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Evaluation of preclinical animal models in bone tissue engineering and their success in clinical translation

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Für meine Eltern

Table of contents

1 Introduction

1.1 The need for bone graft

Trauma, tumor resection and skeletal abnormalities can cause complex bone defects, especially under conditions of compromised healing such as infection, avascular necrosis, atrophic non-union as well as osteoporosis, entailing a significant burden for the patient. When self-repair mechanisms of the bone reach their limits, osseous reconstruction requires a considerable quantity of bone graft in order to recreate form and function of the affected bone and, eventually, to restore the patient's quality of life (Dimitriou, Jones et al. 2011, Marsell and Einhorn 2011).

With bone being the second most transplanted tissue after blood, there has been a considerable number of attempts to reconstruct bone in order to ensure structural and functional integrity (Henkel, Woodruff et al. 2013). Autologous and allogeneic bone graft materials, synthetic bone substitutes, the use of growth factors and living cells, distraction osteogenesis or the Masquelet technique represent current clinical strategies with relatively satisfactory outcome for defect restoration with limited intrinsic regenerative potential (Giannoudis, Dinopoulos et al. 2005, Dimitriou, Jones et al. 2011, Henkel, Woodruff et al. 2013).

Although autologous bone graft material represents the gold standard in daily clinical routine, it certainly has, like the others, its disadvantages regarding costs, efficacy and limitation in availability (Dimitriou, Jones et al. 2011), which will be described in more detail below (see chapter 1.3). In order to overcome these apparently insurmountable limits of present bone graft materials and to finally recreate bone that is indistinguishable from the initially uninjured bone, the interdisciplinary field of bone tissue engineering has emerged and research in this area is under intense examination. A very promising field that, once it has made its way to the clinics, will clearly lead to numerous possibilities for tissue regeneration and repair (Dimitriou, Jones et al. 2011, Henkel, Woodruff et al. 2013).

1.2 Biology of bone

Bone maintains important functions that include the protective support for surrounding organs, metabolic tasks as well as biomechanical strength and locomotion. Its capacity to rebuild and renew itself throughout lifetime makes bone an unique tissue (Feng 2009). This chapter provides information about the osseous composition and structure of the human skeleton, about bone development and the potential regenerative capacity in case of fracture.

1.2.1 Composition and structure

Bone is a complex composite structure consisting of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (mineral or inorganic phase, ~60%), collagen and non-collagenous proteins such as: albumin, fetuin-A, growth factors, proteoglycans, glycosylated proteins and γ-carboxylated proteins (organic phase, ~30%) and water (Feng 2009, van Blitterswijk and De Boer 2015).

The human skeleton consists of bones of different sizes and shapes. Long bones (e.g. femur, tibia, radius, humerus, ulna) can be partitioned into three parts. The middle part of the bone is called diaphysis and contains the bone marrow in the medullary cavity. The diaphysis then merges into the metaphysis on both sides where growth processes take place (see chapter 1.2.2) and for what it is also called growth plate. The end zone is formed by a proximal and distal epiphysis. The periosteum, a double layered membrane containing collagen fibers, skeletal cells, blood and lymphatic vessels and nerve fibers, surrounds most parts of the bone. It is essential for bone nutrition, bone healing and bone growth (Fig.1) (Spence 1986, Schulte, Schumacher et al. 2011, van Blitterswijk and De Boer 2015).

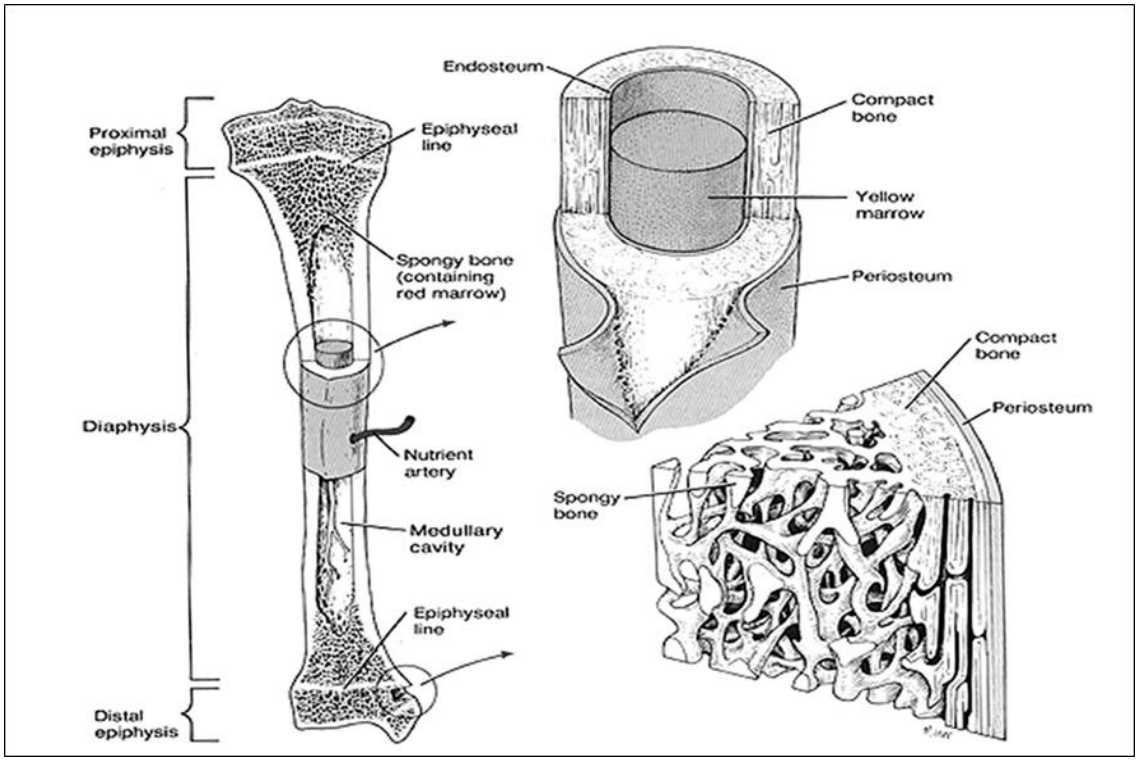


Fig. 1 The architecture of long bones. SPENCE, BASIC HUMAN ANATOMY, 3rd, ©1991. Reprinted by permission of Pearson Education, Inc., New York, New York.

Furthermore, bone tissue consists of a highly organized structure which can be classified by a hierarchical architecture: a macrostructure, a microstructure and a nanostructure (Fig.2) (Rho, Kuhn-Spearing et al. 1998, Henkel, Woodruff et al. 2013).

The bony macrostructure consists of two main types: the cortical and the cancellous bone. The cortical bone (or compact bone) forms the diaphysis of long bones. Since up to 90% of the cortical bone is calcified, it provides mechanical strength and protective function. The epiphysis and metaphysis, on the other hand, mainly consist of cancellous bone (also called trabecular or spongy bone), ensuring the metabolic function of bone and comprising only about 25% of calcified bone. Cancellous bone is hence a metabolically more active structure than cortical bone and remodels itself more often. However, also a small amount of cortical bone can be found at the epiphyseal and metaphyseal part of long bones (Rho, Kuhn-Spearing et al. 1998, van Blitterswijk and De Boer 2015).

The microscopic scale of cortical bone demonstrates layers of mineralized collagen fibers, forming sheets that are called lamellae (~3-7 µm). They wrap in

concentric layers around the Haversian canal with its nerves and blood vessels and form a unit called Osteon or Haversian system (~250 µm). The microstructure of cancellous bone resembles an interconnected framework of rod-rod, rod-plate or plate-plate shaped trabeculae (Rho, Kuhn-Spearing et al. 1998).

Eventually, the nanostructure of bone compromises collagen proteins that are surrounded and reinforced by minerals. The fibrillar substructure of collagen (~200 nm in length) is once again subdivided into fibrils (~500 nm in width) consisting of collagen molecules and bone crystals (Rho, Kuhn-Spearing et al. 1998, Kane and Ma 2013).

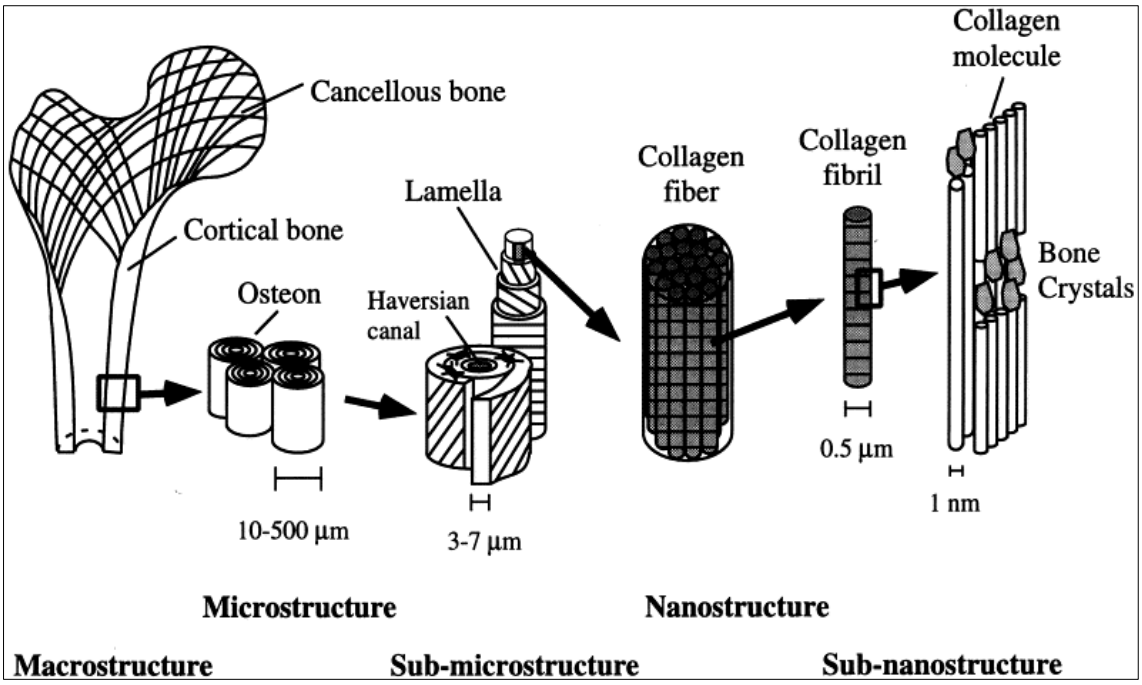


Fig. 2 Schematic representation of the bone structure. Bone can be divided into a macrostructure (cortical and cancellous bone), a microstructure (osteon formed by the Haversian system) and a nanostructure (collagen proteins). Reprinted from Medical Engineering & Physics, Vol: 20, Issue: 2, Rho, J. Y., Kuhn-Spearing, L., Zioupos, P., Mechanical properties and the hierarchical structure of bone, Page: 92-102, Publication Year: 1998, Copyright © 1998 Published by Elsevier Ltd., with permission from Elsevier.

1.2.2 Bone development

Before ossification of human bone begins between the 6th and 7th week of pregnancy, the skeleton of the human embryo consists mainly of hyaline cartilage and fibrous membrane which form a sort of guiding structure (https://opentextbc.ca/anatomyandphysiology/, August 28, 2018). As bone is a replacement tissue, it can only grow by replacing a template. For the de novo formation of cancellous or compact bone, this template can consist of membrane (intramembranous ossification), cartilage (endochondral ossification) or preexisting bone itself which serves as guide for healing bone that has been fractured. Endochondral and intramembranous ossification thereby represent two different pathways that conclude in one and the same structure: calcified bone (van Blitterswijk and De Boer 2015).

1.2.2.1 Intramembranous ossification

Intramembranous ossification gives rise to flat bones of the skull, most of the cranial bones and medial clavicles (Ornitz and Marie 2002). Furthermore, it represents a crucial step during natural fracture healing of bone (see chapter 1.2.3.) (Einhorn and Gerstenfeld 2015).

Therefore, mesenchymal cells differentiate into osteoblasts and synthesize osteoid, an uncalcified bone matrix that mineralizes subsequently. Due to the mineralization process, osteoblasts are entrapped and differentiate into osteocytes. The secretion of osteoid continues and forms a trabecular matrix starting to interconnect (Lüllmann-Rauch 2003). Compact mesenchymal cells surround the trabeculae and form the periosteum. Osteoblasts on the inner surface of the periosteum secrete more osteoid and thereby layers of bone are formed parallel to the existing trabeculae (Gilbert 2000).

1.2.2.2 Endochondral ossification

Long bones, some facial bones, lateral medial clavicles and vertebrae develop by endochondral ossification (Ornitz and Marie 2002). Whereas intramembranous ossification creates bone directly from mesenchymal cells, endochondral ossification achieves this by adding an intermediate step: an avascular cartilaginous template that is gradually being replaced by highly vascularized new bone. Therefore, mesenchymal cells cluster in order to shape the cartilage scaffold, also called anlage (Ornitz and Marie 2002, van Blitterswijk and De Boer 2015). For longitudinal growth, chondrocytes then proliferate, become hypertrophic and finally mineralize, leaving an extracellular cartilage matrix in which osteoclasts, osteoblasts and blood vessels invade in order to eventually transform cartilage into bone (Mackie, Ahmed et al. 2008).

Since bone is a dynamic tissue, it is able to maintain its functions throughout the lifetime of a healthy human skeleton by constant remodeling processes through assembly and disassembly. Thereby, the biochemical and biomechanical surrounding stimulates old bone to disaggregate by the help of osteoblasts, whereas osteoblasts subsequently recreate renewed bone (Feng 2009). However, profuse overload and external force application may result in bone fractures, especially if the osseous structure is already weakened by age related changes in the hormonal milieu and associated diseases such as osteoporosis. (Jakob, Ebert et al. 2012, Zhang, Richardson et al. 2012). Fragility fractures of the vertebrae, the radius and the hip are prone to occur and even minimal trauma may already result in critical bone fractures that impede physiological bone healing and regeneration (Jakob, Ebert et al. 2013). This would hence imply the necessity of accurate bone graft materials or bone tissue engineered constructs, if available.

1.2.3 Fracture Healing

Bone regeneration in normal fracture healing is a remarkable, yet complex physiological complex, involving different cell types, cytokines, growth factors and the extracellular matrix, all responding to mechanical stimuli of the environment (Zhang, Richardson et al. 2012, van Blitterswijk and De Boer 2015).

There are two different types of fracture healing that exist: the direct (intramembranous) and the indirect (intramembranous and endochondral) type. Since direct fracture healing demands rigidly contact of the bone fragments and absolute stability, most of the fractures heal by indirect bone formation. This involves different stages: inflammation, soft callus formation, hard callus formation and bone remodeling (Marsell and Einhorn 2011).

During the acute inflammatory phase, an initial hematoma is transformed into a fibrinous thrombus that is then reorganized into granulation tissue. This process is driven by cytokines and growth factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-11 (IL-11) and interleukin-18 (IL-18), transforming growth factor beta (TGF-β), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (Marsell and Einhorn 2011, van Blitterswijk and De Boer 2015). Although both intramembranous and endochondral ossification are involved, the cartilaginous callus represents a special key characteristic in the process of indirect fracture healing. Mesenchymal stem cells (MSCs) cluster and differentiate into chondrocytes. Together with fibroblasts, they remodel the granulation tissue between the fracture endings and external to the periosteum into a cartilaginous template that forms a soft callus to stabilize the fracture, whereby the adjacent subperiostal tissue is transformed into a hard callus by intramembranous ossification (Marsell and Einhorn 2011). Due to endochondral ossification, the soft callus is transformed into a hard bony callus (woven bone) which osteoblasts and osteoclasts will then subsequently restructure into normal, more solid and fully regenerated bone (Marsell and Einhorn 2011, Einhorn and Gerstenfeld 2015, van Blitterswijk and De Boer 2015).

However, there are limits to the capacity of bone to heal by itself if certain defect sizes are reached. When sufficient stabilization is provided, up to a finite defect size, bone tissue is able to truly regenerate in a physiological way, without the development of fibrotic scar tissue (Henkel, Woodruff et al. 2013). This process can take years to be entirely completed and, depending on age, vascularization and external circumstances may never be fully achieved. Atrophic fibrous non-unions and pseudarthrosis (inaccurate joint forming and development of a synovial membrane) can then occur as a result of insufficient bone repair. There are two types of non-unions: hypertrophic non-unions (with callus formation at the fracture ends), often in case of defaulting on sufficient fracture stabilization, and atrophic non-unions (without callus formation) (Marsell and Einhorn 2011, Garcia, Histing et al. 2013).

1.3 Current treatment of bone defects

As already mentioned, the human skeleton is able to repair bone defects effectively in most cases (see chapter 1.2.3). However, limitations in bone healing occur if certain defect sizes are reached. Therefore, therapeutic strategies needed to arise in order to support and promote physiological bone healing. This chapter provides information about the different characteristics of skeletal defects and current clinical methods applied to treat such critical defects.

1.3.1 Skeletal defects

Seen from an anatomical point of view, skeletal defects can be classified into diaphyseal, metaphyseal or articular defects. The anatomical location of the defect has high impact on the prognosis since blood supply and osteogenic potential varies depending on the location. Considering the extent of the defect, it is important to specify whether the involved bone includes partial or segmental circumferential loss (Keating, Simpson et al. 2005).
A critical-size defect has been defined as a segmental bone defect with either a length that exceeds 2 to 2,5 times the diameter of the affected bone (Gugala,

Lindsey et al. 2007) or a distance bigger than 2 cm between the bony ends, including more than 50 percent of the bone tissue circumference and moreover, would not heal spontaneously in spite of surgical stabilization (Keating, Simpson et al. 2005). For animal models used in preclinical studies, however, the term critical-size defect has also been used differently. Schmitz and Hollinger defined it as the smallest intraosseous defect which cannot heal spontaneously during the lifetime of the animal or a defect that has less than 10% regeneration during lifetime (Hollinger and Kleinschmidt 1990, Gugala and Gogolewski 1999, Schemitsch 2017).

This means that the size that renders a defect "critical" is not very well set (Lindsey, Gugala et al. 2006). Moreover, in addition to the abovementioned classifications, it is important to consider other influences that may have impact on the healing process: the dimension of injured soft tissue surrounding the bone, the condition of the periosteum, the age of the patient, the presence of underlying diseases and the consumption of tobacco, alcohol and medications (Keating, Simpson et al. 2005). According to that, defining a critical-size defect across anatomical locations and species becomes difficult and therefore hard to apply clinically.

1.3.2 Bone graft materials

Muschler defined the term "bone graft" as any implanted material that is capable of bone healing by inducing osteoconductivity, osteoinductivity or osteogenesis (Bauer and Muschler 2000).

Osteogenesis is the ability to form new bone. Bone graft materials containing living cells such as mesenchymal stem cells or preosteoblasts (precursor cells), which are able to give rise to bone generating cells, are able to induce osteogenesis. Osteoinductive materials can stimulate cells in the surrounding tissue to proliferate and differentiate from precursor cells into bone generating osteoblasts. Osteoinductivity is regulated by complex mechanisms that are controlled by different proteins such as bone morphogenetic proteins (BMPs) and cytokines. Osteoconductive materials supply a suitable scaffold in which the

surrounding bone tissue can grow in. Bone graft materials providing osteoconductivity are hence not able to form bone but serve as a guiding structure (Schwenzer and Ehrenfeld 2000, Albrektsson and Johansson 2001).
The ideal bone graft material should therefore contribute to sufficient mechanical properties and vascularization and provide biocompatibility, osteogenesis, osteoinductivity as well as osteoconductivity all in one. It should be easy to sterilize and to operate, while at the same time, cost effective and easily available in a wide range of quantities (Damien and Parsons 1991, Campana, Milano et al. 2014).

1.3.2.1 Autologous bone

Autologous graft is bone tissue harvested from the same individual as it is implanted in (Bauer and Muschler 2000). This means that donor and recipient are one and the same person (Schwenzer and Ehrenfeld 2000). Thus, autologous bone shows histocompatibility and is non-immunogenic (Dimitriou, Jones et al. 2011). According to that, the incorporation of the graft to the host is accomplished more predictably since the chance of rejection due to immunological response or transmission of infections is most unlikely. Depending on the harvested tissue type (cancellous bone, cortical bone, vascularized graft, aspirated bone marrow), autologous bone supports osteogenesis, osteoinduction and osteoconduction all in one (Bauer and Muschler 2000) and therefore represents today's gold standard bone graft material. Jupiter et al. described a method to reconstruct large bone defects by transferring vascularized fibular autograft to the defect zone (Jupiter, Bour et al. 1987). Also the iliac crest and ribs have been used as vascularized bone graft material (Giannoudis, Calori et al. 2013). However, shortage of quantity and shape of the harvested bone graft and severe donor site morbidity due to the second surgical intervention cause major drawbacks that outweigh the benefits of today's material of choice (Damien and Parsons 1991). Furthermore, it has been shown that defects larger than 5 cm are not suitable for conventional autologous bone grafting. At this point, any grafting technique is predisposed to failure (Giannoudis, Calori et al. 2013). These defects, however, are far from

being a rarity. That is why, today, more than ever, the necessity for an adequate strategy, able to heal such bone defects, is crucial (Giannoudis, Calori et al. 2013).

1.3.2.2 Masquelet technique

Since autologous bone graft alone may be associated with significant drawbacks, the interest in alternative techniques has increased. In 1986, Alain Masquelet et al. developed a novel strategy describing a two-stage technique for the treatment of large diaphyseal bone defects (Masquelet, Fitoussi et al. 2000). Firstly, a cement spacer (polymethyl methacrylate) is inserted into the defect in which radical debridement of the surrounding necrotic bone and soft tissue was performed. The defect is then stabilized by an external fixation system. The first step allows the formation of an induced pseudosynovial membrane that provides adequate vascularization, secretion of growth factors and has been shown to prevent the degradation of the subsequently inserted graft. Secondly, after 6 to 8 weeks, the spacer gets removed and the reconstruction of the defect is operated by implanting great amounts of autologous cancellous bone graft (Giannoudis, Faour et al. 2011, Henkel, Woodruff et al. 2013).

The Masquelet technique represents a well-established and successful technique. However, limited quantity of bone and the risk of possible complications due to the necessary step of harvesting autologous bone still remain and hence encourage the development of alternative bone substitutes (Giannoudis, Faour et al. 2011).

1.3.2.3 Allogeneic bone

An allograft is a tissue that has been harvested from one organism and implanted into another organism of the same species (Bauer and Muschler 2000). It can be harvested from living patients or from non-living donors. Therefore, it represents an attractive alternative to autologous bone since inconveniences such as additional surgical interventions and quantity restrictions are being bypassed

(Dimitriou, Jones et al. 2011). To circumvent the risk of rejection reactions due to immunological host response and to limit the possible infective transmission of pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), or bacteria to the recipient, allograft material needs to undergo different processes of sterilization (ethanol, gamma irradiation, freezing or freeze-drying etc.). Accordingly, allogeneic bone is not osteogenic and depending on the preparation process, detrimental effects on osteoconductive, osteoinductive and mechanical properties may occur (Roberts and Rosenbaum 2012, Campana, Milano et al. 2014). Moreover, high costs and the remaining concern of viral or bacterial contamination make allograft an acceptable but imperfect alternative to autologous bone graft material (Roberts and Rosenbaum 2012).

