.3.3 Animal models for osteoporosis research

Depending on the research aspects of osteoporosis, animal models must be carefully chosen: on the one hand, animals are used to investigate anti-resorptive drugs (e.g., bisphosphonates), and on the other hand, bone fracture healing and novel treatment options (e.g., pharmaceutical, implants, etc.) for bone fractures are investigated in vivo [32, 33].

Before choosing the ideal animal model, one must consider different aspects in bone physiology. In general, there are different procedures to induce osteoporosis: on the one hand, surgical manipulation by ovariectomy, hypovasectomy, orchidectomy, and parathyroidectomy can be performed; on the other hand, diet modifications, drugs (e.g., steroids), and immobilization have been used to induce osteoporosis. Another possibility is to use aged animals or genetic modification to reflect senile osteoporosis. However, there have been several studies that demonstrated the relevance of rodent models to study post-menopausal (primary osteoporosis type 1) and senile osteoporosis (primary osteoporosis type 2). For example, the comparability of life time expectancy and closure of the epiphyseal growth plate is similar in mice and humans with about 20% in age ratio, and it markedly differs in rats with 30% as well as in sheep and dogs with 5–10% [2]. Moreover, the genetic uniformity in inbred rodents allows a smaller number of animals compared to outbred strains. Another important aspect has to be taken into account when conducting bone fracture studies: humans are mainly affected by metaphyseal fractures [34].

Mice: The average life span of laboratory mice is between 2 and 3 years, and after 8 months, BALB/c and C57BL/L mice show an age-depended decline in bone quality and mass (mice lack the Haversian remodeling), but aged animals show resorption cavities which are comparable to humans' Haversian canals [35]. The popular laboratory mouse strains, C57BL/L and BALB/c, develop senile

osteoporosis-like bone phenotype with decreased bone mass and quality [36, 37]. For example, senescence-accelerated mouse (SAM) lines are reasonable models to study senile osteoporosis, because the aging phenotype is apparent even after 6–8 months [38].

Rats: In osteoporosis research, the rat model is most commonly used, especially for research on post-menopausal osteoporosis. Considering the bone physiology, the transition of modeling to remodeling occurs at 6–9 months of age in the proximal metaphysis of the tibia and at 12 months of age in the cortical bone in rat. In aged rats, Haversian canals are present, and at the age over 12 months, rats represent a good model for senile osteoporosis. However, the major issue with the rat model is that ovariectomy induces changes predominantly in the trabecular bone (**Figure 1**), and rats are preferably used to study late stages of bone fracture healing [39]. Another advantage when compared to mice is that this model is larger, which simplifies surgical procedures and investigation of mechanical properties.

Large animal models: Bone mass is only marginally reduced in dogs following ovariectomy and sheep exhibit plexiform bone arrangements in which age-related osteopenia does not occur. However, in general, sheep and mini-pig represent the most appropriate animal model for both post-menopausal and senile osteoporosis (>9 years of age). Nevertheless, extensive costs associated with housing and the variability of sheep regarding the aging process is a notable disadvantage for this large animal model.

A major disadvantage in aged large animals is that osteoporosis with low bone turnover develops only 24 months after hypothalamic-pituitary disconnection. Moreover, the typically ovariectomy-induced osteoclast recruitment has not been observed with this surgical method [40].

3.4 Osteoarthritis

3.4.1 Clinical significance

The definition of osteoarthritis (OA) depends on the way, how the disease was diagnosed including radiography, symptoms, self- or physician-diagnosed. Accordingly, the incident and prevalent numbers of OA dramatically vary and are also connected to OA with or without symptoms. OA is mainly characterized by deteriorated cartilage in joints, thereby resulting in rubbing of the bones leading to pain, stiffness, and impaired movement [10]. However, OA predominantly affects hands, feet, knees, and spine. OA is an age-depended disease, which is closely associated with several risk factors such as less physical activity, obesity, bone density, trauma, and gender [41, 42]. Especially, due to the age-related aspect of OA, it has been estimated that 15% (130 million) of people over 60 (20% of the population estimated by 2050) will exhibit OA-depending symptoms and one-third of those will be severely disabled (40 millions) [42]. Diagnostic tools for OA include magnetic resonance imaging (MRI), X-ray, and arthroscopy. However, the major problem associated with OA is non-modifiable risk factors such as age, gender, and genetics.