1.3.2.4 Xenograft bone

Xenogeneic bone originates from a genetically different, non-human species such as bovine and porcine bone or coral based materials. Xenograft bone is usually used as a calcified matrix that has been deproteinized, demineralized or freeze-dried (Campana, Milano et al. 2014) . Thereby, porcine bone represents a very suitable xenograft for transplantation since simplified breeding and genetic manipulations of pigs are possible (Sprangers, Waer et al. 2008). Other approaches for the establishment of a suitable xenograft material involve the use of bovine bone mineral (Bio-Oss, Osteohealth, Shirley, NY) which has been shown to effectively heal defects in oral surgery (Kao and Scott 2007). Coralline xenografts consist of calcium carbonate that shows accurate resorption when used as graft but, in contrast to human bone, does not involve hydroxyapatite. In order to adjust the material to human bone, the use of coral may imply its transformation into hydroxyapatite which then again influences its resorption rate (Campana, Milano et al. 2014). Generally, the potential complications concerning disease transmission, immunological responses and resorption (Kim, Kim et al. 2016) as well as the loss of cells and growth factors due to the preventive

treatments of the graft, minimize the favorable use of xenogeneic materials over autograft bone (Henkel, Woodruff et al. 2013).

1.3.3 Distraction osteogenesis

In response to mechanical stretch stimuli induced by an external fixator device, new bone is formed. This phenomenon is the key to the process of distraction osteogenesis or also called callus distraction. A process that was described by Professor Ilizarov in the second half of the 20th century (Ilizarov, Lediaev et al. 1969).

This ability of bone to regenerate by distraction using an external fixator system consisting of rings, rods and wires is also used to elongate bone. In a first step, corticotomy (cutting only the outer layer of the bone and leaving the periosteum and endosteum intact) is performed in order to obtain two bone fragments. In a second step, gradual distraction on both sides of the two bone fragments is exerted using the external fixation apparatus (daily distraction rate of about 1mm). Due to opposed axial traction, revascularization, callus formation and eventually de novo bone formation occurs; a so-called "Tension-Stress-Effect" (Raschke, Ficke et al. 1993, Gubin, Borzunov et al. 2016).

Although this method has several advantages (minimal-invasive surgery, weight bearing during healing phase), severe disadvantages have to be considered (complicated technique for surgeons, long and painful treatment as well as pin site infections) (Raschke, Ficke et al. 1993, Gubin, Borzunov et al. 2016).

1.3.4 Bone substitutes combined with growth factors and living cells

The complications associated with the aforementioned clinical approaches for bone defect reconstructions have led to the idea of substituting bone tissue in order to promote bone regeneration and to replace autologous and allogeneic bone grafts. The development of organic (biological) and inorganic (synthetic) materials involves demineralized bone matrix, growth factors, ceramics, polyester

and composites (Schlickewei and Schlickewei 2007). High versatility, lower costs, optimized acceptance rate and safety conditions as well as the ease of handling make bone substitute materials an attractive alternative to conventional methods (Pryor, Gage et al. 2009). However, the requirement of osteoconductivity, osteoinductivity and osteogenesis make great demands on the establishment of an ideal bone substitute material and therefore implies the combination of several materials (Henkel, Woodruff et al. 2013). Therefore, progress has been made in the design of scaffold-based tissue engineered products that seek to combine a three dimensional structure (scaffold) showing osteoinductivity with embedded osteogenic and osteoinductive components, paving the way to current bone tissue engineering strategies (Giannoudis, Einhorn et al. 2007, Henkel, Woodruff et al. 2013). Thereby, BMPs, VEGFs, PDGFs, insulin-like growth factor-1 (IGF-1) and TGF-β play an important role since they have important physiological influence on bone healing by effecting cell development, proliferation and differentiation (Minuth, Strehl et al. 2002, Henkel, Woodruff et al. 2013).

In 1981, based on the work of Marshall R. Urist, Sampath and Reddi finally managed to prove the existence of BMPs as a large heterogeneous protein family within the TGF-β superfamily, possessing a wide range of skeletogenic functions. (Urist 1965, Sampath and Reddi 1981, Campana, Milano et al. 2014). Ever since, industries and researchers are working on these proteins capable of inducing bone formation in non-bony sites by promoting the differentiation of MSCs into osteogenic cells, stimulating vascularization and alkaline phosphatase functions (Sampath and Reddi 1981, Wozney and Rosen 1998, Campana, Milano et al. 2014, van Blitterswijk and De Boer 2015).

Altogether, 15 different types of human BMPs have been investigated and the clinical use of BMP-2 and BMP-7 is authorized for certain indications in Europe and the Unites States following the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approval (Dabra, Chhina et al. 2012, Campana, Milano et al. 2014, Gothard, Smith et al. 2014).

Although today's surgical techniques for bone reconstruction have made large progress over the last years and many promising research approaches continue to be intensively studied, they are still not sophisticated enough to overcome

practical limits. Driven by the present need for well-developed and applicable models for bone regeneration, the field of bone tissue engineering has emerged and searches its way into clinical application.

1.4 Bone tissue engineering

Langer and Vacanti described tissue engineering as an interdisciplinary field using the principles of biology and engineering in order to develop functional biological substitutes that reconstruct damaged tissue and maintain tissue function (Langer and Vacanti 1993). Today, nearly every human tissue has been intensively studied for the possible replacement with engineered structures (Langer and Vacanti 2016). The goal is to provide a cell-driven, living construct that is able to interact with the surrounding tissue (Rose and Oreffo 2002). Therefore, acquired knowledge from various interdisciplinary fields (medicine, engineering, material science, quantum physics, molecular and cell biology, polymer chemistry) is involved into one research project.

Bone, however, represents a very complicated tissue to reconstruct in its former structure and function (see chapter 1.2). It consists of a unique structure with key functions to structural support and protection. Furthermore, bone is involved in mineral homeostasis and the provision of bioactive molecules and hematopoietic cells (van Blitterswijk and De Boer 2015). This being the case, the magnitude of its tasks will necessitate the consideration of a multitude of factors when constructing a bone tissue engineered construct. The combination of certain basic elements is thereby involved:

- a three dimensional and biocompatible scaffold conducive to cell attachment
- skeletal stem or precursor cells such as MSCs
- bioactive molecules that induce differentiation and tissue formation (growth factors)
- a host or culture medium
- mechanical stimulation (Giannoudis, Einhorn et al. 2007)

Thereby, autologous cells should be used for the engineered construct in order to avoid vehement reactions of the host's immune system and the underlying risk of rejection (Minuth, Strehl et al. 2002).

The applied scaffold is expected to provide a three-dimensional interconnected pore network with surface properties that ensure cell attachment, cell proliferation, cell differentiation, cell migration as well as metabolic processes, vascularization and mechanical support. Biocompatibility and controllable biodegradability are important for the formation and the remodeling of cells and tissue. For a mechanical scaffold design, geometrical shape, size, porosity, stiffness and strength of the material have to be taken into account, considering furthermore the different size, location and type of bony defects (Woodruff, Lange et al. 2012, Henkel, Woodruff et al. 2013).

The use of MSCs as well as periosteal cells and osteoblasts represent promising strategies for the regeneration of new bone (Henkel, Woodruff et al. 2013). Multipotent progenitor cells, MSCs, are able to transform themselves into various osteogenic, chondrogenic and adipogenic tissues and therefore have the potential to regenerate bone. Furthermore, they are relatively easy to isolate (bone marrow aspiration) and to expand. However, the in vivo comportment in humans and long-term effects remain little known (Berner, Reichert et al. 2012, Lin, Sohn et al. 2018, Toosi, Behravan et al. 2018, Mousaei Ghasroldasht, Matin et al. 2019).

Furthermore, bone tissue engineering requires an exact recapitulation of signaling cascades for osteogenesis, chondrogenesis and angiogenesis in order to heal properly (Gothard, Smith et al. 2014).

The controllable application of osteoinductive growth factors like fibroblast growth factors (FGFs), PDGFs, IGF-1s, VEGFs and especially BMPs (see chapter 1.3.2) has great impact on the mechanisms of the bone healing cascade and therein performed cell differentiations (Sundelacruz and Kaplan 2009, Berner, Reichert et al. 2012, Gothard, Smith et al. 2014). It has been demonstrated that the use of growth factors as signaling molecules can increase bone formation and therefore optimizes any tissue engineering strategy, whereby coordinated release ensures their survival and bioactivity. However, the spatiotemporal orchestration (dosage,

dynamics) is not yet completely understood (Rose and Oreffo 2002, Gothard, Smith et al. 2014) and therefore decelerates the immediate implementation.

Additionally, a proper vascular network is crucial for an adequate integration and the maintenance of the tissue engineered products (van Blitterswijk and De Boer 2015). One of the major difficulties constitutes the provision of sufficient blood supply and hence oxygen delivery that is crucial for the long-term survival of cells. The centered cells of such engineered constructs quickly decline before vital blood supply is available (Santos and Reis 2010, Laschke and Menger 2012). Since the process of necessary blood vessel ingrowth is too slow to ensure the survival of the tissue engineered construct, Laschke et al. investigated two novel strategies focusing on either the promotion of angiogenesis by blood vessels provided by the construct surrounding host cells or the stimulation of vascularization provided by preformed angiogenic networks that have already been included into the implanted tissue engineered construct (Laschke and Menger 2012).

Tissue engineered constructs represent a highly promising alternative to current standard therapies. Unfortunately, they do not completely fulfill all the requirements to become a routine in reconstructive surgery yet. There is still a major discrepancy between research in tissue engineering of over 30 years and its mainstream clinical application. Bara and Herrmann et al. recently published survey data addressing the current obstacles of the clinical translation of cell-based therapies in orthopedics, emphasizing the need for better comprehension of the underlying mechanisms, for enhanced economic support mandatory for fundamental research and for deeper transparency of regulatory processes (Bara, Herrmann et al. 2016). Moreover, studies need to be further refined, focusing on the establishment of standardized in vivo preclinical animal models allowing comparison and reproducibility (Reichert, Saifzadeh et al. 2009). The profound research of biological, physical, chemical, clinical and translational scientists as well as representatives from industry might then pave the way to its daily clinical application (Woodruff, Lange et al. 2012, Verrier, Alini et al. 2016).

1.5 Preclinical animal models for bone tissue engineering

The use of appropriate bone defect animal models in preclinical research is crucial for understanding of the physiology behind bone-healing cascades and for testing of therapeutic strategies for the treatment of non-unions and critical-size defects. To enable a reliable interpretation, precisely designed models need to imitate the human physiology as effectively as possible. Furthermore, standardization and reliability of research parameters as well as reproducibility in between studies were described as preconditions for valid quantitative and qualitative comparison and eventually translation into clinical application (O'Loughlin, Morr et al. 2008). Therefore, the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines have been developed. They intend to ameliorate and maximize the description of utilized animals for research, including a checklist of necessary facts to be indicated for published research on animals (e.g. animal species, strain, gender, genetic background, conditions of animal husbandry and housing, observation methods) (Kilkenny, Browne et al. 2010).

The following chapter provides information about parameters that should be considered for the establishment of preclinical animal models in the field of bone tissue engineering.

1.5.1 The animal choice

Various factors matter when it comes to imitating human conditions in bone healing as close as possible. Thereby, the choice of the animal species, strain, age and gender constitute important factors that need to be considered.

1.5.1.1 The species of the animal

Choosing the right animal species is already challenging when taking all conceivable options into consideration (Tab.1) (O'Loughlin, Morr et al. 2008, Reichert, Saifzadeh et al. 2009, Gothard, Smith et al. 2014).

Tab. 1 Factors affecting the choice of the animal species.

Aspects for animal model selection	
Age	Gender
Animal breeding and farming	Genetic aspects
Animal size	Growth behavior (growth plate characteristics)
Comparability with humans	Immunosuppression necessities and methods
Costs	State of health and metabolic condition
Ethical values	Time of observation

In a review published in 2008, O'Loughlin et al. reported a prominent preference towards the use of rodent animal models in fracture healing research (rat 38%, rabbit 19%, mouse 15%) compared to large animal models (sheep 11%, dog 9%, goat 4%, other 4%). This is not very surprising given the fact that small animal models are mentioned to be easily available and more convenient to house, to show faster healing rates than large animal models and to be genetically manipulable. Furthermore, mice and rats exhibit higher bone turnover and remodeling rates than large animals (O'Loughlin, Morr et al. 2008), whereby rats are used very frequently since they were reported to show bigger girth compared to mice, making surgical procedures and biomechanical testing more feasible. Rabbits, however, exhibit even longer bones and larger joints compared with other rodents and they were mentioned to quickly attain skeletal maturity. On the other hand, the different structure and high metabolic activity of rabbit bone was reported to have adverse effects on practical applicability since it might complicate the extrapolation of study results onto results that could be expected in human beings (O'Loughlin, Morr et al. 2008, Mapara, Thomas et al. 2012, Gothard, Smith et al. 2014).

Moreover, small animal models were described to show differences compared to large animal models in terms of mechanical load as well as stress conditions and even though both endochondral and intramembranous bone formation (see chapter 1.2.2) was mentioned to occur in rodent animal models, endochondral ossification dominates (O'Loughlin, Morr et al. 2008).

In addition, when it comes to imitating human conditions in bone healing as closely as possible, fixation methods and biomechanics can be better recapitulated in large animals. In comparison with human properties, large

animals provide more appropriate body weight and bone mineral density (Pearce, Richards et al. 2007). Sheep, for instance, possess a body weight, a bone mineral composition and remodeling rates that can be comparable to the one of adult humans (Reichert, Saifzadeh et al. 2009). Furthermore, the macrostructure of their long bones and the mechanical environment allows relatable comparison to humans after the implantation of suitable constructs (Reichert, Epari et al. 2010). However, cost-intensive husbandry for large animal models and the considerable amount of time necessary to reach an appropriate age for research generally complicates their standard use (Aerssens, Boonen et al. 1998, Pearce, Richards et al. 2007, Gothard, Smith et al. 2014).

1.5.1.2 The strain of the animal

The work with small animal models demands special attention to the animal strain. Depending on the strain, there are major differences in skeletal morphology, bone mineral density, mechanical properties and cellular metabolism (Beamer, Donahue et al. 1996, Ignatius, Röntgen et al. 2011).

Inbred mice and rats are the result of at least 20 consecutive generations of brother-sister mating. Emerging from generations of inbreeding, each animal represents the genetic clone of all other animals in the same strain and therefore was reported to provide standardization by eliminating the disruptive risk of genetic variability (Beck, Lloyd et al. 2000). Outbred stocks, on the other hand, are the result from a closed population of at least 4 generations and show an increased variability in genetic characteristics. They therefore were referred to as genetically unique individuals with maximum heterozygosity (Chia, Achilli et al. 2005).

Among rats, Sprague Dawley or Lewis rats represent commonly used outbred stocks that were mentioned to be easily accessible, manageable, and cost-effective. However, the femur bone of outbred Wistar rats has been reported to reflect human bone characteristics more likely than Sprague Dawley rats and therefore may represent a more suitable model to imitate bone regeneration (Drosse, Volkmer et al. 2008).

Transgenic mouse (and recently rat) lines were mentioned to enable the study of the influence of specific genes in the healing process (Cheung, Kaluarachi et al. 2003).

Since the application of tissue engineered constructs might entail the use of non-autologous cells and materials and thus the release of a host immunological response that might provoke the rejection of the implanted construct (Crupi, Costa et al. 2015), a reduced immune response can be crucial. Nude mice and rats are characterized by the absence of the thymus, which consequently leads to an inhibited adaptive immune system. Therefore, nude mice were shown to be able to accept different types of human tissue and cells without an immune mediated rejection response (Belizário 2009).

1.5.1.3 The age of the animal

Furthermore, the animal's age represents an important factor in the bone healing process. Healing time and healing rate vary with the age of the different animal species. Bone fractures in young rats, for instance, were shown to heal within five weeks by formation of an external periosteal callus, whereas medullary callus formation was hardly reported to occur (O'Loughlin, Morr et al. 2008). Meyer and Meyer demonstrated that rats considered as young (6 weeks old), as adult (26 weeks old) and as old (52 weeks old) showed significant differences in the amount of time needed for bone formation after induced fracture. Thereby, the age-related modification of mRNA expression of genes responsible for bone formation was mentioned to be one of the reasons (Meyer and Meyer 2007)(O'Loughlin, Morr et al. 2008).

The bone volume of 9-year-old ewes was shown to be comparable to the one of men and women at the age of around 60 to 70 years. From a histological point of view, however, ovine bone comprises mainly primary bone structure until the age of 7 to 9 years, followed by a secondary bone structure with higher bone density and strength compared to human bone (Pearce, Richards et al. 2007, Reichert, Epari et al. 2010).

In context of the growth characteristics, also the biology of the growth plate must be taken into account. A fully completed closure of the growth plate signifies skeletal maturity and hence represents an important parameter for bone healing (Kilborn, Trudel et al. 2002). The time of growth plate closure in different mouse strains, for instance, was mentioned to vary between an age of 3 to an age of 12 months, whereas other studies described growth plates in rodent animals to remain disclosed throughout adulthood (Beamer, Donahue et al. 1996, Kilborn, Trudel et al. 2002, Ignatius, Röntgen et al. 2011, Schindeler, Mills et al. 2018).
Moreover, previous studies demonstrated age associated slow-down in bone defect bridging as well as delayed bone-healing rates in female mice, showing that also the animal's gender may have influence on the outcome of the healing process (Lu, Hansen et al. 2008, Garcia, Histing et al. 2013).
In conclusion, it can be said that the provision of animal models that enable studies with reliable comparison for the total bone healing process in humans is nearly impossible. In fact, each animal model corresponds to a particular research question addressed to different sections in the bone-healing cascade (O'Loughlin, Morr et al. 2008).

1.5.2 The surgical design

After choosing the animal model, it is important to define a surgical protocol which, depending on the different defect designs, may have considerable impact on the outcome of the healing process. Thereby, specifications concerning the defect type (simple fracture, open fracture), the defect size, the defect form (segmental defect, drill hole), the defect model (critical-size defect, non-union etc.), the defect localization (long bone, flat bone etc.) and the potential fixation device need to be considered.
Simple fractures, also called closed fractures, do not penetrate the skin. Compound fractures or open fractures, on the other hand, cause tissue and periosteal damage. The rate of healing success in open fractures is lower than in simple fractures due to risk of infection with pathogens that may easily enter through the open wound (Schulte, Schumacher et al. 2011).

The presence and the size of a gap in between the bone fragments represent an important parameter. Bone fractures that do not show the presence of a gap heal much faster and are not suitable for testing tissue engineered constructs (O'Loughlin, Morr et al. 2008). However, the creation of critical-size defects can be challenging regarding the many variables that need to be considered (Tab.2) (Rimondini, Nicoli-Aldini et al. 2005, Lindsey, Gugala et al. 2006, Reichert, Saifzadeh et al. 2009, Gothard, Smith et al. 2014).

Tab. 2 Factors affecting the quality of critical-size defects.

Aspects for characterizing a critical-size defect	
Size of the defect	Fixation method
Bone location	Mechanical loading
Bone structure	Animal species and strain
Vascularization and nutrition	Immune status
Fracture type	Age
Surrounding tissue and periosteum	Gender

As already mentioned above, controllable fixation is important when it comes to obtaining reliable results in callus formation, callus size and composition. Fracture geometry and biomechanical conditions have great impact on the healing process, whereby load-bearing parameters in calvarian defects may be ignored. The fixation of bony defects in long bones can be achieved using internal or external fixation devices including different methods and materials. Internal fixation represents the most commonly used fixation method and can be performed using plates, intramedullary screws, wires, pins or nails made of metal or polyethylene (Drosse, Volkmer et al. 2008, O'Loughlin, Morr et al. 2008). Thereby, stabilization can be obtained using a rigid or dynamic (allowing interfragmental movement) fixation design. However, fixation methods that do not allow any interfragmental movement at all happen to show low bone healing stimulation (Ignatius, Röntgen et al. 2011). On the other hand, too much movement may retard the healing process by inducing a larger callus formation with lower mechanical stiffness and can therefore sometimes lead to pseudarthrosis (Claes, Augat et al. 1997, Drosse, Volkmer et al. 2008, Ignatius, Röntgen et al. 2011). Moreover, the use of wires alone does not provide suitable rotational stability and therefore represents an inadequate fixation device for

segmental defects. Drosse et al. compared different fixation methods (external fixation devices and internal plates) in small animal models with regard to their surgical and biomechanical fitness. Even though all devices showed satisfying outcome in terms of bone bridging, the use of external fixations, compared to internal plates, presented a technical more challenging surgical procedure and complicated the post-operative manipulation (infection, fixation loss, higher costs) (Drosse, Volkmer et al. 2008).

Additionally, Schindeler et al. recently reviewed studies on preclinical rodent animals for bone tissue engineering, highlighting commonly employed fracture models and therefore applied fixation methods. Thereby, fixation of critically sized segmental defects in the femur, described as the gold standard model for studying bone healing, was suggested, inter alia, with polyacetyl plate systems combined with Kirschner wires (Schindeler, Mills et al. 2018).

Garcia et al reviewed previous studies that described surgical interventions in rodent animal models resulting in delayed fracture healing or the creation of non-unions, whereby the impact of different stabilization methods was discussed (Garcia, Histing et al. 2013). Of note, quite apart from ethical aspects, poor or no stabilization means also to enlarge the variability of callus size and composition due to inaccuracy in fracture gap size, in localization of the bone fragments and in interfragmental moving processes and hence should be considered conscientiously, whether it be in the work with large animal models or with small ones (Ignatius, Röntgen et al. 2011, Garcia, Histing et al. 2013).

Moreover, the use of different fixation methods can influence the outcome of biomechanical tests (three-point bending tests, four-point bending tests, torsion tests) that may be conducted to investigate the mechanical properties of the healing bone (Manigrasso and O'Connor 2004).

The establishment of preclinical animal models to test bone tissue engineered constructs demands hence the consideration of several factors and represents a challenging scientific mission. Researchers need to choose within a broad variety of possible defect designs, including the selection of an appropriate animal species, animal strain, age, gender and at the same time applicable observation methods. This can be complicated given the fact that currently no concrete

guideline exists, which might be also one of the reasons for the persistent gap between research studies and their clinical translation.

1.6 Objective of the thesis

For over thirty years, scientists from all over the world have been striving for tissue engineered strategies to replace damaged bone and the number of published papers addressing this matter has increased dramatically since 1985, starting with less than 250 published papers on “Pubmed” between 1985 and 1987 and more than 4000 papers in 2011 (Amini, Laurencin et al. 2012). Scientific research in bone tissue engineering has made great progress with promising results. However, the number of tissue engineered constructs with actual clinical relevance remains low. Knowledge gaps persist referring to limitations in the understanding of complex immunological aspects, potential side effects in the host tissue, the selection of the most effective combination of cells, scaffolds and bioactive molecules as well as the appropriate evaluation of the osseous quality and functionality (Amini, Laurencin et al. 2012). Despite intense research activity, clinical translation has so far been slower than originally expected and remains a major hurdle that needs to be bridged. This so called "Valley of Death" (Hollister 2009, Summers-Trio, Hayes-Conroy et al. 2019) stands on immense costs and a distinct lack of predictive preclinical models that would allow the routine clinical application. Thereby, the necessity of designing animal experiments should be mentioned as a motivation to streamline experimental procedures in order to replace, reduce, and refine (ethical principle named “3Rs”) animal experiments (https://www.bfr.bund.de/de/3r_prinzip-193970.html, July 26,2019) and in the sense of the Max Planck society add a fourth “R” for responsibility (https://www.mpg.de/10885134/3rs, July 26,2019). Moreover, Summers-Trio et al. recently mentioned the importance of considering a patient’s biography and biology in order to surmount the translational gap between research advances and their application in the clinics (Summers-Trio, Hayes-Conroy et al. 2019). Undoubtedly, once this valley will be bridged, medicine will find itself with ceaseless possibilities for bone repair (Henkel, Woodruff et al. 2013).

Therefore, this thesis aimed to evaluate the efficiency of currently used preclinical models from the perspective of scientists, clinicians and clinical scientists from all over the world. A survey was conducted addressing the matter of whether preclinical animal models reflect the current need for bone replacements and how researchers assess their potential clinical application. Furthermore, the survey was designed to elucidate the most frequently used and most satisfying models as well as their limitations. Additionally, an electronic literature research was conducted in order to review the currently used study designs to test bone tissue engineered constructs on preclinical animal models.

2 Materials and methods

This chapter provides information about the survey development and the literature research design for this thesis.

2.1 Introduction

The aim of this thesis was to compile a comprehensive survey that shows the perceptions from both surgeons and scientists on currently used preclinical animal models in the field of bone tissue engineering.
The study was designed in cooperation with the specialized "Junior Research Group Tissue Regeneration in Musculoskeletal Diseases" of the IZKF Würzburg, which is led by Dr. Marietta Herrmann (http://www.med.uni-wuerzburg.de/mcw/forschung/experimentelle-forschung/izkf-nachwuchsgruppe/, 28 August,2018) and the preclinical surgery department of the AO Research Institute Davos.
Apart from that, a systemic overview of accessible literature in the field of bone tissue engineering was compiled and compared to the data obtained with the survey. Literature research was focused on preclinical in vivo studies testing bone tissue engineered constructs for bone regeneration.

Analyses of the questionnaire and literature research outcome was performed using “Microsoft Excel” (Microsoft-Office 365 by Microsoft Corporation). The software was used for the description and the graphical representations of the obtained data. Additionally, the scientific 2D graphing software “GraphPad” (GraphPad Software, Inc.) was used.

2.2 Survey design

2.2.1 Procedure of the survey

The targeted participants for this study were, on the one hand, surgeons with professional background in orthopedic, trauma, craniomaxillofacial or veterinary surgery and, on the other hand, scientists who do research in the field of bone biology and regeneration.

Generally, surveys can be conducted using a wide range of distribution possibilities such as written or oral forms and electronic media. For this thesis, a web-based electronic survey was mainly used as it provides economical advantage. Therefore, the survey was conducted by “soscisurvey.de”, an online tool that enables the user to design and to apply online questionnaires (https://www.soscisurvey.de/, 28 August 2018). The questionnaire was distributed via the respective web link and the collected outcome of the survey was stored into a database automatically. A pilot study was performed in order to pretest the survey and hence to reduce problems of misreading or misinterpretation. The actual survey period extended from July 2017 to April 2018.

An invitation mail containing the link to the online questionnaire (shown in supplementary figure S.1) was written to potential scientists and surgeons from all over the world to inform them about the survey. Therefore, email-addresses from surgeons working in orthopedic, veterinary and craniomaxillofacial clinics in Germany, Switzerland, Austria, Italia, England and Spain were collected. Furthermore, literature research in the field of bone tissue engineering was conducted and therein indicated correspondence addresses from the authors were contacted.

Additionally, a short article appeared in the “News and Views” section of the Alternatives to Laboratory Animals (ATLA) journal to outline the issue of absent clinical translation for bone tissue engineering and to draw attention to the online survey project. Therefore, the article contained the survey link provided by “soscisurvey” (Herrmann 2017).

Moreover, the survey was handed out in paper form on the „Tissue Engineering and Regenerative Medicine International Society“ EU Conference 2017 in Davos, Switzerland (TERMIS, https://www.termis.org/, 28 August 2018).
The participation in the survey was voluntary. All the recruited respondents were assured anonymity regarding their data and numerical coding was applied by “soscisurvey” in order to differentiate the questionnaires.

2.2.2 Structure of the survey

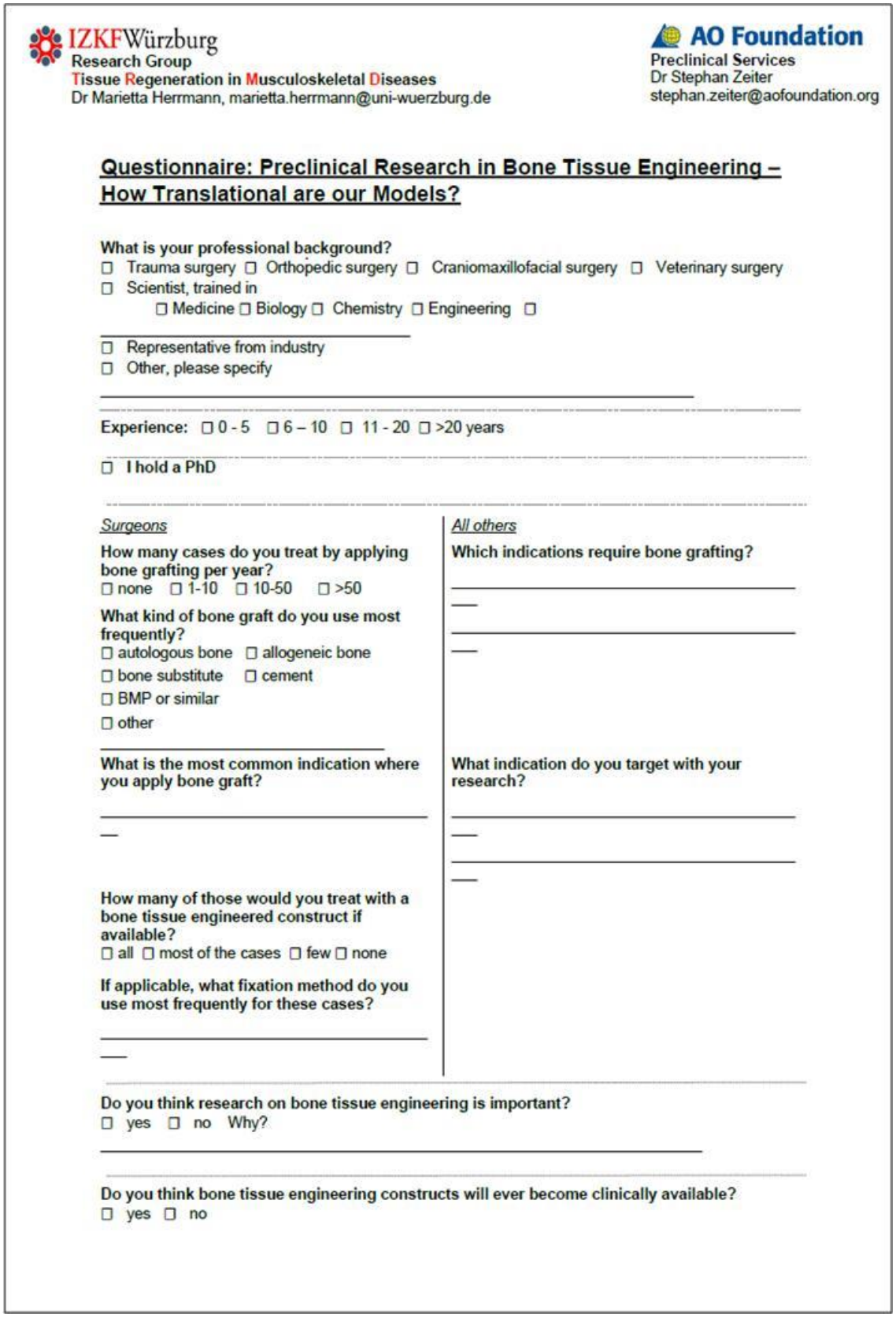
The survey was clearly structured and different question types with different response alternatives were used (Fig.3 and Fig.4). Selection questions permitted only one answer, whereas multiple-choice questions allowed more than one response. Text input was used when participants were asked to enter text.

The survey was divided into four broad categories. Firstly, three questions asking about the professional background, the experience level and whether the award of a PhD existed, were put in the survey. This category allowed the classification of the participants according to their specializations and experience level.
Secondly, surgeons and scientists were surveyed separately about their work. Thereby, surgeons were asked to indicate their number of surgical cases with bone graft substitutes per year (ranging from none to more than 50 cases) and to indicate what kind of bone graft they were thereby applying (autologous bone, allogeneic bone, bone substitute, cement, none morphogenetic proteins or other). Furthermore, two open text fields allowed surgeons to write down the most common indications for bone grafting and possible fixation devices for such defects. In addition, a question asking about how many cases surgeons would treat with bone tissue engineered constructs if available (ranging from none to all of their cases) provided information about their general attitude towards bone tissue engineering. Additionally, scientists were asked to write down the indications that they felt to require bone grafting and the indications targeted with their research into open text fields.

Thirdly, the general assessment of bone tissue engineering was queried, again addressing all participants. To evaluate the participants' perception, the first item asked whether the participants thought that research on bone tissue engineering was important or not. The respondents were asked to justify their answer (yes, no) in an open text field. The following questions inquired whether the respondents thought that bone tissue engineered constructs would ever become clinically available or not (yes, no) and if yes, how long it would take (ranging from 5 to more than 20 years). Furthermore, the participants' feeling about currently used preclinical models for research on bone tissue engineering was queried. Thereby, the question investigated the quality of the models, ranging from well-developed and clinically applicable to worthy of optimization.

In a final step, participants doing preclinical research were asked to describe their animal models in detail (animal species, age, gender, strain, observation time and methods, implantation site, defect model, defect size and type, fixation methods) and to differentiate between most and least satisfying model designs. Additionally, researchers were asked to evaluate their animal model (very satisfied, mostly satisfied, not satisfied) and to assess its clinical relevance (yes, no).

Finally, an open text field for comments was provided at the end of the survey.



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Questionnaire: Preclinical Research in Bone Tissue Engineering – How Translational are our Models?

What is your professional background?
☐ Trauma surgery ☐ Orthopedic surgery ☐ Craniomaxillofacial surgery ☐ Veterinary surgery
☐ Scientist, trained in
☐ Medicine ☐ Biology ☐ Chemistry ☐ Engineering ☐
☐ Representative from industry
☐ Other, please specify

Experience: ☐ 0 - 5 ☐ 6 – 10 ☐ 11 - 20 ☐ >20 years

☐ I hold a PhD

Surgeons

How many cases do you treat by applying bone grafting per year?
☐ none ☐ 1-10 ☐ 10-50 ☐ >50

What kind of bone graft do you use most frequently?
☐ autologous bone ☐ allogeneic bone
☐ bone substitute ☐ cement
☐ BMP or similar
☐ other

What is the most common indication where you apply bone graft?

How many of those would you treat with a bone tissue engineered construct if available?
☐ all ☐ most of the cases ☐ few ☐ none

If applicable, what fixation method do you use most frequently for these cases?

All others

Which indications require bone grafting?

What indication do you target with your research?

Do you think research on bone tissue engineering is important?
☐ yes ☐ no Why?

Do you think bone tissue engineering constructs will ever become clinically available?
☐ yes ☐ no

Fig. 3 Page one of the survey. The survey was conducted in order to evaluate scientists' and surgeons' general assessment of bone tissue engineering and preclinical animal models.

If yes, how long will it take?
approx. ☐ 5 ☐ 10 ☐ 20 ☐ >20 years

What is your feeling about the preclinical models, which are currently used to test bone tissue engineered constructs?

☐ The models are well developed, reproducible and results translate well in the clinic
☐ The models are well developed and reproducible but do not translate in the clinic
☐ The models need optimization
☐ The models are poor
☐ I don´t have experience with preclinical models.

If you do preclinical research, which model are you currently using to test the success of bone tissue engineered constructs? (If you have used several models, please provide information for the two models with which you are (1.) most satisfied and (2.) least satisfied.)

1. Species	Age	Gender	Strain / Genotype	Duration of Observation	Analysis Methods
☐ sheep					
☐ rabbit					
☐ rat					
☐ mouse					
☐ other:					

Implantation site: ☐ ectopic ☐ orthotopic **Please specify site:** _____

Defect: ☐ segmental defect ☐ drill hole ☐ simple fracture ☐ _____

Defect size: _____
if applicable, is the model: ☐ a critical size model ☐ non-union model or ☐ normal healing model
Fixation: ☐ none ☐ external fixator ☐ internal plate ☐ intramedullary nail ☐ _____
How satisfied are you with the model? ☐ very satisfied ☐ mostly satisfied ☐ not satisfied
Is the model clinically relevant? ☐ yes ☐ no

2. Species	Age	Gender	Strain / Genotype	Duration of Observation	Analysis Methods
☐ sheep					
☐ rabbit					
☐ rat					
☐ mouse					
☐ other:					

Implantation site: ☐ ectopic ☐ orthotopic **Please specify site:** _____

Defect: ☐ segmental defect ☐ drill hole ☐ simple fracture ☐ _____

Defect size: _____
Is the model: ☐ a critical size model ☐ non-union model or ☐ normal healing model
Fixation: ☐ none ☐ external fixator ☐ internal plate ☐ intramedullary nail ☐ _____
How satisfied are you with the model? ☐ very satisfied ☐ mostly satisfied ☐ not satisfied
Is the model clinically relevant? ☐ yes ☐ no

Comments:

Fig. 4 Page two of the survey

2.3 Literature research design

2.3.1 Paper selection process

An online literature research was conducted in order to evaluate currently used preclinical animal models for testing of bone tissue engineering constructs on their different study designs and frequency. Therefore, the electronic database “Pubmed” was searched for relevant anglophone literature of the last ten years spanning from January 2008 to May 2018. The inclusion criteria were determined and applied on “Pubmed” as follows:

Search**:** "tissue engineering" AND "bone" AND ("bone defect" OR "fracture") AND ("tibia" OR "femur" OR "cranium" OR "calvaria" OR "radius" OR "humerus" OR "ulna" OR "maxilla" OR "mandible") AND ("in vivo" OR "animal model" OR "mouse" OR "mice" OR "rat" OR "sheep" OR "goat" OR " rabbit" OR "horse" OR "pig" OR "dog" OR “mural” OR "equine" OR "porcine" OR "canine" OR "ovine" OR "rodent")

The key words "tissue engineering" and "bone" were included separately in the research field in order to minimize the specification and to obtain more research results. The aim was to evaluate orthotopic models including a "bone defect" or "fracture" within the most commonly used bones, namely "tibia", or "femur", or "cranium", or "calvaria" (as synonym) or "radius", or "humerus", or "ulna", or "maxilla", or "mandible". Furthermore, animal species were determined following the general usage of different kinds of large and small animal models. Thereby, adding the terms "in vivo" and "animal models" was meant to expand the outcome range of literature research on “Pubmed”.
Reviews were excluded from the search such as articles describing pure in vitro studies or in vivo studies utilizing ectopic models.

2.3.2 Literature review

Obtained data on preclinical animal models was tabulated and summarized graphically. First, the authors name and the target of the research design described in the selected paper were noted, followed by the indicated information about the applied animal model specifications. Therefore, details about the animal species, the animal age as well as its gender and strain were searched and listed. Furthermore, information about the surgical procedures within the preclinical animal models was collected, generally including details about the anatomical location of the defect, the defect size and form, the defect model as well as the applied fixation method. The papers were then searched for indications of whether a negative control was conducted or not and if yes, whether bone bridging occurred in the empty defect or not. After the description of the surgical design, the paper usually indicated the observation time and observation method which was also extracted for evaluation.

If the paper did not provide any information about one of the research details listed above, the missing information was replaced with "/", when listed in an “Excel” table. The age of the animal was sporadically described as "adult" or "skeletally mature". This information was then classified as "not defined". The same applies to the healing of the empty defects if neither bone bridging nor the remaining of a gap were indicated precisely.

3 Results

3.1 Outcome of the survey

This chapter presents the results from the survey analysis. Supplementary table 1 (Tab-S.1) describes the necessary variables to decode the full set of the online collected data from the survey which is provided in supplementary table 2 (Tab-S.2). The table shows the outcome in the way it has been registered by “soscisurvey” for retrieval. Altogether, 70 surveys were filled out thoroughly in paper form or online. Incomplete surveys were excluded from the analysis.

3.1.1 Background and experience of the participants

Among the participants, 51% were surgeons (specialized in trauma, orthopedic, craniomaxillofacial or veterinary surgery) and 42% of the respondents were scientists trained in medicine, biology, chemistry, engineering or representatives from industry. Participants working as both surgeon and scientist (7%) are referred to as clinical scientists (Fig.5A). Fig.5B represents the percentage distribution of scientists and surgeons in the different specializations.

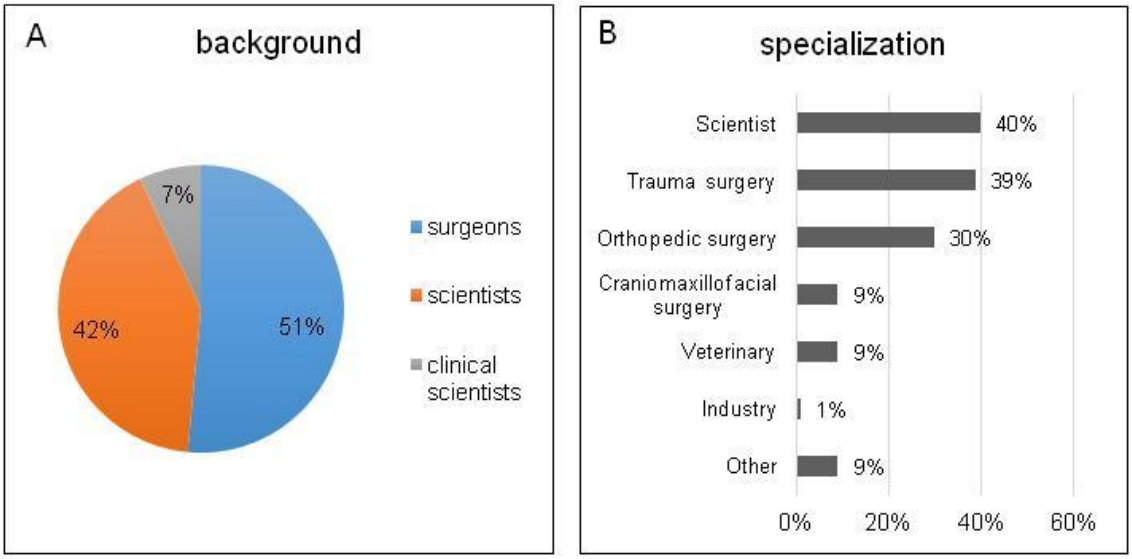

Fig. 5 Professional background of the participants in the survey, n=70. A. The graph shows the professional background of the respondents. B. The diagram indicates the professional background of surgeons subdivided into specializations; several answers were possible.

To evaluate the experience and education level of the participants, questions concerning the number of working years and the obtainment of a PhD degree were posed. In addition, for the surgeons, the number of with bone graft treated cases provided a reference of their experience level. Clinical scientists will hereinafter be ranked among the surgeons as well as the scientists.

Thereby, 22% of surgeons stated to have 0 to 5, 11 to 20 or more than 20 years of work experience, respectively, and 34% indicated to have 6 to 10 years of work experience (Fig.6A). Among the surveyed surgeons, 49% hold a PhD (Fig.6B), making reference to their experience level in research.

When asking scientists about their experience, 25% stated to have 0 to 5 years of work experience, 28% 6 to 10 years, 19% 11 to 20 years, and 28% more than 20 years (Fig.6C).

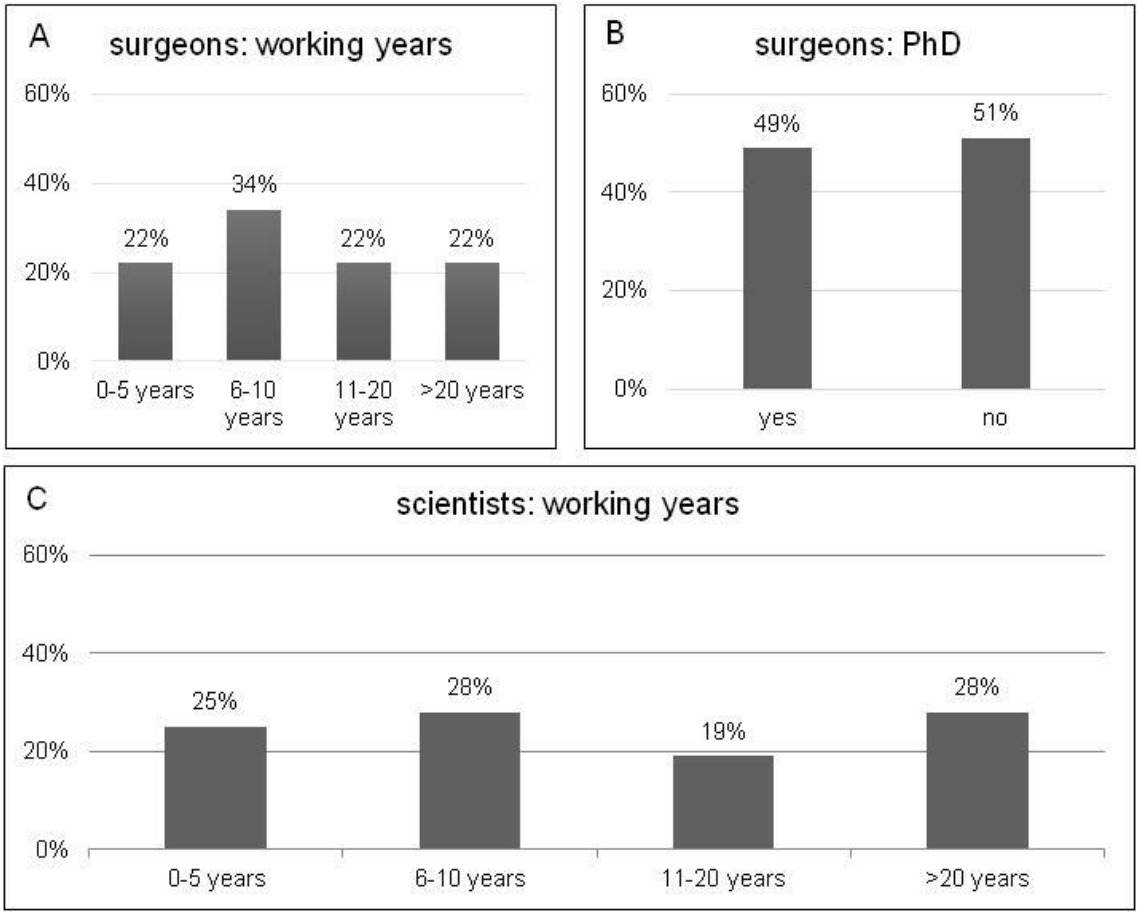

Fig. 6 Experience level of the participants in the survey. A. The graph shows the number of years that surgeons have worked in their domain, n=41. B. The graph shows the number of surgeons with PhD degree, n=41. C. The graph indicates the number of scientists' working years, n=33.