Hence, the disease must be properly understood to develop novel therapies to either stop or reverse the OA progression.

3.4.2 Osteoarthritis: pathogenesis and classification

This pathology leads to cartilage degradation, inflammation of joints, and abnormal bone formation [43]. Under healthy conditions, the meniscus, synovial membrane, subchondral bone, and articular cartilage support the joint: the

meniscus is composed of type I collagen (also less amount of type II, III, V, and VI collagen), proteoglycans, and water and takes over several functions such as load bearing in the knee joint [44]; the synovial joints need the articular cartilage to move and latter one is composed of type II collagens and proteoglycans; the joint and the articular cartilage are nourished by synovial fluid, which is produced by the synovial membrane [45]; and the subchondral bone built up from mineralized type I cartilages serves to support the joint. The progression of OA can be stimulated by different factors; for example, mechanical abrasion tremendously degenerates type I and II collagen within the meniscus in the knee and further results in a pro-inflammatory situation with increased release of tumor-necrosis factor alpha (TNFα), IL-1, IL-4 or IL-13 and enzymes such as matrix-metalloproteinases (MMPs) might trigger the OA progress [46]. Due to MMP release, the collagen matrix is degraded, leading to articular cartilage degradation and in parallel, and the chondrocytes are not even more able to for new cartilage. Hence, abnormal remodeling of the subchondral bone, making the calcified cartilage and bone interface more acceptable to invades and leading to pain [10]. To date, novel treatment strategies are based on cytokines and the inflammatory situation, such as anti-rheumatic drugs [47]. Additionally, other treatment options such as scaffolds or lifestyle modifications might play a future role.

Similar to osteoporosis, OA was originally classified in primary and secondary OA: while primary OA was to be naturally occurring in either one (localized) or more (generalized) joints, secondary OA was associated with risk factors including diseases of bone or metabolism, trauma or others. However, there have been several debates on the classification of OA, which has been replaced based on recommendations and includes five phenotypes depending on aging, metabolism, genetics, trauma, and pain. On the basis of these phenotypes, the following ways to induce OA, including advantages and disadvantages have been proposed (**Table 1**):

3.4.3 Animal models to study osteoarthritis

In order to study the pathophysiology, pathogenesis, and therapeutic efficiency of novel treatment options for OA, there are several in vivo animal models [49]. The variability of this disease and the different outcomes for the patients make the choice of the ideal in vivo model much more difficult. While pathogenetic studies require naturally occurring OA models, molecular biological studies make use of genetic models. However, to test therapeutic strategies, surgical models are preferred (**Table 1**) [48]. Somebody has also to consider the morphology of the lesion and the pathogenesis-involved mediators, especially when testing pharmaceuticals [50].

Mice: Murine models are currently used to study primary OA, which is naturally occurring and is associated with the time consuming OA development [51]. The major disadvantage is huge husbandry costs due to the slow progression (**Table 1**), whereas the translatability to the human situation is given [48]. Genetic models, such as the prominent transgenetic model STR/ort with increased oxidative stress leading to the naturally development of OA, are particularly useful to investigate genes and their interaction with tissue components [52]. Transgenic mice are extensively used to both, induce and worsen OA progression, or to protect from the disease; to investigate molecular aspects underlying OA, inflammation and genetic contribution to OA. Surgical intervention in the knee of mice can be performed to induce OA: medial collateral ligament transection with partial medial meniscectomy [53] leads to moderate or severe medial cartilage degeneration with comparable lesion development in rats. Anterior cruciate ligament (ACL) has been described to result in severe lesions. However, the combination of a genetic