Since the number of treated cases per year may also provide information about the experience level, a representation of the performed surgeries applying bone graft materials per year is shown in Fig.7. Hereby, 23% of the surgeons and clinical scientists stated to perform less than 10 cases per year, 49% operated 10 to 50 cases and 28% of the participants performed more than 50 cases per year.

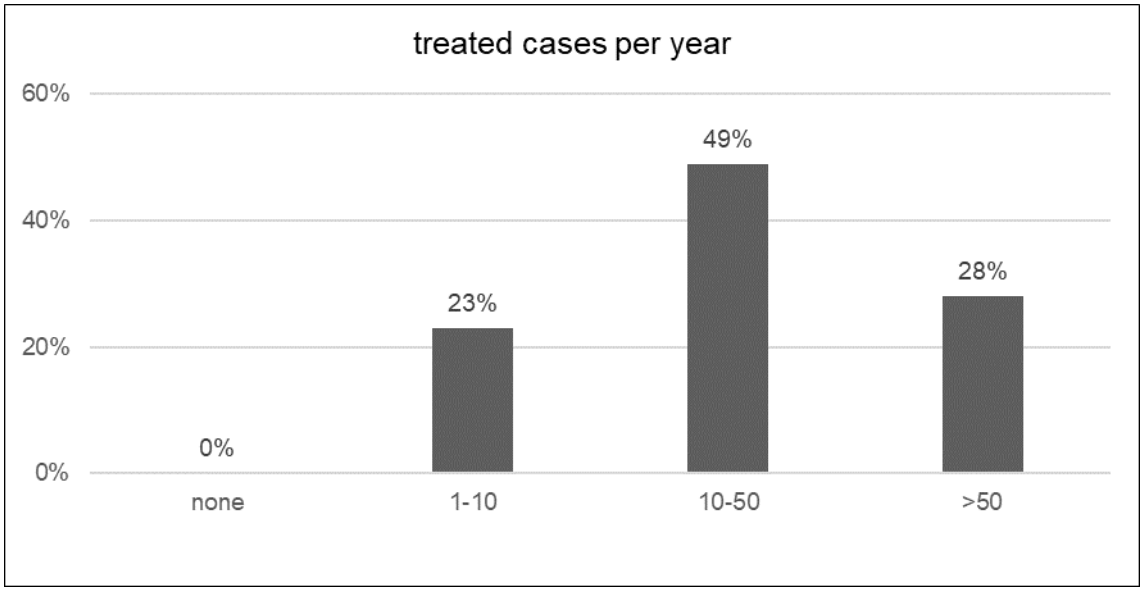

Fig. 7 The number of cases that participants treated with bone graft materials per year, n=34.

3.1.2 Surgeons

In this chapter, the survey outcome obtained by surgeons shall be assessed individually. The results from the questioned scientists will be detailed later (see chapter 3.1.3).

3.1.2.1 Application of bone graft materials

Fig.8 represents bone graft materials that are currently used by the surveyed surgeons in clinical routine. In the first place, all primarily applied bone graft materials were evaluated, whereby autologous bone represented the most commonly used bone graft material (58%) next to allogeneic bone (19%) (Fig.8A). Secondly, bone graft materials used by surgeons that treat more than 10 cases per year (Fig.8B) were evaluated, revealing almost the same distribution for the utilized bone graft materials.

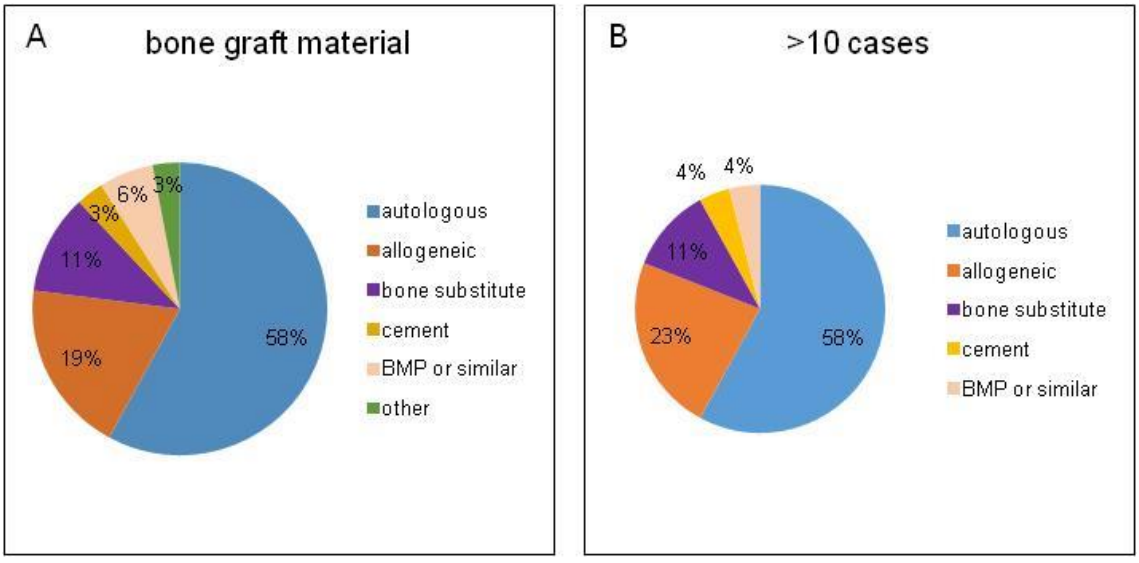

Fig. 8 The currently used bone graft materials by the surveyed surgeons. A. The graph shows an overview of the applied bone graft materials, n=36. B. The graph indicates the bone graft materials used by surgeons who treat more than 10 cases per year, n=26.

Thirdly, autologous and allogeneic materials were quantified and imaged individually depending on the experience level of the surveyed surgeons (working experience) since they represent the majority of the utilized bone grafts, whereas bone substitute, cement, bone morphogenetic protein, hydroxyapatite and ß-tricalcium phosphate constitute only a minor part and were therefore summarized into a sums list (other) (Fig.9).

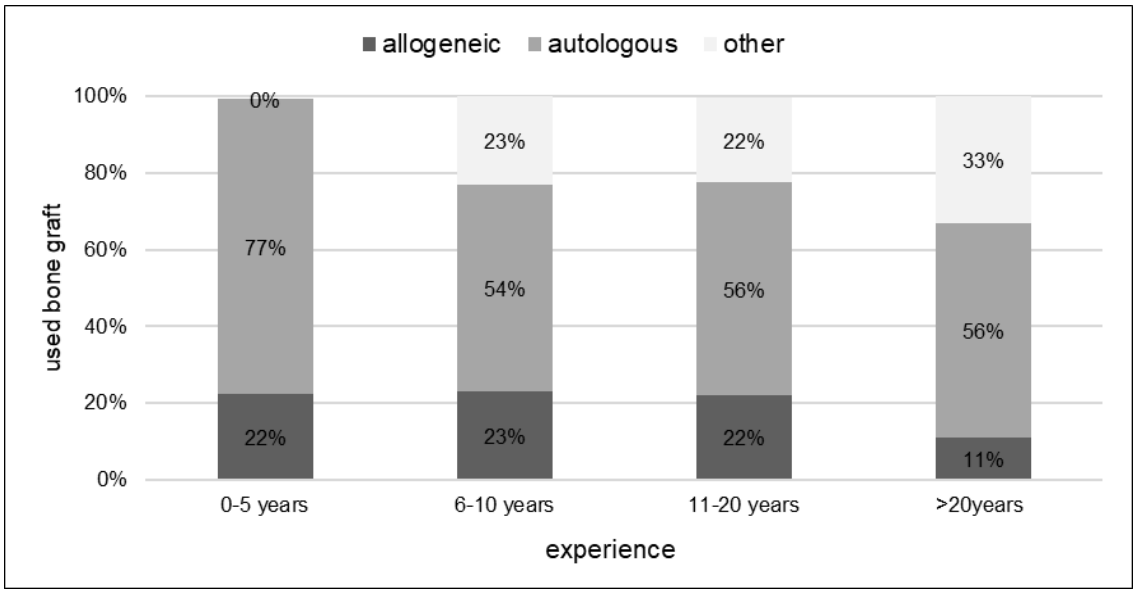

Fig. 9 The most commonly applied bone graft materials by surgeons depending on their experience level, n=36.

3.1.2.2 Indications for bone graft materials

Regarding the indications for bone graft application, 50% of the surgeons named large bone defects to be one of the most common indications requiring bone graft materials. The application of bone graft for non-union defects was mentioned by 30% and the remaining participants implied other indications such as hip replacement or vertebral fusion surgery (spondylodesis) (Fig.10). For defect restoration, 71% of large bone defects (Fig.11A) as well as 67% of all non-unions (Fig.11B) are treated with autologous bone by the respondents in the survey. Allogeneic bone and other bone grafts such as bone substitute, cement or bone morphogenetic protein, represent less than half of all bone graft materials applied for defect restorations.

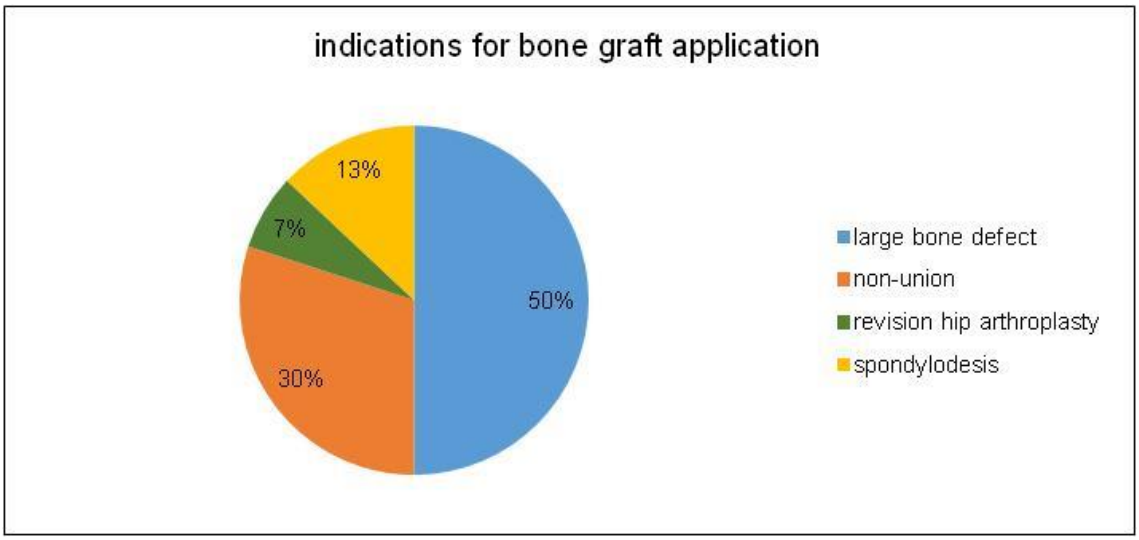

Fig. 10 The most common indications that surgeons mentioned to require the application of bone graft material, n=34.

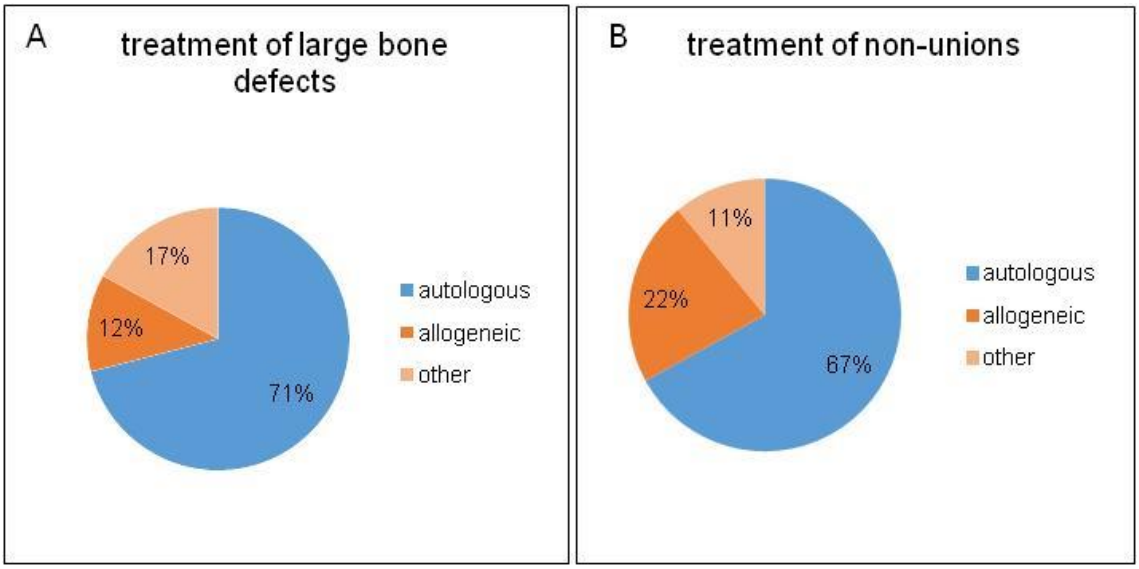

Fig. 11 The restoration of common bone defects with different bone graft materials. A. The graph indicates the number of bone graft applications for the treatment of large bone defects, n=17. B. The diagram shows the number of bone graft applications for the treatment of non-unions, n=11.

3.1.2.3 Surgeons' opinion on bone tissue engineering and preclinical animal models

In terms of attitude towards bone tissue engineering, surgeons were asked how many of their cases they would treat with a bone tissue engineered construct, if available. A 4-point scale with the answer options of "all of the cases" up to "none of the cases" was used, whereby 62% of the surgeons affirmed that they would treat all or most their cases with an engineered construct, if available, and 38% only a few or none of their cases (Fig.12).

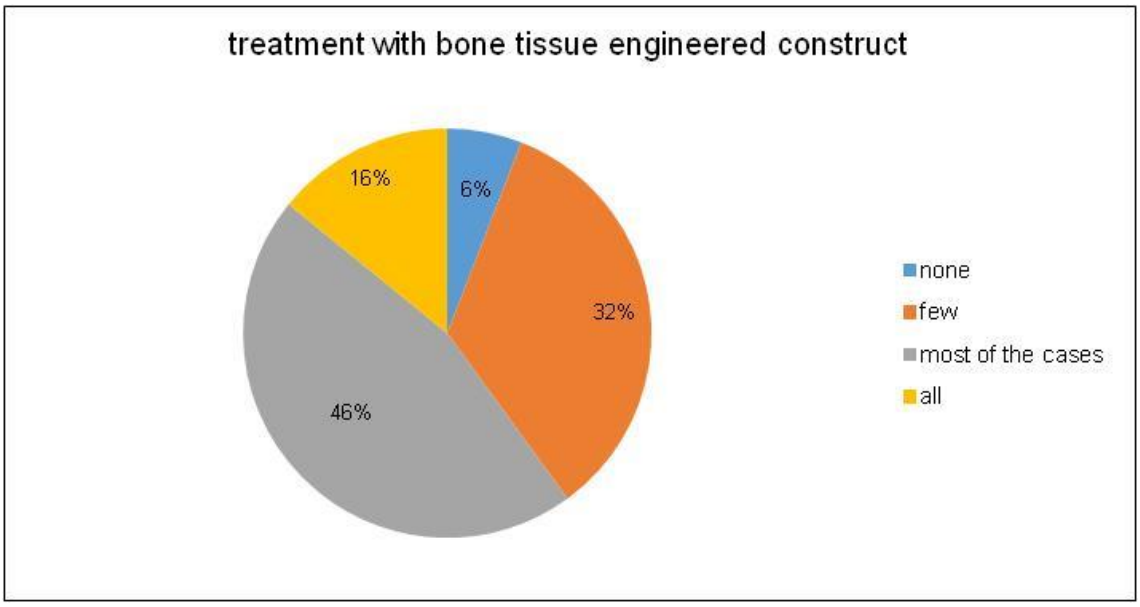

Fig. 12 Number of cases that surgeons would treat with a bone tissue engineered construct if available, n=31.

Additionally, Fig. 13 shows the different kinds of bone defects as indicated by the surveyed surgeons and their answer to the question of whether they would treat such defects with a bone tissue engineered construct or not. In the case of large bone defects, 54% of the surgeons would use a tissue engineered construct for all or most of their cases, whereas 46% would use such a construct for only few or none of them. When treating non-unions, 33% of surgeons would apply an engineered construct, if available, on all or most of the defects and 67% on only few or none.

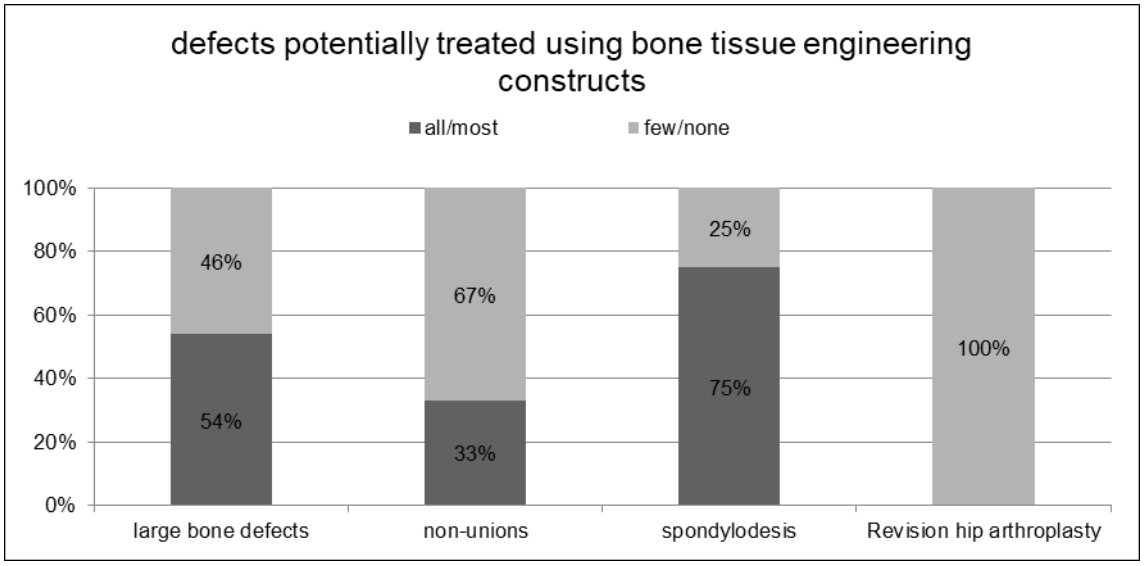

Fig. 13 The potential usage of bone tissue engineered constructs as indicated by the surveyed surgeons, n=31. The graph depicts how many of the respective cases surveyed surgeons would treat with bone tissue engineered constructs if available.

Furthermore, to evaluate surgeon's perception of preclinical models designed to test bone tissue engineered constructs, they were asked to rate the quality of such models. Their general assessment was that preclinical models for bone tissue engineering are well developed, reproducible but do not translate well in the clinic (45%) and that models need optimization (32%). None of the surgical respondents thought that models were poor and 13% had no experience with such constructs (Fig.14).

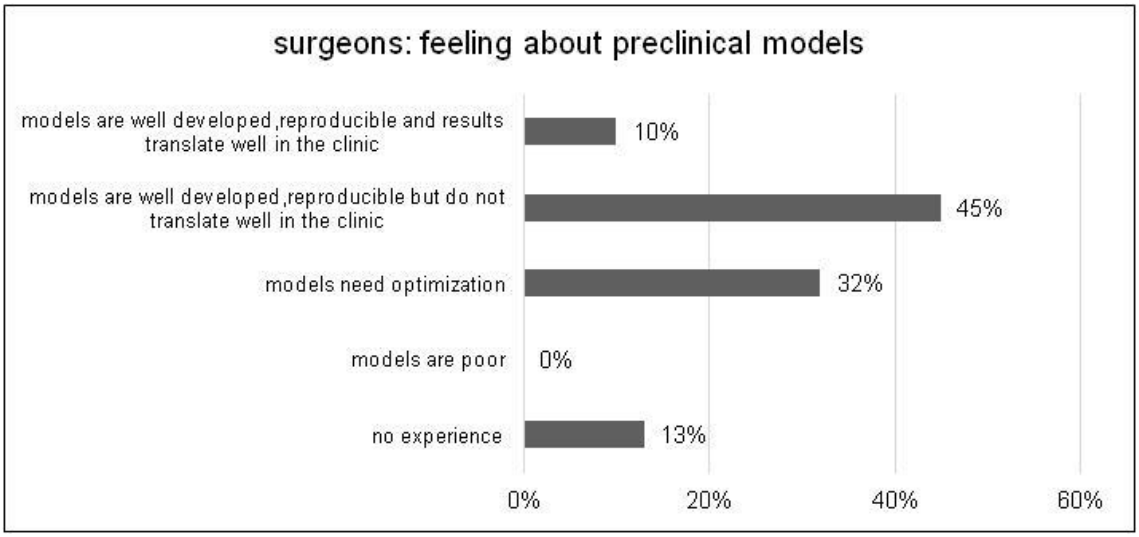

Fig. 14 Surgeons‘ feeling about preclinical models, n=31. Several answer options were possible to choose for this question.

Surgeons with more professional routine were then particularly taken into observation in order to get a profound point of reference (Fig.15). Additionally, surgeons working more than 10 years had been compared to those who have been operating for less than 10 years in their specialization (Fig.15 and Fig.16).

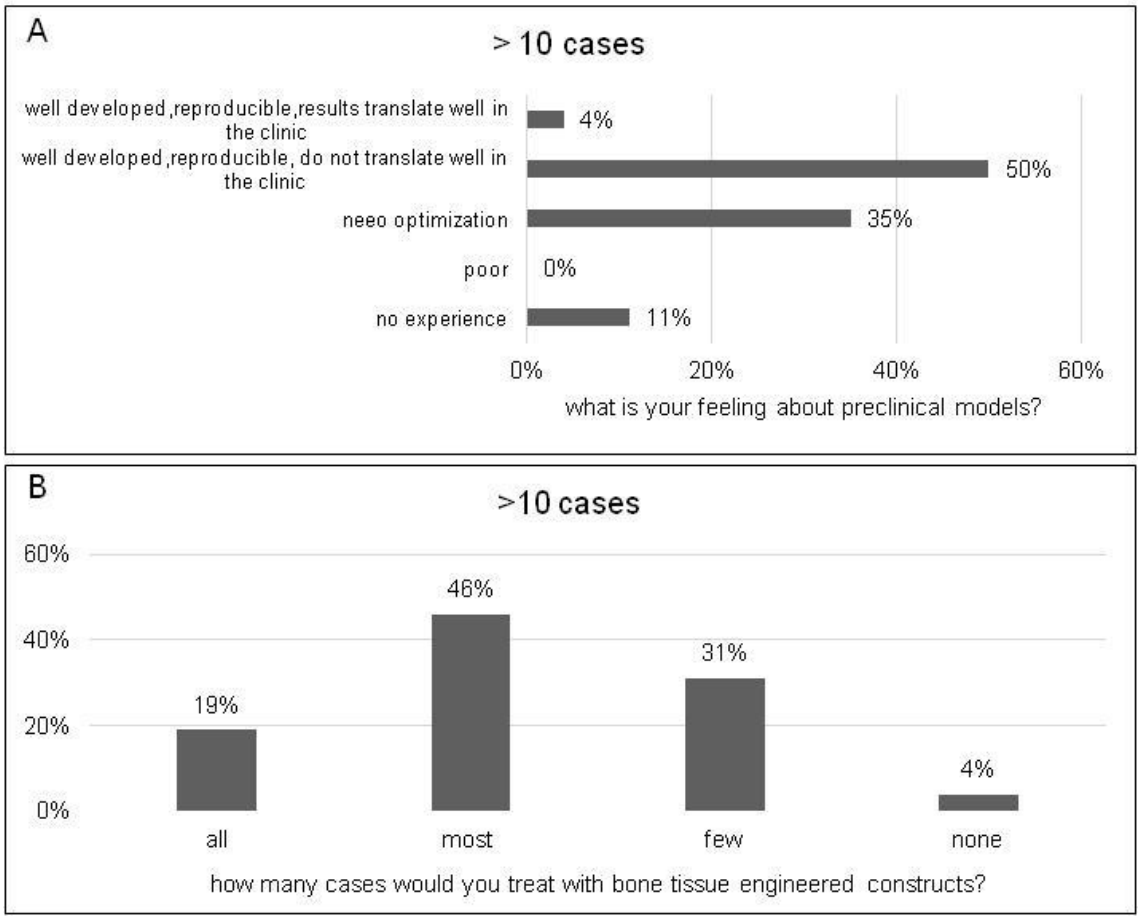

Fig. 15 Surgeons treating more than 10 cases per year and their perception of preclinical models and bone tissue engineering, n=26. A. The graph shows surgeons' opinion on the currently available preclinical models. B. The graph shows the number of cases surgeons would treat with a bone tissue engineered construct if available.

Among the surgeons who treat more than 10 cases per year, 50% thought that preclinical models for bone tissue engineering showed reproducible, though not translatable outcome for the clinics and 35% marked that models needed further development (Fig.15A). Furthermore, 65% of them would use bone tissue engineered constructs for all or most of their cases, whereas 35% would use only few or even none of the constructs (Fig.15B). Hence, numbers did not differentiate remarkably between the general feeling about preclinical models and

the one that surgeons with more work routine showed (compare Fig.14). However, the general disposition to actually apply new tissue engineered constructs on all or most of the cases in the clinics was less supported among surgeons with fewer experience than among those with more experience, namely by only 43% compared to 65%.

Among the surgeons with a longtime career background, 63% would treat all or most of their large bone defects with a bone tissue engineered construct, whereas only 43% of those with less than 10 years of experience would do so (Fig.16A). When treating non-unions, 40% of surgeons with more experience and 25% of those with less experience would treat their cases with a tissue engineered construct (Fig.16B).

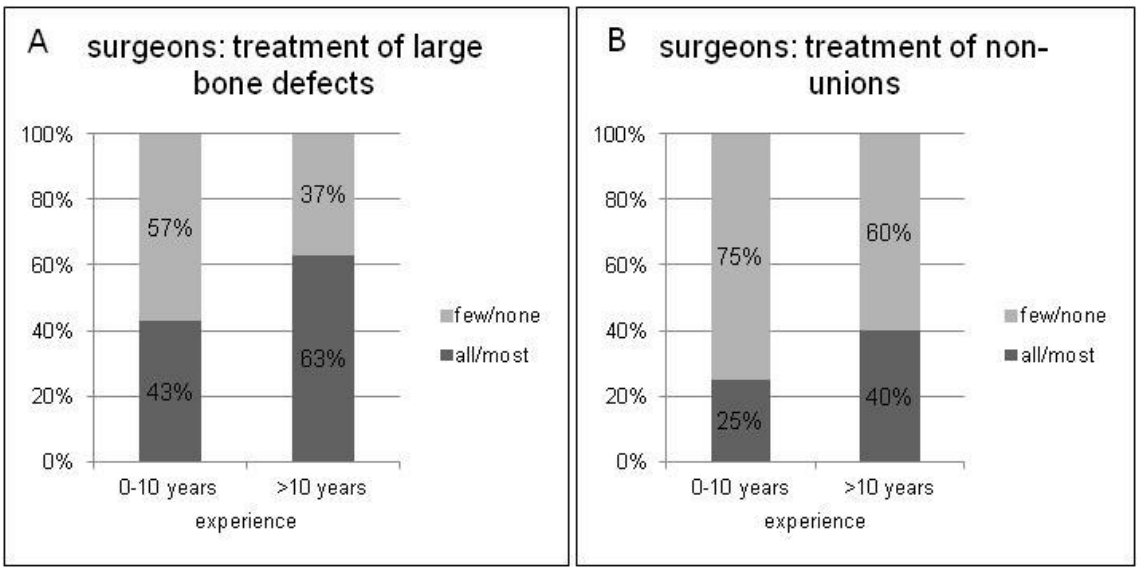

Fig. 16 Surgeons and their attitude towards bone tissue engineered constructs for osseous defects by considering their experience level. The graph shows surgeons with more and less professional experience and their willingness to treat large bone defects (A, n=17) or non-unions (B, n=11) with bone tissue engineered constructs, if available.

In the survey, autologous bone graft was mentioned most often to be applied by surgeons for the repair of bone defects. It represents the most popular material among the surveyed surgeons, whether it was for surgeons who had more than ten years of experience or less. Therefore, autologous graft was investigated individually and compared to the potential use of bone tissue engineered constructs. In case of large bone defects, 67% of surgeons with more experience and 60% of surgeons with less experience would use a tissue engineered

construct, if available, for cases they currently treat with autologous bone graft (Fig.17A). Among the surgeons who have been working for over 10 years and who currently use autologous bone graft material for non-unions, 33% would use a tissue engineered construct for most or all of their cases, whereas 67% would not rely on bone tissue engineered constructs for most of their cases. When asking surgeons with less than 10 years of experience, 75% would only treat few or none of their non-union cases with bone tissue engineered constructs (Fig.17B).

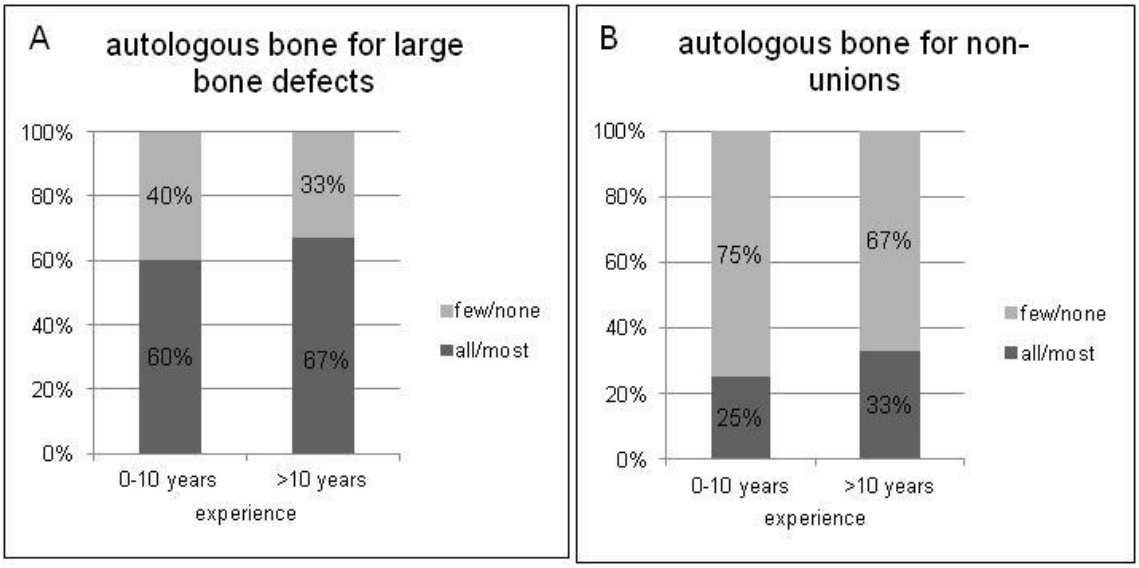

Fig. 17 Surgeons who use mainly autologous bone graft materials for the repair of bone defects and their attitude towards bone tissue engineered constructs by considering their experience level. A. The diagram presents the potential use of bone tissue engineered constructs by surgeons who mainly use autologous bone to repair large bone defects and who have been working more than or less than 10 years, n=12. B. The diagram presents the potential use of bone tissue engineered constructs by surgeons who mainly use autologous bone for non-unions and who have been working more than or less than 10 years, n=7.

Furthermore, the aim was to explore the underlying motives for surgeons' different perception and attitude towards the potential use of bone tissue engineered constructs in the future. Therefore, Fig.18 represents the current surgical use of bone graft materials and surgeons' feeling about preclinical models. All surgeons were divided into two groups, one representing surgeons who would treat few or no cases (Fig.18A) and the other one showing surgeons who would treat all or most of their cases with bone tissue engineered constructs, if available (Fig.18B).

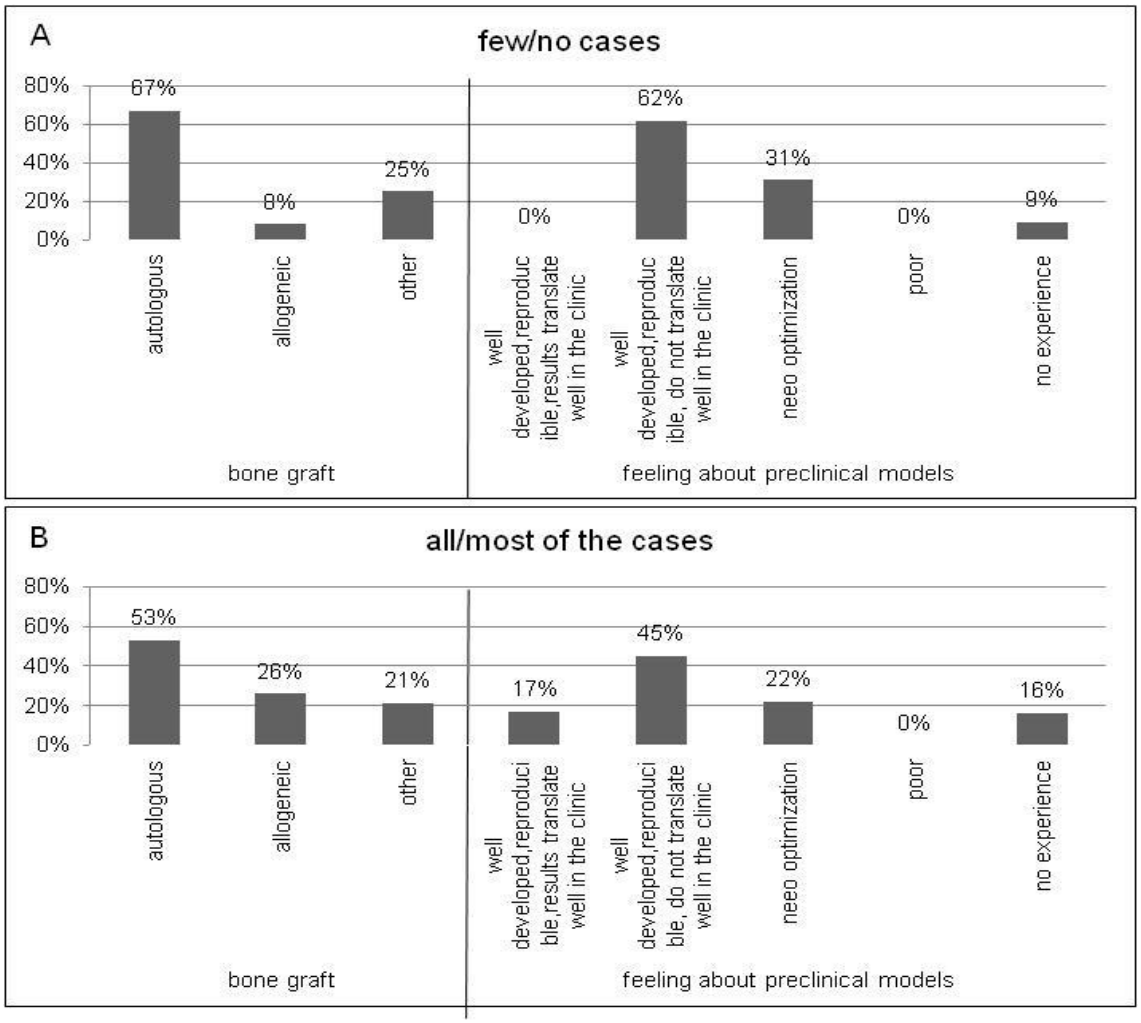

Fig. 18 Potential application of bone tissue engineered constructs by surgeons considering what they currently use as material for bone grafting and their perception of preclinical models. A. The graph represents the number of surgeons who would treat few or no cases with such constructs, n=13. The left part of the graph shows the currently used bone graft material and the right part surgeons' assessment of present animal models. B. The diagram outlines surgeons who would treat all or most of their cases with bone tissue engineered constructs, n=22. On the left, bone graft materials which they usually apply are represented and on the right surgeons' feeling about current preclinical research using animal models.

3.1.3 Scientists

Whereas the previous chapter outlined the obtained data from the survey when filled out by surgeons, the following chapter evaluates the outcome of the survey and the perception of preclinical bone healing from a scientific point of view. Therefore, the obtained answers from scientists and clinical scientists were evaluated.

3.1.3.1 Indications for bone tissue engineered constructs

Concerning the targeted indications for bone tissue engineered constructs, 37% of the researchers referred to large bone defects, 19% to critical-size defects and 41% mentioned non-unions to represent a major indication targeted with bone tissue engineered constructs. Some scientists referred to spinal fusions as targeted objective for research, although it represented only a small percentage (3%) of the indications mentioned in the survey (Fig.19).

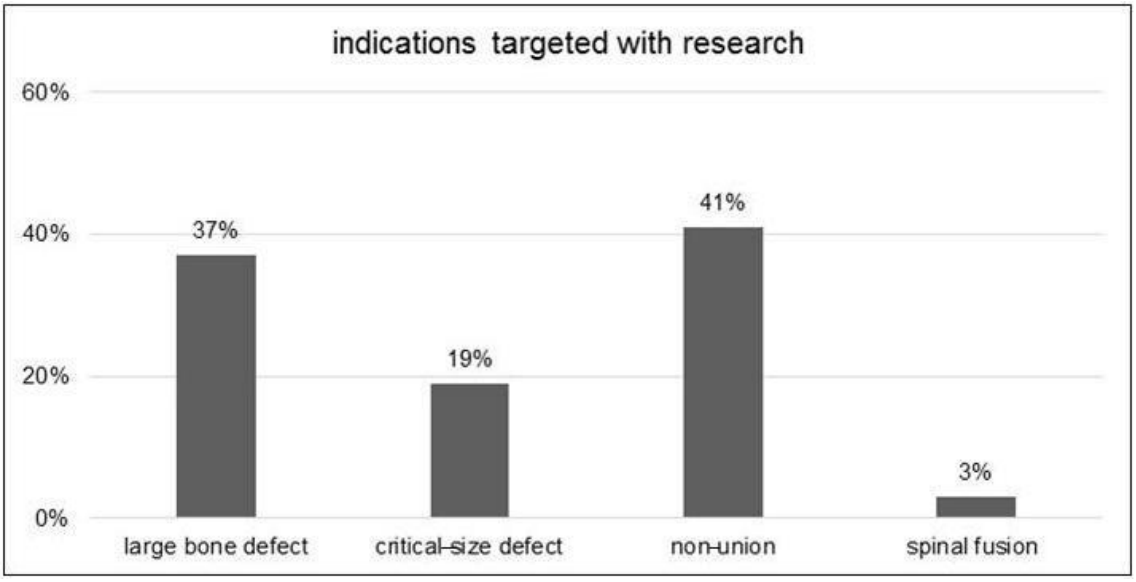

Fig. 19 The graph represents the different indications for bone tissue engineering targeted with the research conducted by the participants of the survey, n=25. Large bone defects were referred to defects associated with tumor excision, trauma, infections, osteonecrosis or dental implantology. Non-unions were referred to pseudarthrosis or delay of consolation.

3.1.3.2 Scientists' opinion on bone tissue engineering and preclinical animal models

As already performed on the answers of the surgeons, scientists were divided into two groups by taking their experience level into account. The first group represents scientists with less than 10 years of professional experience and the second group scientists with more than 10 years of experience. Among those with less experience, 26% thought that preclinical models are well developed but do not translate well in the clinics, whereas 11% indicated that models do translate well in the clinics. Moreover, 53% replied that models need optimization and 5% thought that they are poor. The remaining respondents did not have any experience with research on animal models (Fig.20A). Among the scientists having more than 10 years of experience, 35% considered models as not well translated in clinics. In comparison, 6% of them responded the contrary and 47% thought that they need optimization, while 12% described the models as poor (Fig.20B).

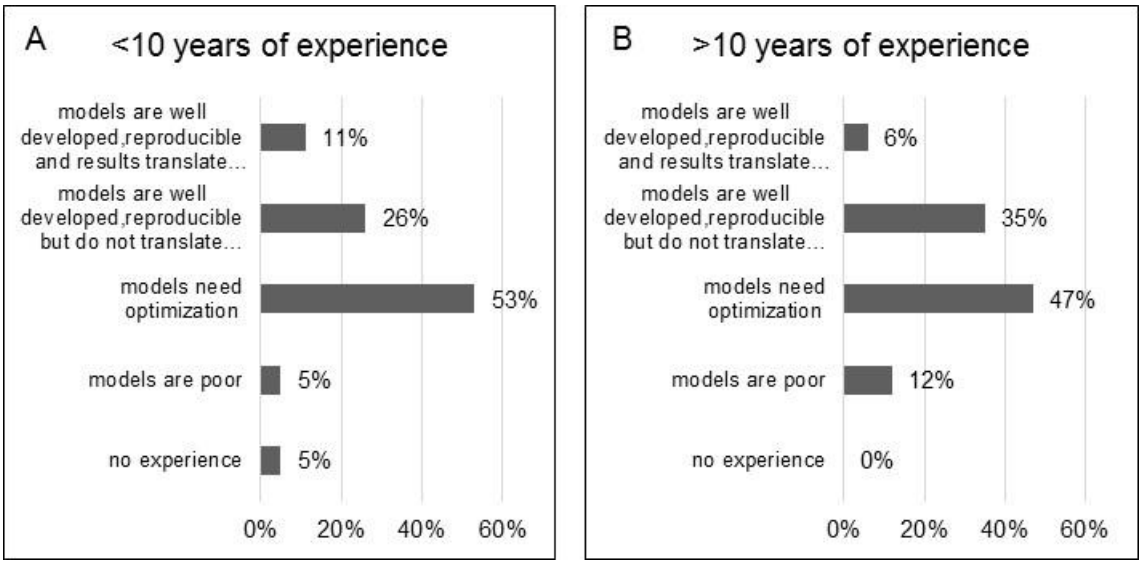

Fig. 20 Scientists and their attitude towards preclinical models considering their work experience. The diagram shows scientists' feeling about preclinical models when having less than 10 years of professional experience (A, n=19) and more than 10 years of professional experience (B, n=15).

3.1.4 Evaluation of preclinical animal models indicated in the survey

One of the main objectives of this thesis was to explore what kind of animal models are currently used in the research field for bone tissue engineering and to evaluate these models regarding their study outcome and translational success.

To evaluate currently available preclinical animal models, scientists were asked to describe the models that they are using and to indicate, whether they were satisfied with the outcome or not.

3.1.4.1 Establishment of animal models for bone tissue engineering

The participants were asked to describe the animal models that they were or are currently using for research on bone tissue engineering. The question was constructed as a table with open text spaces to fill in the answers. Fig.21 shows the percentage distribution of all animal species, gender, implantation sites and defects that were used in the preclinical research models by the survey participants.

Most of the studies were conducted in small animals, namely rats (36%), mice (22%) and rabbits (11%). Large animal models such as sheep and goats were used in 28% of the described studies. The remaining 3% were performed on minipigs (Fig.21A).

In terms of gender, 28% of the studies were conducted in male animals, 28% in female animals and 44% in animals of both sexes (Fig.21B).

The implantation sites indicated in the survey varied mainly between femur (55%), tibia (30%) and cranium (6%). The remaining defects (other) were performed either in the jaw (mandible) or in the metatarsus (Fig.21C).

When researchers were asked to define the defect model they were working with, 21% described their defect as a simple fracture, 10% as a large bone defect, 50% as a critical-sized defect and 12% as a non-union. If created defects were indicated in the survey but could not be clearly assigned to one of the defect

definitions represented in the graph, they were put in the category named "other"(Fig.21D).