Model induction	Use	Advantage	Disadvantage	Animal model
Naturally occurring – no intervention needed	• Study pathogenesis of degenerative OA	• Variable disease like in humans	• Long time for disease development • Time consuming • High costs	Mouse, sheep, dog
Surgical intervention	• Test therapeutic efficacy of treatment options • Examine OA lesions and stages	• Rapid progression • Reproducibility • Severe lesions • Induction of traumatic OA	• Due to surgery, inappropriate for pathogenesis of degenerative OA	All
Chemical intervention	• Test therapeutic efficacy of treatment options • Examine OA lesions and stages	• Most rapid progression of OA • Less invasive • Easy implementation	• Not correlated to any type of human OA	All
Genetic intervention	• Test genetics of OA	• Genomic intervention	• Additional cartilage abnormalities or embryonic lethal deletions • High cost	Mouse

Table has been adapted from [48].

Table 1.
Methods to induce OA including the use, advantages, and disadvantages as well as most prominent animal models used according to the OA induction method.

model with a surgical intervention will be also beneficial to study detrimental factors or prophylactic effects of pharmaceuticals during different stages of OA [54]. However, chemical intervention using bacterial collagenase by intra-articular injection induces OA lesions which vary in severity [55]. Injections must be done carefully, otherwise damaging the cruciate ligaments thereby resulting in unwarranted OA lesions.

Rats: OA can only be induced surgically or chemically in rats, since there are only rare cases in which minimal focal areas of degenerative tibia can be seen [56]. However, OA in rats can be induced via medial meniscal tear or injection of iodoacetate, followed by ACL transection. After unilateral medial meniscal tear, OA-associated cartilage degeneration rapidly progresses [57] and large lesions can be observed. The major disadvantage of this model is the rapid degeneration of the cartilage thereby being difficult to observe protective effects. Importantly, toxicologic testing is the major advantage of the rat OA model, since efficacy of therapeutic interventions can be obtained easily and in a short duration and rats consistently respond to the surgery [50]. The intra-articular single-dose injection of iodoacetic acid (25–50 μl of 10 mg/ml) sufficiently kills chondrocytes by inhibition of aerobic glycolysis. The outcome on bone is remarkable and forms the basis for the development of cartilaginous lesions [58]. ACL transection in mature rats also leads to progressive changes, especially in the medial joint. In comparison to the meniscal tear model, OA progresses much slower after ACL transection and results obtained after ACL transection are comparable between rats and dogs. However, due to the slower progression, ACL transection is preferred when testing therapeutic interventions.

Disadvantages of this method are comparable to those in dogs, including variable severity of lesions and lesion locations [50].

Sheep: This large animal model is also used to study naturally occurring OA (primary OA) with similar advantages compared to mice. For example, sheep have been successfully used to study early changes of cartilage degeneration, meniscus changes, and treatment options [59].

Dogs: Dogs have been shown to be natural models to study therapeutic interventions: cranial cruciate ligament transection has been demonstrated to induce naturally occurring OA and serves as an interesting model to evaluate structural and functional benefits of treatment strategies that will give a better prediction for clinics [60]. Moreover, established canine OA models usually undergo ACL transection or partial medial meniscectomy. The major disadvantage is that dogs need large runs or plenty of exercise, otherwise resulting in mild, variable lesions. Additionally, surgical procedures must be carefully performed to avoid traumatic lesions. However, if surgical procedures are performed appropriately, OA lesions are consistent thereby allowing a relatively small number of animals per group (12–15 animals per treatment group). Another major advantage is a short screening and testing duration of 1 month [50].

Currently, there is no "gold-standardized OA model," and the most appropriate animal has to be chosen individually, depending on the research question. Moreover, extensive work is needed and advantages and disadvantages of the models must be clearly outlined in the future.

4. Orthopedic in vivo implant research

In vivo studies are essential to investigate novel implant materials and cannot be fully covered by in vitro testing. Preliminary safety tests with new implant materials using in vitro models give some information on acute toxicity and cytocompatibility. Nevertheless, some studies use the term of biocompatibility when testing implant material in vitro. However, biocompatibility tests need living organisms such as animals and humans; therefore, cytocompatibility needs to be correctly used when testing in vitro.