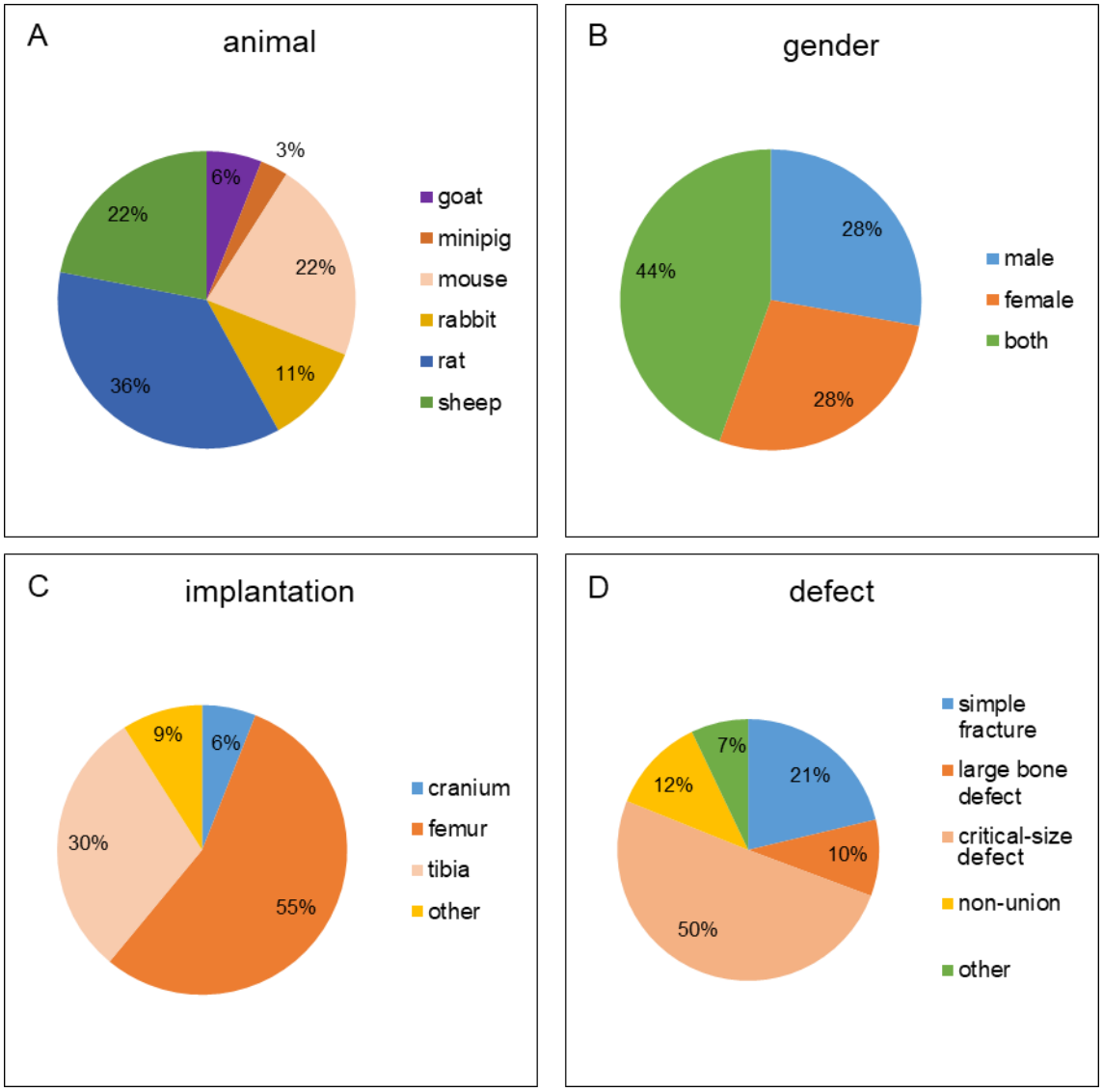

Fig. 21 Models that are currently used as indicated in the survey. If participants mentioned several models in the same survey, they are listed separately A. The graph pictures all animal species used in the studies, n=36. B. The chart represents the animal gender that researchers were working with, n=36. C. The diagram shows the different implantation sites for the defects, n=33. D. The graph indicates the defect types created for testing the bone tissue engineered constructs, n=33.

When using the femur bone as implantation site, 80% of the defects were designed as segmental defects and 20% as drill holes (Fig.22A). Tibial defects were in 69% of segmental design and in 31% drill hole defects (Fig.22B).

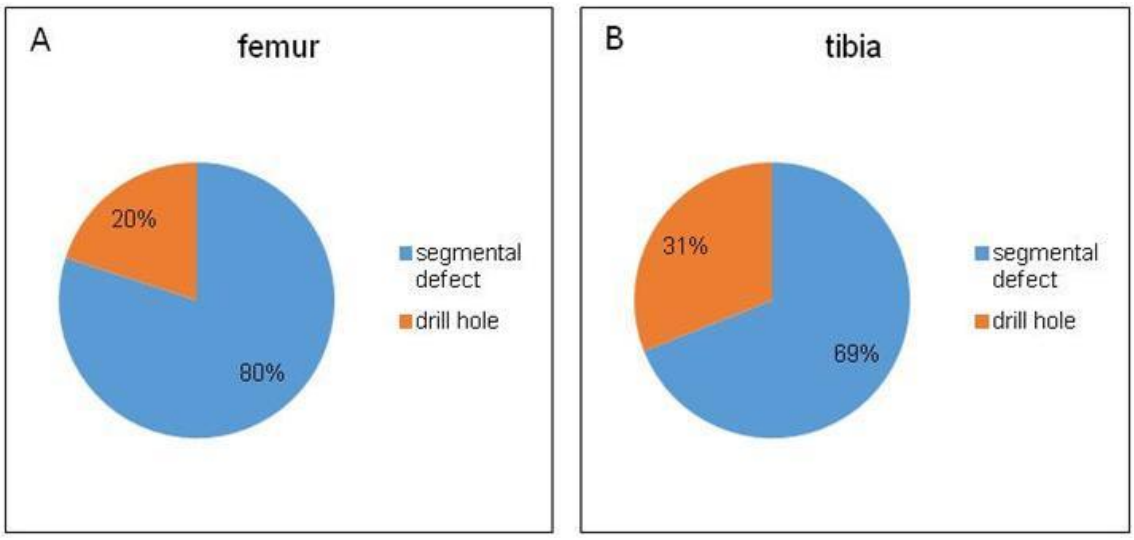

Fig. 22 Defect models in femur and tibia. The graph shows the different surgical designs for the defects that were created in the femur of the animals (A, n=20) and in the tibia of the animals, (B, n=13).

3.1.4.2 Satisfaction with the research outcome

Next, the outcome of the reported studies utilizing different preclinical animal models was evaluated. Therefore, scientists and clinical scientists were asked to classify the research outcome of their animal model design according to their satisfaction with it.

Research with goats, mice and rabbits as animal models was described with equal frequencies in the category most and least satisfied, whereas the work with rats and sheep was described proportionally more often in the category most satisfied (Fig.23A). Half of the defects conducted in the cranium, 58% of the femoral defects and 80% of the tibial defects were mentioned in the category of satisfying models (Fig.23B). Furthermore, half of the large bone defects, 56% of the simple fractures, 71% of the critical-sized defects and 80% of the non-unions provided satisfying outcome (Fig.23C).

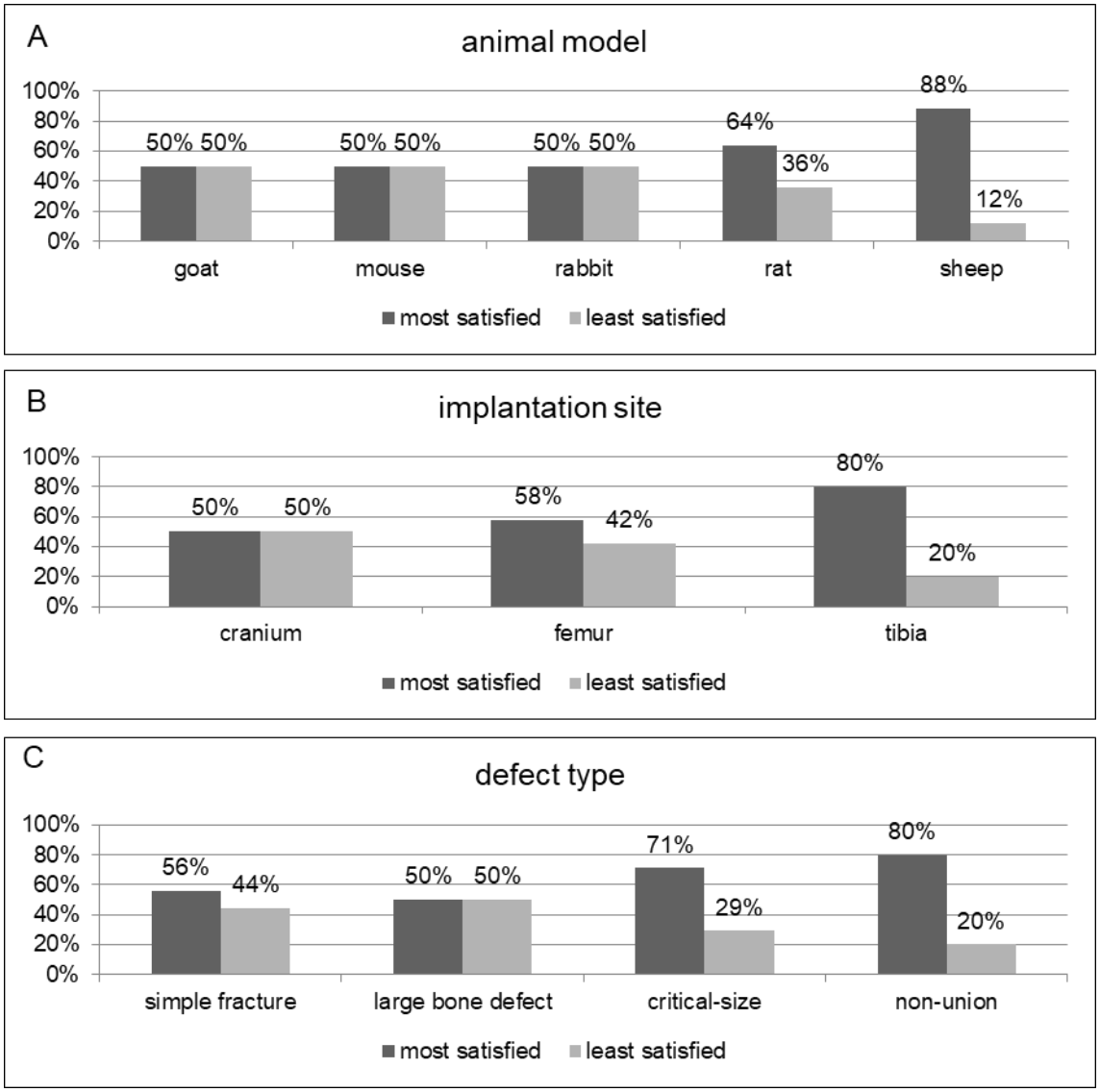

Fig. 23 Researchers' satisfaction with the outcome of currently used animal models. Data computing was performed using the total count of answers in one category (animal species, implantation site, defect type) as reference for calculating afterwards what percentage of the total amount represents the most and least satisfied outcome. The graph shows the outcome for the two categories, most and least satisfied, when using different animal species, (A, n=36), different implantation sites (B, n=33) and different defect types (C, n=42).

Segmental defects in the femur were mentioned with 63% in the category most satisfying outcome, whereas drill holes in the femur were exclusively mentioned in this category (Fig.24A). In comparison, drill holes in the tibia showed only 75% of satisfying outcome, whereas segmental defects convinced with 89% in the positive outcome category (Fig.24B).

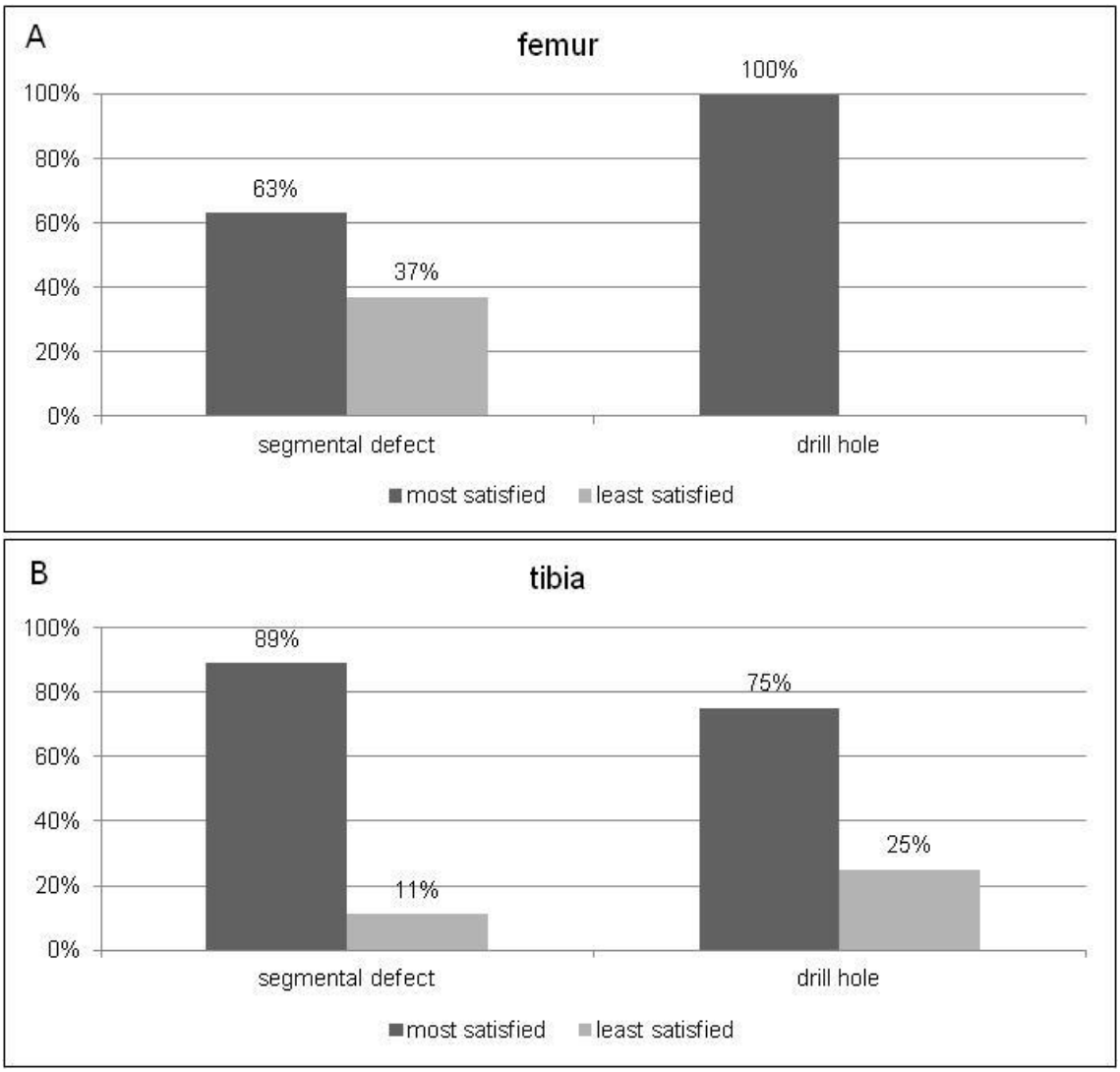

Fig. 24 Researchers' satisfaction with the experimental outcome when using different types of defects in the femur and tibia. Data computing was performed using the total count of answers in one category (segmental defects/drill holes in the femur and tibia) as reference for calculating afterwards what percentage of the total amount represents the most and least satisfied outcome. The graph shows the outcome for the two categories most and least satisfied when using segmental defects and drill holes in the femur (A, n=20) and segmental defects and drill holes in the tibia (B, n=13).

The surgical procedure of creating a bony defect in animal models may, depending on the defect site, include the application of different fixation methods, which may themselves influence the outcome of a study. Participants of the survey were therefore asked to describe their fixation device, if applied. The obtained answers from researchers showed that segmental defects in the femur were mainly fixated with plates (53%) or intramedullary nails (26%), whereas most of the segmental defects in the tibia were stabilized by plates (45%) or

external fixators (33%) (Fig.25A and Fig.25B). Femoral drill hole defects were performed without fixation in 75% of the cases (Fig.25C). The same applies to drill holes in the tibia (Fig.25D).

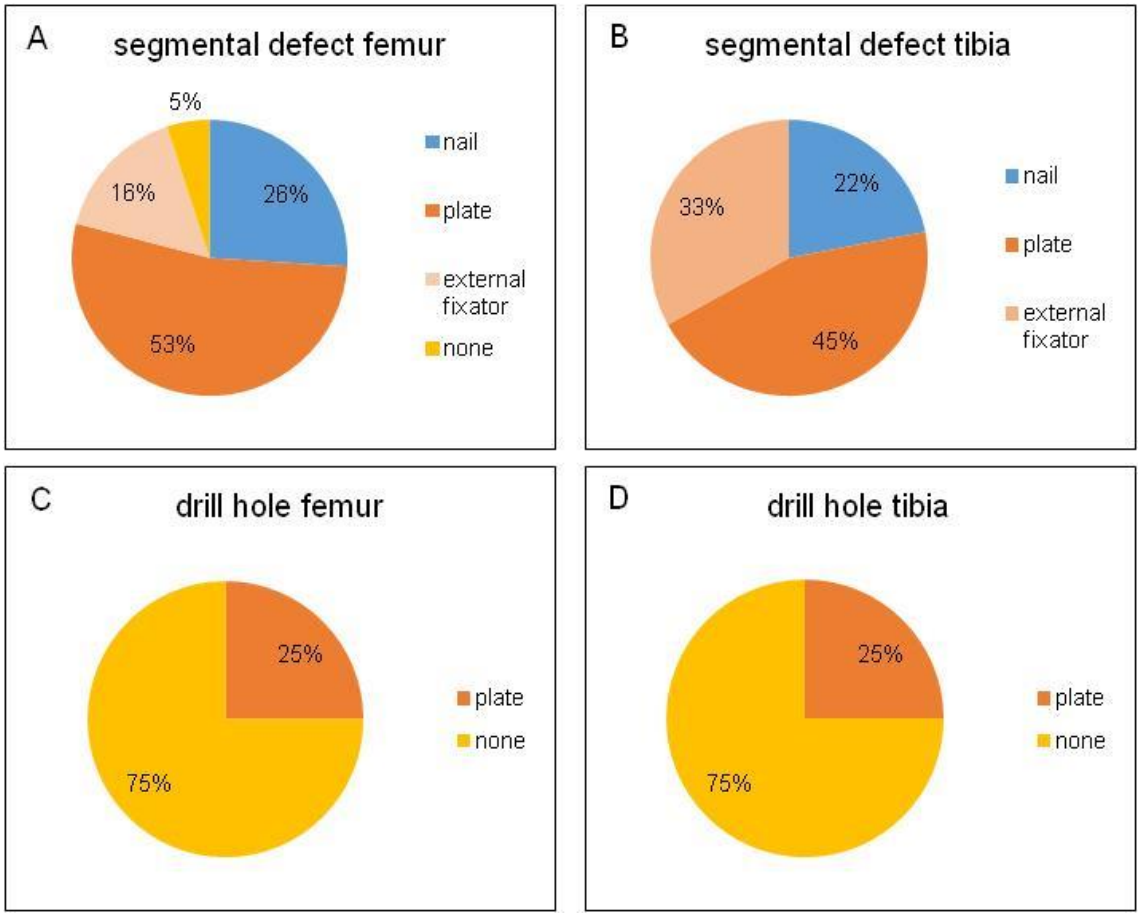

Fig. 25 Fixation methods for different kinds of defects in the femur and tibia. The graph shows the fixation methods used for segmental defects in the femur (A, n=19), segmental defects in the tibia (B, n=9), drill holes in the femur (C, n=4) and drill holes in the tibia (D, n=4).

The different animal models described in the survey were once again evaluated individually with regard to the applied fixation methods and the satisfaction of researchers with the models. Thereby, femoral and tibial segmental defects stabilized by nails, plates and external fixators were most commonly mentioned to be satisfying in more than half of the cases. Unfixed segmental femoral defects, however, were only mentioned in the not satisfactory category, but represent only one case in the survey (Fig.26A and Fig.26C). Drill holes in the femur provided satisfactory outcome regardless of whether plates had been used or not

(Fig.26B), whereas drill holes in the tibia did not satisfy in 33% of the cases in which no fixation stabilized the defect (Fig.26D).

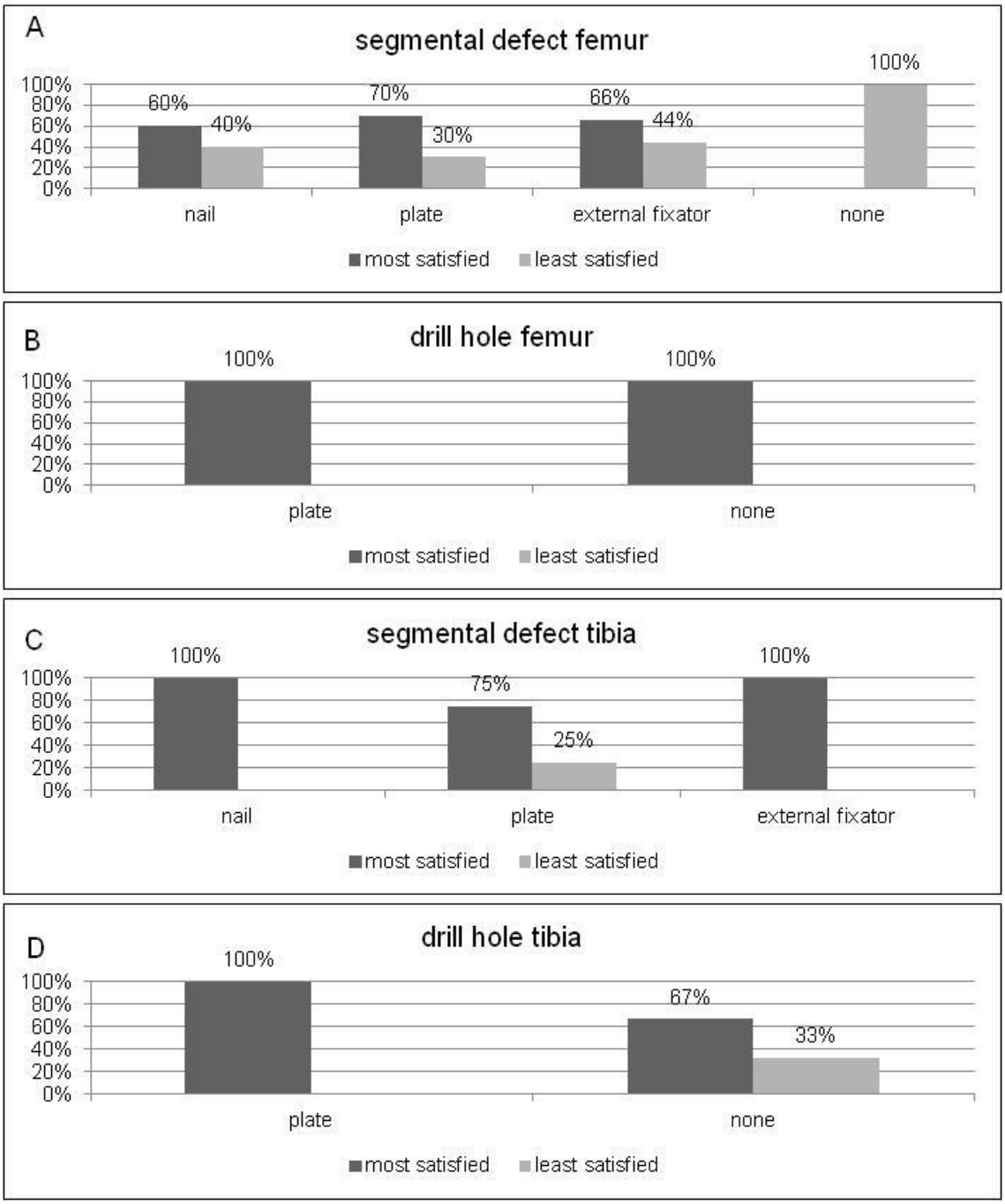

Fig. 26 Fixation methods used in femoral and tibial defects and researchers' satisfaction with them. The graph shows researchers' satisfaction with the fixation methods used in femoral segmental defects (A, n=19), femoral drill holes (B, n=4), tibial segmental defects (C, n=9) and tibial drill holes (D, n=4).