In order to test implant safety, adverse tissue reactions as well as corrosion and wear resistance need to be investigated to guarantee its long-term application in clinics. Hence, in vitro and in vivo tests are essential to evaluate new implant materials regarding cytocompatibility, biocompatibility, and mechanical stability.

The development of bioresorbable metal implants is one of the major goals in orthopedic and trauma surgery. Apparently, the advantages are the unnecessity to remove the implant due to material resorption and the associated avoiding of narcosis, mandatory for the second removal surgery. Since there is an increasing number of patients with metal sensitivity to permanent implants such as titanium (Ti), and long-term complications associated with currently available metal implants cannot be foreseen to date, there is a high demand to develop novel biocompatible and bioresorbable implants with good mechanical properties to stabilize bone fractures.

4.1 Implant design

To test bioresorbable orthopedic implants in animal models, the implant design and dimension is of utmost importance. Moreover, the implant number and size directly influences the number and species of animals used to test a research hypothesis. The most common implant designs used in small animal models such as mice and rats are cylindrical-shaped pins [61], whereas screws are the commonly

used designs in large animal models such as sheep [62] (**Figure 2**). However, there are much more designs that are less commonly used such as plates and discs. More importantly, the size of the implant must be adjusted to the size of the animal which is comparable to common implant sizes in humans. In small animal models, cylindrical rods are used with a simple geometry that makes it easier to analyze implant degradation and behavior. However, these rods have to exactly fit ("press-fit"), otherwise the implants will become unstable and will be lost during investigation. Screws are more reliable when it comes to comparison with humans, since screws are commonly used to stabilize fractures or fix plates in humans [63]. However, the geometry is more complicated which makes the analysis more difficult.

Dimensions of implants differ according to the sizes of the animals. For example, the most appropriate dimension for cylindrical implants in rabbits is 6 mm in length and 2 mm in diameter, whereas the ideal dimension for large animals including goat, dog, and rabbit is 12 mm in length and 4 mm in diameter, according to ISO guidelines. Proper controls have to be chosen to investigate new implant material. According to ISO standards, it is recommended to use currently certified materials, which are already used in clinics, as a control [64]. In order to properly examine implant material, primary outcomes have to be specified: to test mechanical properties, bone tissue with implants are harvested and undergo pull-out/push-out tests (cylindrical implants) and torque removal tests (screws) (**Figure 2**). This test usually demonstrates proper integration of the implant in bone [1]. In case of resorbable biomaterials, degradation behavior and bone in-growth are the major primary outcomes besides mechanical properties. Real-time imaging techniques, such as in vivo micro-computed tomography (μCT) in small animals and clinical CT in large animals are used to observe material changes (degradation, bone in-growth, etc.) over the entire study duration. After reconstruction, 3-dimensional (3D) images can be reconstructed, and implant volume loss and bone formation can be calculated [61].

Other studies aim to investigate effects of implant surface modifications on bone formation and bone-implant interaction. To obtain accurate results, surface characteristics including chemical composition and surface topography must be determined. Therefore, visual observation (scanning electron microscopy, μCT) and numerical analysis (energy dispersive X-ray microscopy, profilometry) must be performed [65].

4.2 Implant material

Conventional alloys currently used in the treatment of fractures include Ti and stainless steel, which are more rigid with desirable advantages including biocompatibility, good resistance to material corrosion, and most importantly, these alloys do not show severe toxic effects on various immune cells and can bear weight soon

Figure 2.
Screws (left image) and cylindrical pins (right image) are often used in orthopedic and trauma research.

after implantation. However, Ti and stainless steel implants have a higher e-modulus (Ti: e-modulus 100–124 GPa; steel: e-modulus 200 GPa) than bone (e-modulus 6–24 GPa). Moreover, permanent implants can cause "stress-shielding" leading to loss of bone under the plate or between bone and implant, thereby increasing the risk of refractures, designated as peri-prosthetic or peri-implant fractures [29, 31]. Moreover, the FDA has already described possible metallic sensitivity or allergic reactions linked to Ti-based alloys, such as Ti-6Al-4V [66], and studies using vanadium have also shown adverse effects [67]. However, resorbable materials exhibit functional properties by supporting bone formation and in-growth on a molecular level. To date there are only few resorbable materials used in cardiac, dental and neuro-surgery and some not weight-bearing application in orthopedic surgery, but adequate materials for orthopedic and trauma surgeries need good mechanical properties. Therefore, the main focus in biomaterial research is to evolve materials and tools to develop the optimal implant for the respective bone condition under the necessity to bear weight.

4.2.1 Polymers

Poly-L-lactic acid (PLLA) and poly-lactic-co-glycolic acid (PLGA) are the most commonly used polymers considered for their use as osteosynthesis and bone grafts [68]. Disadvantages of polymers include poor mechanical properties (low strength and stiffness, high brittleness) and osteoconductivity. Degradation behavior depends on monomers and can be very slow thereby increasing the risk of adverse effects such as sclerotic areas in bone and fibrous encapsulation [69].

Alternatively, polyhydroxybutyrate (PHB) can serve as a polymeric implant material, which is produced by microorganisms, especially bacteria. However, PHB can induce toxicological effects. However, these effects have been reduced dramatically, raising the potential for its application in clinics. Nevertheless, functional properties (i.e., osteoinductivity or osteoconductivity) of polymeric implant material have not been discovered yet.

4.2.2 Ceramics

Ceramics are synthetic bone replacement materials with good biocompatibility and osteoconductivity, thereby showing good osseointegrative and non-immunogenic effects. Composed of hydroxyapatite (HA), or alpha (α)- and beta (β)-tricalcium phosphates (TCP), ceramics exhibit poor mechanical properties including low yield strength and high brittleness, which make them unattractive for their application in load-bearing regions.

4.2.3 Bioresorbable metals

In comparison to polymers and ceramics, iron (Fe), magnesium (Mg), and zinc (Zn) are more stable, tensile, and load-bearing, respectively. To process Fe-based alloys, the low melting point of Fe constitutes an interesting property. However, Hofstetter et al. demonstrated that limited access to oxygen was associated with slow degradation rates [70]. Metal implants based on Zn display several disadvantages including low rigidity and deformability, as well as corrosion inhibition. Therefore, Zn is likely more suited as an alloying element in combination with other materials. Finally, Mg-based alloys exhibit several advantages including good biocompatibility, resorbability, and favorable biomechanical properties. Moreover, some studies have demonstrated Mg's associated functional properties, especially its ability to support bone fracture healing [33]. For example, recent studies using

26 Mg isotope pins in a rat model demonstrated high Mg content in the bone-implant interface [71]. Good bone in-growth and a tight interface between bone and implant were observed. Additionally, drilled hole bone fractures showed full recovery after complete degradation of Mg implants. The serum concentration of Mg indicated a high tolerance of increased Mg levels which was controlled by urine excretion. Bone formation has been observed after implantation of XHP-Mg-0.45Zn-0.45Ca implants in young, growing small and large animal models [61].

5. Conclusion

Here, we summarized fundamental differences in small and large animal models concerning bone quality, composition as well as their individual advantages and disadvantages. Focusing on two major complications in orthopedics and traumatology, we wanted to underline the merits of an animal model by supporting with scientific results obtained from our intensive literature recherché. Implant research is a hot topic in orthopedics and trauma surgery. Based on our expertise, we wanted to give insights into implant technology, materials, and designs. Currently, permanent implants are the state-of-the-art material used to stabilize bone fractures in orthopedics and trauma surgery. However, to develop the ideal implant for a certain bone condition (e.g., osteoporosis and osteoarthritis), the underlying disease and the detrimental outcome on bone (e.g., bone mass, fracture risk, and bone density) have to be taken into account when choosing the implant material (e.g., Ti-, Mg-, Fe-based implants), design (e.g., pin, screw, plate, and scaffold), material properties (e.g., tensile strength, non- or bio-resorbable), and implantation site (e.g., knee, femur, and tibia).

Hence, it is of utmost importance to choose the most appropriate animal model according to the research question and warranted primary outcome measures.

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