3.1.5 The assessment of bone tissue engineering from a scientific and surgical point of view

Main focus of this thesis was to evaluate the assessment and general perception of scientists and surgeons on bone tissue engineering and its potential way in the clinics. A particular interest laid in its future development – i.e., the evaluation of how researchers try to adapt and to potentially renew the design of preclinical animal models in order to achieve clinical translation of bone tissue engineered constructs. Therefore, the survey included the question of whether bone tissue engineered constructs would ever become relevant in clinical practice and if yes, how long it would take.

When asked whether research on bone tissue engineering was important, nobody answered in the negative. A designated open text field was part of the question for answering why research on bone tissue engineering was considered important. Here, aspects concerning donor site morbidity, costs and the need for alternatives to conventional bone grafts have been mentioned repetitively. In particular, the need of an alternative to today's gold standard, autologous bone, was noted. Autologous bone was described to be often limited in quantity and not capable to heal in a satisfactory manner, especially when it comes to larger bone defects.

The assessment of preclinical models for testing bone tissue engineered constructs had already been illustrated individually for surgeons and scientists (Fig.14 and Fig.20). Now, Fig.27 represents surgeons' and scientists' feeling about such models in comparison. Generally, scientists showed a more pessimistic perception of the models than surgeons. The broad assessment of surgeons was that preclinical models for bone tissue engineering were well developed and reproducible (45%) but do not translate well in the clinic, whereas scientists confirmed this statement in only 30 % of the cases. Further optimization for the models was underlined to be necessary by 32% of the surgeons and 50% of the scientists. The minority of both surgeons and scientists reported that models were sufficient and adequate for clinical translation. However, again, this opinion had a greater presence among the surgical respondents (10%) than among the scientists (8%). Moreover, none of the surgical respondents thought

that models are poor, whereas 9% of scientists marked this answer with a cross. Surgeons who had no experience with such models occurred naturally more often (13%) than scientists (3%).

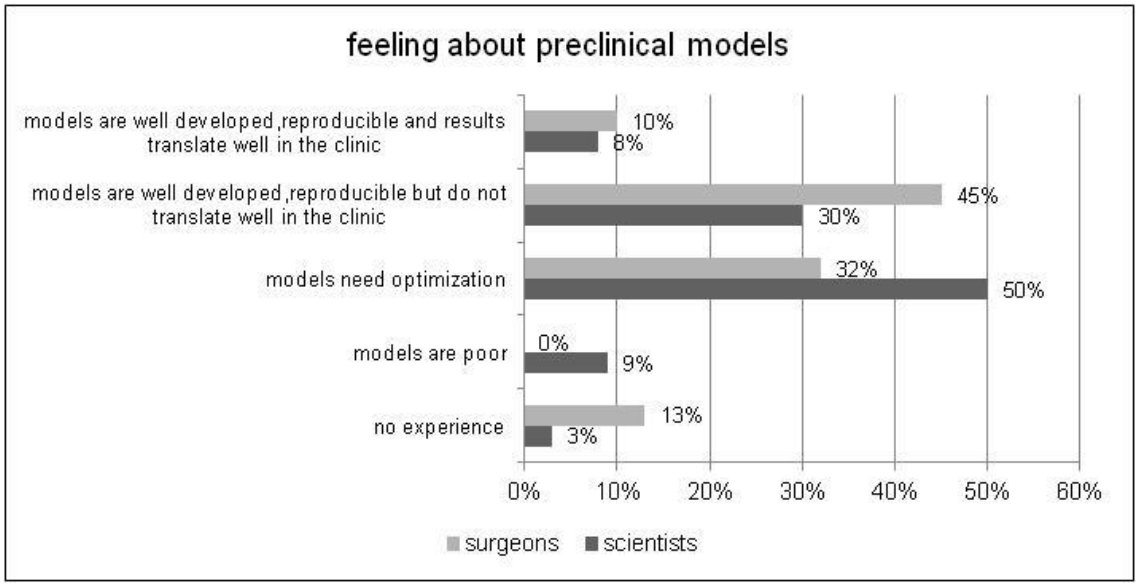

Fig. 27 Respondents' feeling about preclinical animal models; several answers were possible, scientists: n=34, surgeons: n=31.

Fig. 28 summarizes the perception of the surveyed scientists and clinical scientists on preclinical research models for bone tissue engineering. Furthermore, it reflects the opinion of the survey respondents on the possible breakthrough of bone tissue engineering in today's clinical practice. When asking scientists whether the applied animal models were clinically relevant or not, 77% of the surveyed researchers affirmed the models as relevant (Fig.28A). In this context, all participants in the survey were asked if they could envisage the actual clinical translation of bone tissue engineered constructs in the future. This was affirmed by 98% of all respondents (Fig.28B).

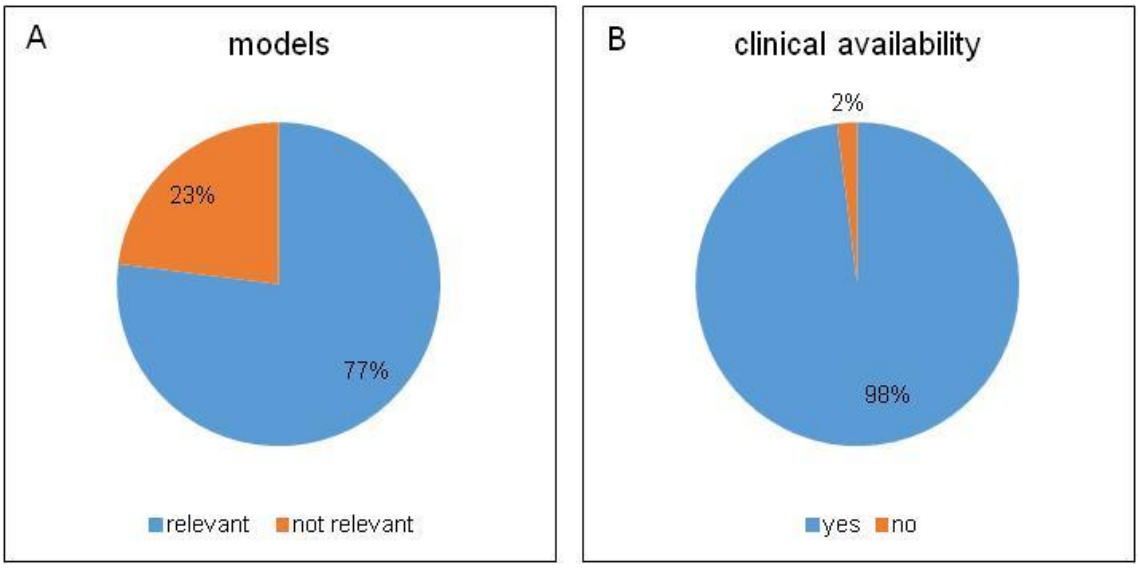

Fig. 28 The assessment of preclinical animal models and the potential clinical availability of bone tissue engineered constructs in the future. A. The graph represents the relevance of currently used animal models, n=31. B. The graph indicates the possibility for bone tissue engineered constructs to become clinically available (scientists and surgeons point of view), n=67.

After confirming the possible translation of bone tissue engineering in the clinics by almost all participants, the point of interest was then to perceive an idea of how long it would take. Therefore, a question concerning the estimated period of time required to achieve clinical application of bone tissue engineered constructs realistically was part of the survey. Thereby, the participants did not always estimate actual clinical translation as imminent. However, only 5 years until clinical application were indicated to be necessary by 31% of the scientists and 19% of the surgeons. 10 years for achieving clinical translation were assumed by 61% of the surgeons and 41% of the scientists, showing a slightly greater optimism from the surgical side. The remaining smaller part of the participants (28% of scientists and 20% of surgeons) marked 20 years or more to be realistic for actual clinical application. Nevertheless, the majority of both surgeons (80%) and scientists (72%) deemed clinical translation in the nearer future (5 to 10 years) possible, whereby surgeons affirmed a slightly more optimistic assumption (Fig.29A and Fig.29B).

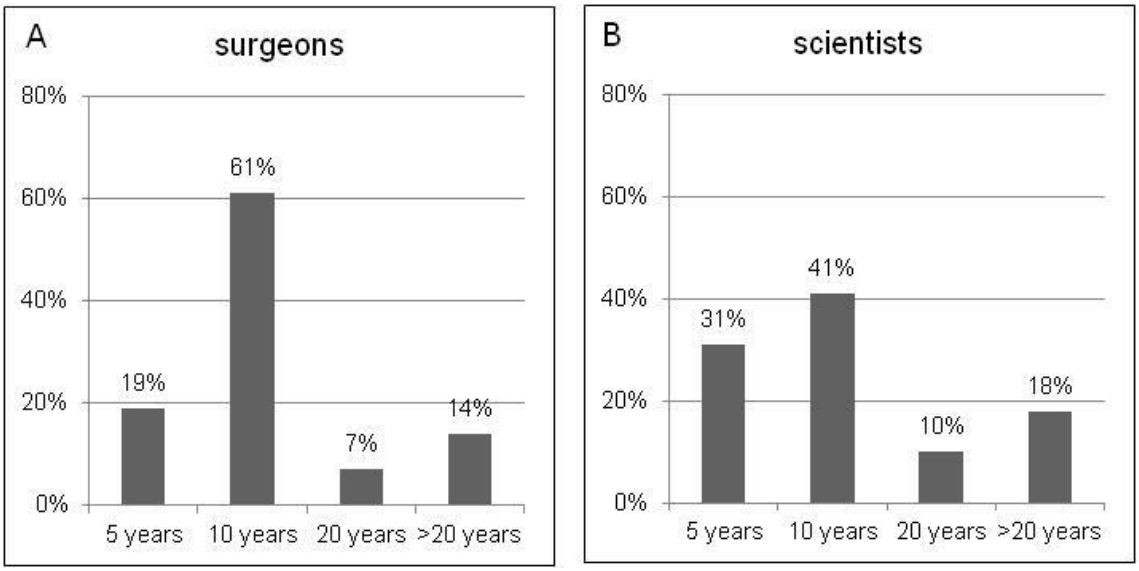

Fig. 29 The estimated time for bone tissue engineered constructs to become clinically applicable. The graph indicates the duration (years) as estimated by surgeons (A, n=28) and scientists (B, n=29).

3.1.6 Issues for further discussion

At the end of the survey, respondents were asked to comment their reflections, suggestions and opinion on preclinical animal models for bone tissue engineering. Therefore, the last question of the survey provided an open text field for the respondents to fill out.

The following are the comments that participants had put in the field designated for discussion:

- "Need for better non-union models which are difficult to treat and lead to ischemic bone wounds."
- "Small animal models are appropriate research questions, but they are used too often for trying to directly translate into the clinical situation. Large bone defect models have quite a variability which is not described or taken into account appropriately."
- "The immunological aspect of tissue regeneration in vivo is entirely different from what we observe in animal models. We are able to get only an idea of how it will work but the moment the graft is put inside a human body, vascularity plays a crucial role. I believe there is race between tissue

regeneration and apoptotic signals that influences the final outcome of a tissue engineered bone."

- "There is the need of preclinical in vivo model standardization to reduce/refine animal use; also, post-operative management of animals during bone regeneration is to be targeted and standardized."
- "Tissue engineering will always be a domain of academic institutions and not for daily practice."
- "We work with rats for all the obvious reasons but question their clinical relevance because they respond so exuberantly to BMP-2. Humans do not. Also, it's hard to identify new and better osteogenetic agents when BMP2 is so hard to beat as a positive control."
- "In my opinion, and I'm not a bone researcher, personalized 3D bio printing with incorporated bone (allo-or auto-) graft (aspirate or cells) is an interesting research field regarding large sized bone defects."

3.2 Outcome of the literature research

3.2.1 Evaluation of currently used preclinical animal models

The following chapter presents the results from the literature analysis. Out of 260 potential papers, a total of 167 papers fulfilled the search criteria as described above. The papers and a summary of the studies are listed in supplementary table 3 (Tab-S.3). Supplementary table 4 (Tab-S.4) shows a list of paper that had been excluded. The aim was to evaluate the included paper with regard to therein described animal models and thus to obtain a general trend in the current design of preclinical animal models.

The studies described in the papers generally provided information about the utilized animal (species, age, strain), the observation process (methods, time), the implantation site, the defect design (type, classification, size, fixation) and whether an empty defect was part of the investigation or not.

3.2.2 Research target of the studies

Experiments with preclinical animal models were mainly conducted to test new bone tissue engineered constructs (91%) using different kinds of scaffolds, cells and/or bioactive factors. Other studies (9%) aimed to establish new preclinical animal models by testing defects of different sizes and shapes by exploring the appearance of interfragmentary movements and by creating new fixation or observation methods or biomechanical tests.

3.2.3 Animals

3.2.3.1 Species and strain

Preclinical animal models involved large animal models such as dogs (n=6), goats (n=6), horses (n=1), monkeys (n=2), pigs (n=3) and sheep (n=8). However, the predominant majority of the evaluated paper described studies performed on

small animal models, namely mice (n=18), rabbits (n=69) and rats (n=54) (Fig.30).

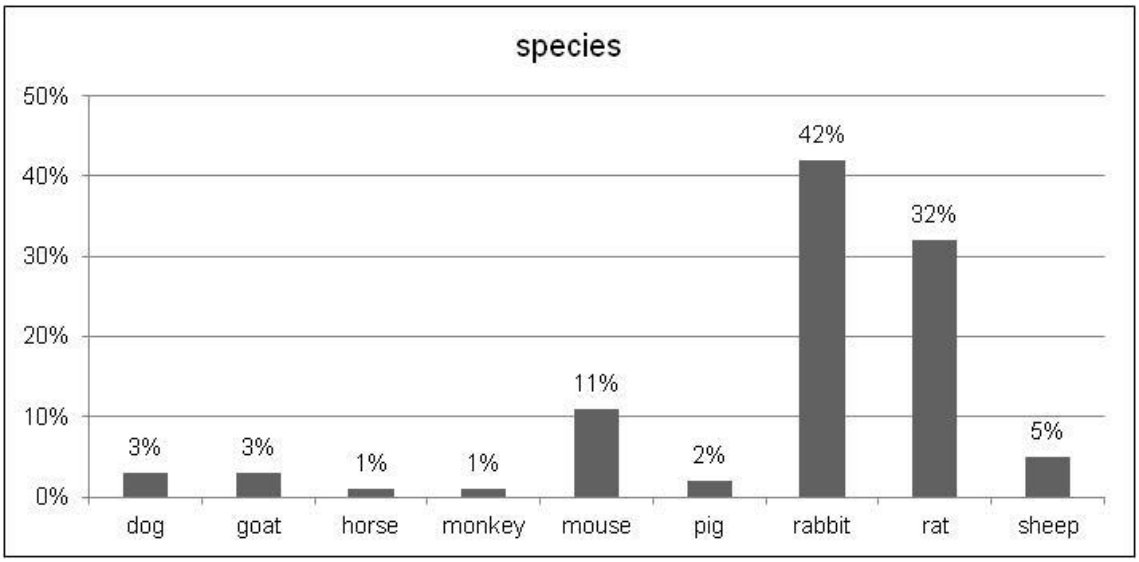

Fig. 30 An overview of the preclinical animal models used in the field of bone tissue engineering over the last 10 years. The graph shows the different kinds of animal species that were used for the studies, n=167.

Table 3 summarizes the different strains that were reported in the publications. Outbred strains (Sprague–Dawley rats, Wistar rats, New Zealand White rabbits) were used in 74% of the studies with small animal models, whereas inbred strains (Lewis rats, Fischer rats, BALB/c mice, C57bl/6J mice, C3H/HeN mice) were used in 16%. All in all, 10% of the studies were conducted using immunodeficient animals (mice or rats) and 3% studies using ovariectomized models.

Tab. 3 Different animal strains used for research in bone tissue engineering.

mouse	rabbit	rat	dog	monkey	pig	sheep
BALB/c C57bl/6J SCID beige C3H/HeN nude genomic-modifications	New Zealand White Japanese White Nihon White Chinchilla-bastard	Wistar Fischer Sprague Dawley Holtzman Lewis nude genomic-modifications	Beagle	Macaca-Fascicularis Rhesus	MGH-miniature Danish Landrace Sus Scrofa	Merino Swiss Alpine Black-face Mountain Bergamasca Latxa Asturian

3.2.3.2 Age and gender

The reported age of small animal models (mouse, rabbit, rat) started at 6 weeks. Thereby, the age of mice ranged between 6 weeks and 3,5 months, whereby the average age was 2,4 months. Rabbits were used between the age of 6 weeks and 12 months and the mean age was 4,5 months. The indicated age of rats varied between 6 weeks and 6 months with a mean age of 2,9 months (Fig.31A). In large animal models (dog, goat, monkey, pig, sheep, horse), the reported age ranged between 5 months and 17 years, whereby dogs were used between the age of 12 and 18 months and with a mean age of 15,6 months. Goats' age varied between 12 months and 3 years and the mean age was 24,5 months. The studies using pigs reported an age range between 5 and 20 months and a mean age of 11,3 months, whereas the age of sheep ranged between 1 year and 9 years, resulting in an average age of 4,4 years (Fig.31B). One study was evaluated using horses between the age of 11 and 17 years. The terms "skeletally mature" and "adult" were used sporadically to describe the animals age. However, they were not included for the evaluation of age since they cannot be defined precisely by an exact age. The same applies for studies indicating the age of the animals when "purchased", "obtained", "provided", "procured", "acquired", "fed or kept in cages", but not indicating the exact age of surgical procedures.

If reported, male animals were used in 57% of the studies for examinations, whereas studies on female animals were described in 31% of the evaluated publications. Experiments on animals of either sex were performed in 12%. (Fig.31C).

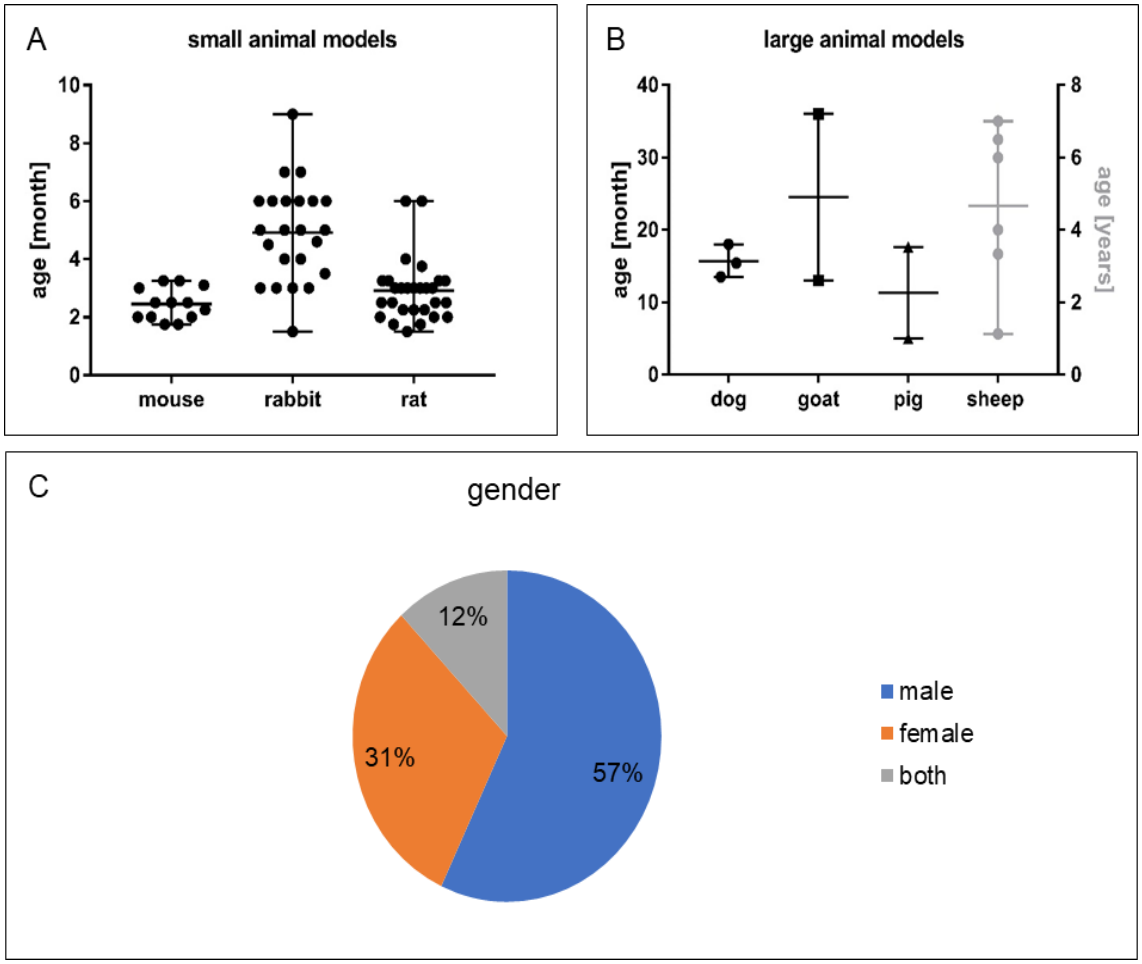

Fig. 31 The animal mean age and gender that researchers were working with, n=119. A. Age of small animals upon study entry. Data is given as mean ± standard deviation, n=63. B. Age of large animals upon study entry. Age is depicted in month for dog, goat, pig and years for sheep. Data is given as mean ± standard deviation, n=14. C. The graph shows details on the sex of animals used for the studies if indicated, n=119.

3.2.4 Defect design

3.2.4.1 Implantation site

Anatomical implantation sites for the defects varied mainly between the cranium (11% of the studies), the femur (38% of the studies), the tibia (14% of the studies), the radius (20% of the studies) and the mandible (11% of the studies). The ulna, the humerus and the maxilla occurred less often and are summarized under the umbrella term "other" (Fig.32).

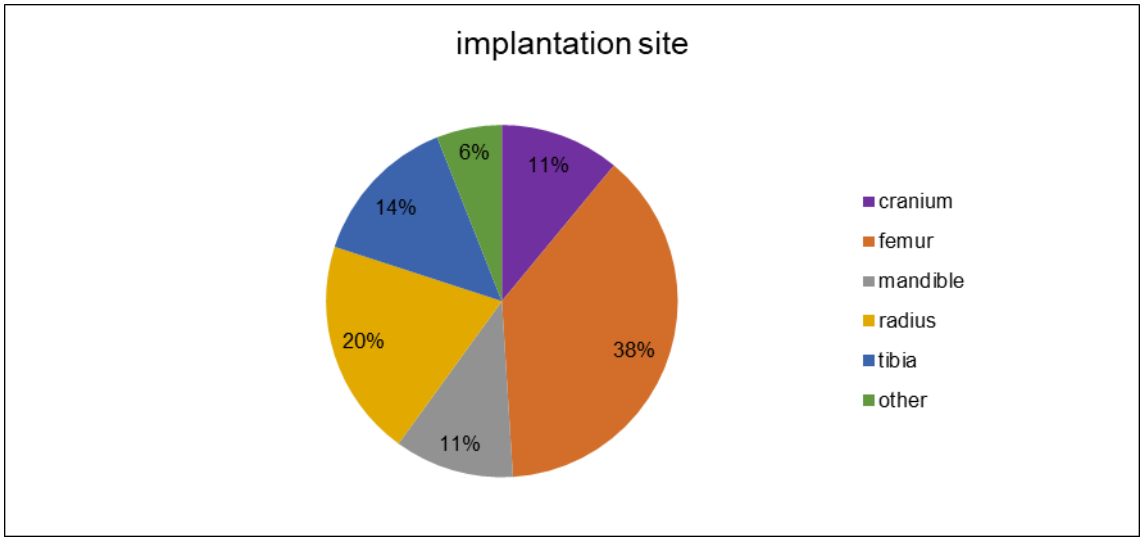

Fig. 32 Anatomical locations of the created defects to test bone tissue engineered constructs, n=171. If several implantation sites were reported in one study, they are listed separately.

In small animal models, 13 % of the defects were induced in the cranium, 40 % in the femur, 8% in the tibia, 24% in the radius, 9% in the mandible and 6% in other anatomical locations (ulna, humerus, maxílla) (Fig.33A)

In large animal models, 31% of the defects were conducted in the femur, 36% in the tibia, 21% in the mandible and 10% in the cranium, humerus, and radius (other) (Fig.33B).

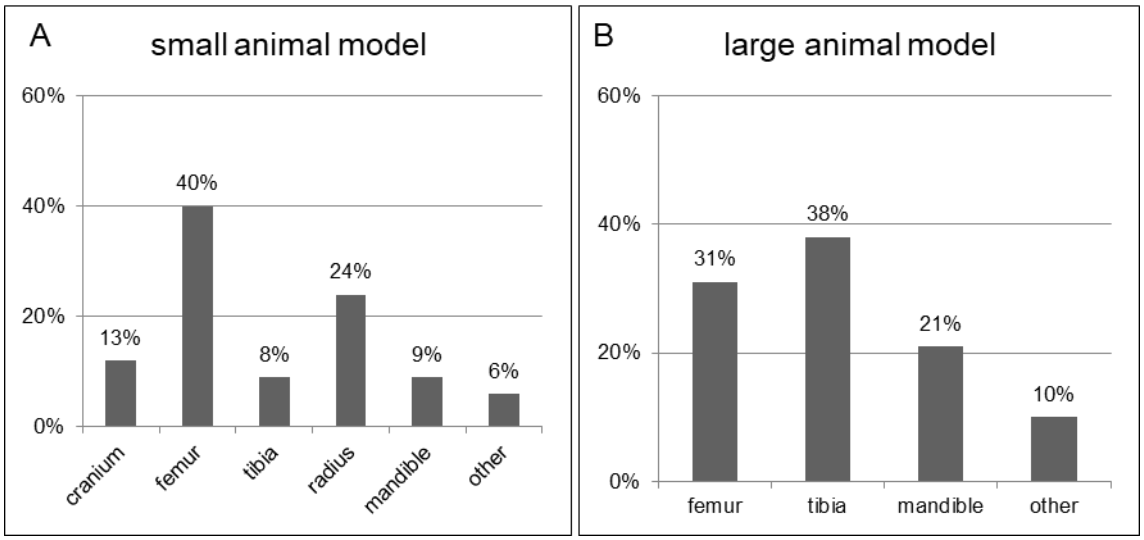

Fig. 33 Sites of orthotopic bone defects in preclinical animal models. A. The graph shows the locations in small animal models, n=142. B. The graph shows the defect locations in large animal models, n=29. If several implantation sites were reported in one study, they are listed separately.

Studies with small animal models involved rabbits, rats and mice. In rabbits, orthotopic defects were mainly created in the radius (42%) and the femur (22%), whereas tibial and mandibular defects occurred less often in the rabbit animal model such as the ulna, the humerus and the cranium (Fig.34A).

Rats were used proportionally more often for the creation of femoral defects (59%) and cranial defects (20%). Furthermore, surgery was conducted less often on the jaw (11%) and on the tibia and radius (5% each) (Fig.34B).

Almost the same applies to mice, albeit used more seldom as preclinical animal model. Half of the defects were induced in the femur and 20 % in the cranium, whereas tibia and radius represented less predominant defect sites (Fig.34C).

In general, surgery on large animals was performed considerably less often to test bone tissue engineered constructs. If, however, researchers did conduct studies on large animal models, sheep represent the most frequently used species among them. Thereby, most defects were created in the tibia (45%) and the femur (36%). The mandible and the humerus were used only once (Fig.34D).

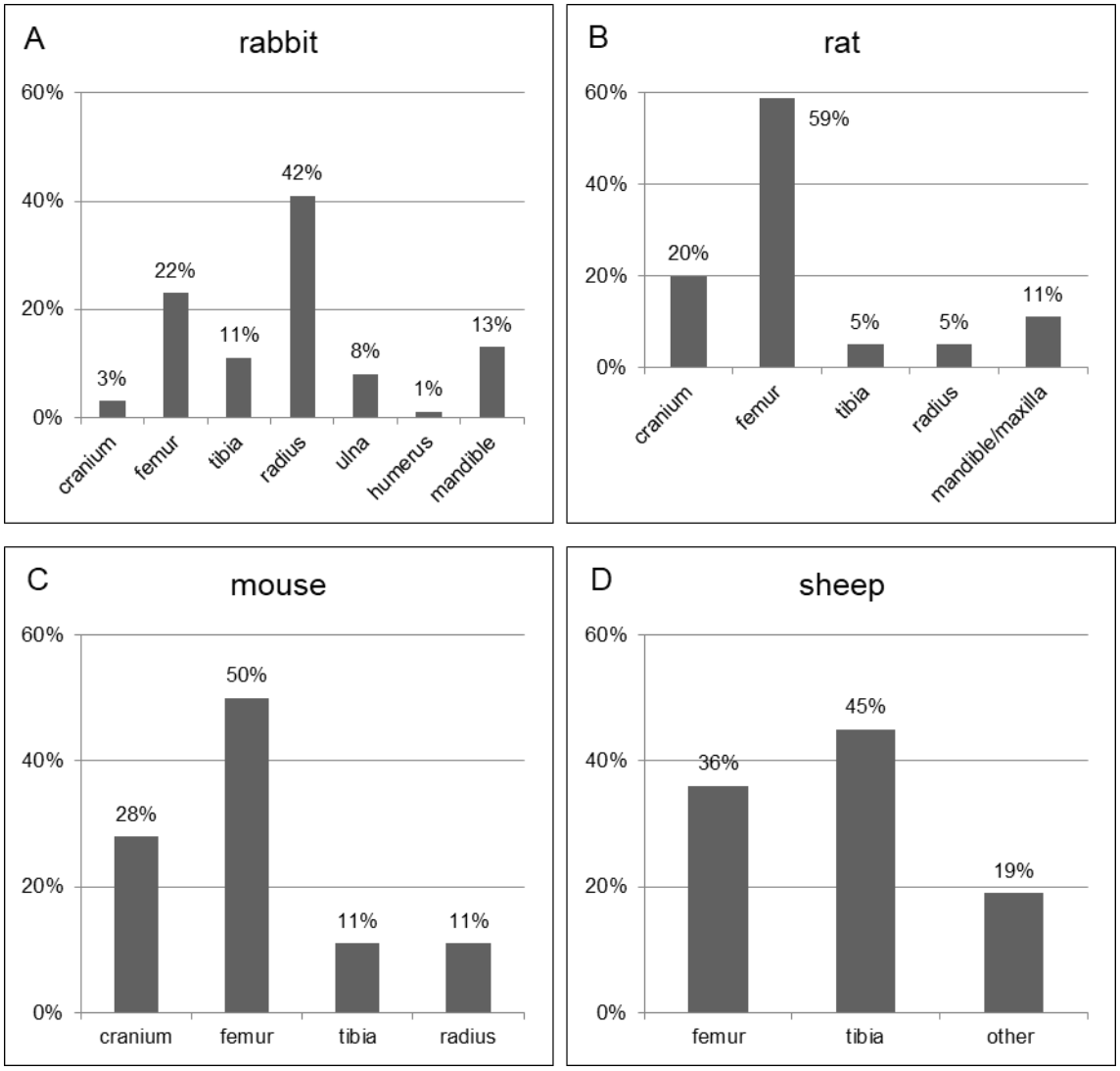

Fig. 34 Defect sites in preclinical animal models. The graph shows the different anatomical locations for the created defects in rabbits (A, n=69), rats (B, n=55), mice (C, n=18) and sheep (D, n=11). If several implantation sites were reported in one study, they are listed separately.

3.2.4.2 Defect form and defect model

For the creation of orthotopic defects, surgeons mainly utilized dental drills, burs and saws, resulting in different shapes of defects. Segmental defects were created in 59% of the cases (86 % in small animals, 14% in large animals) and drill holes or cubic defects in 32% of the cases (87% in small animals, 13% in large animals). The remaining defects were performed as alveolar defects in the mandible or maxilla, which showed a broad variety of surgical designs: the removal of either cortical plates, trabecular bone and tooth roots ("full thickness bone defect") or solely one side of the plates, trabecular bone and tooth roots ("

partial thickness bone defect") (Young, Bashoura et al. 2008) such as parodontitis induced alveolar bone defects (Fig.35A). One defect was described as bone gap in the mandibular symphysis of a rat, which was not included in the graph (Yagyuu, Kirita et al. 2015) .

Researchers specified their defects as non-critically sized defect (3%), large bone defect (6%), critically sized defect (75%), delayed bone healing defect (2%), non-union defect (7%) or other (simple fracture, experimental periodontitis model, infected bone defect, osteonecrosis model, alveolar cleft, open fracture model, peri-implant osseous defect) (Fig.35B)

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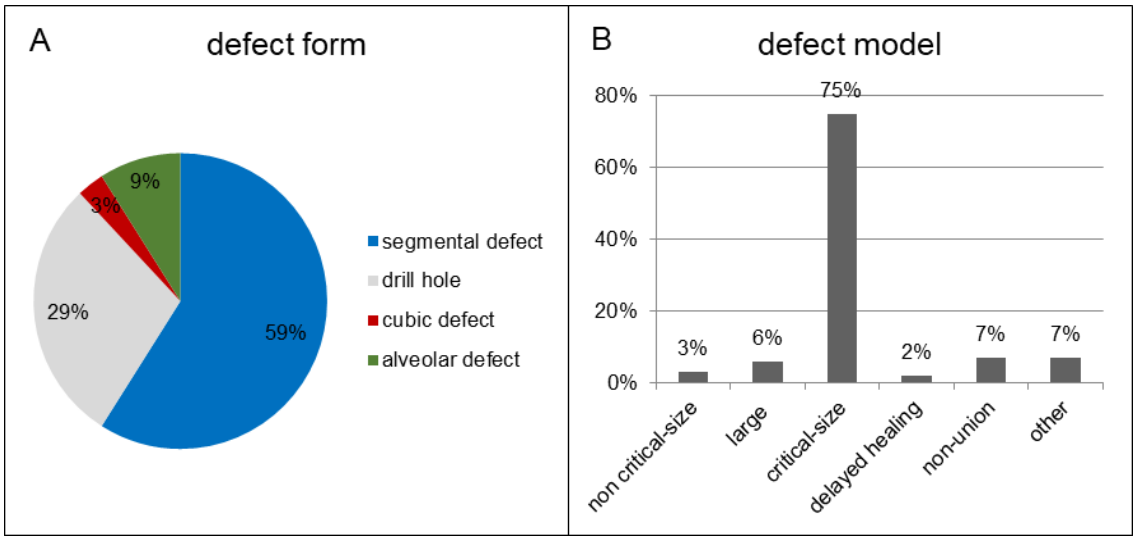

Fig. 35 The different bone defect designs created for research in bone tissue engineering. A. The graph shows the induced defect form, n=167. B. The graph shows the defect model chosen to test, n=126.

3.2.4.3 Defect size

Table 5 shows the different ranges of defect sizes that were reported in the studies of the literature search. One defect in a goat was described as a percentage of the osteotomy length to the whole bone length (Jian, Tian et al. 2010). Defects of the lower and upper jaw bone showed a broad range of indicated defect shapes and are therefore not comparable and were not summarized.

Drill hole defects in the murine calvaria varied between 2,5 mm and 5 mm (diameter) with a mean area of 11,3 mm², in the cranium of rabbits between 12

mm and 15 mm resulting in an average area of 144,8 mm² and in the cranium of rats between 4 mm and 8 mm (28 mm²). Drill hole defects in long bones varied between 4 mm and 8 mm in rabbits (34 mm²) and 0,8 mm and 4 mm (5,3 mm²) in rats. They were all mainly described as critically sized defect models.

Femoral segmental defects ranged between 1 mm and 5 mm in mice (both described as critical-sized defects), in rabbits between 12 mm and 15 mm (described as critical-size defect) and between 1 mm and 10 mm (both described as critical-size defect) in rats. Radial and tibial segmental defects were mainly performed on the rabbit animal model and started at 5 mm in the tibia and 5 mm in the radius (described as critical-size defect) and ranged up to 20 mm.

Most of the defects created in large animal models were conducted in sheep, followed by dogs and goats. Thereby, surgery performed on ovine animal models concluded mainly in tibial and femoral segmental defects that ranged between 20 mm and 30 mm (both critical-size defects). Drill holes in long bones varied between 5 mm and 8 mm (32,7 mm² mean area) and were indicated as critical-size or large defects. Canine models were mainly utilized for defect surgery at the mandible and goats as animal model for critical segmental defects in the tibial bone (25 mm to 30 mm).

Tab. 4 Reported defect sizes and implantation sites in preclinical animal models.
Large animal models shown left, small animal models right. In case several defect sites were reported in one study, these are listed separately in the table. For defect size, diameter is given for drill hole defects, gap size for segmental defects and defect area or volume for other volumetric defects. * due to extreme heterogeneity in defect location and size in mandibular defects no summary possible.

species	defect	mean defect size (range)
dog (n)		
2	femur drill hole	7,5 mm (5-10 mm)
3	mandible variable alveolar defects	*
1	radius segemental defect	15 mm
goat (n)		
2	femur segmental defect	20 mm (20 mm)
1	tibia drill hole	10 mm
3	tibia segmental defect	27,5 mm (25-30 mm),
		20 % of tibia length
pig (n)		
1	cranium drill hole	15 mm
1	femur segmental defect	20 mm
1	mandible alveolar volume defect	3-7 cm^3
sheep (n)		
1	femur segmental defect	25.4 mm
3	femur drill hole	6.3 mm (5-8 mm)
1	humerus drill hole	6 mm
1	mandible segmental defect	30 mm
2	tibia drill hole	6.5 mm (5-8 mm)
3	tibia segmental defect	25 mm (20-30 mm)

species	defect	mean defect size (range)
mouse (n)		
5	cranium drill hole	3.7 mm (2.5-5 mm)
9	femur segmental defect	3 mm (1-5 mm)
2	radius segmental defect	2.5 mm (2.5 mm)
2	tibia segmental defect	2 mm, fracture
rabbit (n)		
2	cranium drill hole	13.5 mm (12-15 mm)
11	femur drill hole	5.8 mm (4-8 mm)
3	femur segmental defect	14 mm (12–15 mm)
1	humerus segmental defect	7 mm
9	mandible variable alveolar defects	*
29	radius segmental defect	14.3 mm (5-20 mm)
4	tibia cubic defects	88.3 mm^2 (45-150 mm^2)
2	tibia segmental defect	5 mm (5 mm)
6	ulna segmental defect	15,8 mm (10-20 mm)
rat (n)		
11	cranium drill hole	5.8 mm (4-8 mm)
2	femur cubic defect	14.2 mm^2 (12.5-15.8 mm^2)
7	femur drill hole	2.2 mm (0.8-4 mm)
23	femur segmental defect	6.5 mm (1-10 mm)
4	mandible variable alveolar defects	*
2	maxilla variable alveolar defects	*
3	radius segmental defect	4.7 mm (4-5 mm)
1	tibia cubic defect	5 mm^2
2	tibia drill hole	2.3 mm (1.6-3 mm)

3.2.4.4 Fixation methods

The different fixation devices that were applied for defect stabilization are shown in Fig.36. Thereby, drill holes, non-load-bearing sites (cranium) as well as implantation sites that are sufficiently stabilized by one another (radius, ulna) were excluded from the evaluation of the fixation methods (see also chapter 4.2.6 and 4.2.8). The graph shows that fixation of segmental defects in the femur, tibia and the jaws was obtained through external fixation systems, plates, dynamic and static intramedullary fixation systems (rods, nails, Kirschner wires, pins) and other fixation methods (gauge needle, orthotopic splint, ligature wire).

When operating on large animal models, stabilization of the defect was most often obtained by plates (73%) of which more than the half occurred in the tibia. Furthermore, dynamic and static intramedullary fixation devices were applied in the femur and the tibia (20%). One external fixation system was applied in the tibia of a goat model.

For defects in small animal models, plates were utilized in 55% of the cases and almost exclusively in the femur. Intramedullary fixation devices were reported in 12% of the studies, whereby either the femur or the mandible were stabilized. Furthermore, external fixation systems, which were reported for both the femur and the tibia were applied in 21% of the reported studies.

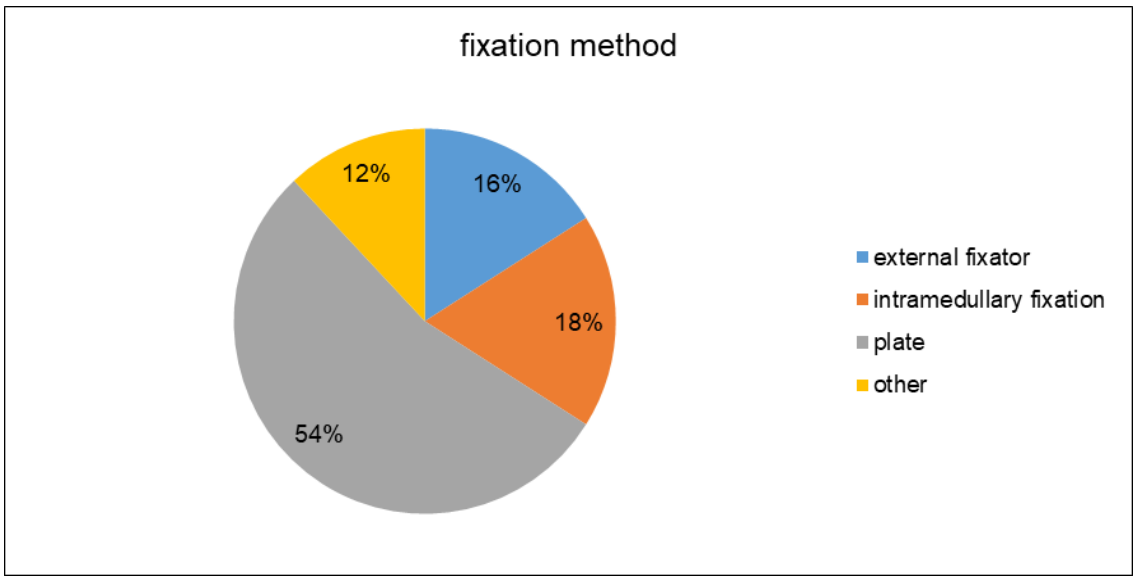

Fig. 36 An overview of the different fixation methods in load-bearing defects applied in animal surgery, n=58.

3.2.4.5 Negative control

To find out whether created defects would have also healed without any intervention or implantation of a bone tissue engineered construct, studies often included the creation of an empty defect, meaning no implantation of any material in a defect of the same size and shape.
The evaluation of such defects was included in 56% of the studies with animal models of which 2% showed bridging, 92% resulted in non-unions and 6% described none reproducible outcomes. The remaining 44% of the studies forwent an empty defect as negative control.

3.2.5 Observation process

Table 4 outlines the different observation methods for quantitative and qualitative analysis and the frequency of occurrence in the evaluated studies. Thereby, the most commonly used combination of methods included the application of radiology (x-ray), tomography (micro-computed tomography) and histology.
The observation time in the publications ranged from 2 weeks to 12 months in small animal models and from 12 days to 10 months in large animal models.

Tab. 5 Observation methods used for preclinical examination.

		small animal models (n=141)	**large animal models** (n=26)	**all** (n=167)
radiology	x-ray, energy dispersive spectral studies	60%	73%	62%
tomography	computed tomography, micro-computed tomography (µ-ct), single photon emission computed tomography (SPECT), cone beam computed tomography, perfusion weighted magnetic resonance imaging (MRI), fluorescence molecular tomography (FMT)	64%	73%	65%
histology	histomorphometry, immunohistochemistry, immunofluorescence, fluorescence microscopy, morphometric analysis, neovasculogenesis analysis	91%	92%	92 %
electron microscopy	scanning electron microscopy, laser electron microscopy	11%	4%	10%
molecular biology	gene expression, reverse transcription polymerase chain reaction, in situ hybridization	11%	8%	10%
biomechanical testing	push-out test, 3 point bending	33%	35%	34%
fluorexon	fluorochrome analysis with calcein	9%	8%	9%
other	in vivo fluorescence imaging, flow cytometry, ultrasonography	6%	8%	7%

4 Discussion

4.1 Introduction

In the last years, the interest in bone tissue engineering with the objective of bone regeneration, bone augmentation, bone repair or bone replacement has tremendously increased. Large bone defects, due to injury or disease, represent a critical clinical and socioeconomic challenge, especially in today's ageing population. The restoration of such defects requires the supply of significant amounts of bone, which is not yet realizable and hence entails a major decrease of life quality and increase of clinical expenses (Rose and Oreffo 2002). In vivo testing of bone tissue engineered constructs incorporates a necessary step half way between laboratory in vitro testing and clinical studies in humans but requires the attention to a multitude of factors. The focus of this thesis was hence to evaluate these factors and to discuss how scientists assess their implementation in research. Currently used preclinical study designs for research were analyzed in order to get an impression of how surgeons and scientists evaluate the current and future role of bone tissue engineering. A particular view was given to the establishment of preclinical animal models in order to develop an understanding of why current engineered constructs still fail to appear in the daily routine of clinics.

The following chapters discuss the design of current studies reported in the survey and described in the evaluated papers from literature research. Furthermore, issues that were raised in the comments from the participants of the survey will be addressed.

4.2 Need for standardization

Although in vitro studies represent helpful strategies to examine different processes of bone tissue regeneration, in vivo studies are crucial and indispensable as they provide a way to imitate underlying biological mechanisms and environments (Peric, Dumic-Cule et al. 2015). Clinical application of bone tissue engineering depends on preclinical studies in animal models and in order

to analyze and to optimize their outcome, the systematic control of objective and quantifiable study parameters is needed (Muschler, Raut et al. 2010). However, the evaluation of the preclinical animal models described in the conducted survey and in the reviewed paper revealed that the experimental set-up of the studies still varies significantly and no official common guideline for the study design could be distinguished. Consequently, a recurrent argument in the evaluated surveys is the need for standardized and reproducible preclinical in vivo models in order to reduce and refine animal use. This ethical principle named "3Rs" (Reduce, Refine and Replace) was first described in 1959 by William Russell and Rex Burch (Russell, Burch et al. 1959) and should represent the basis for the standardization of study designs. Preclinical animal models should be designed by elaborated guidelines, whether it is for choosing the animal species, strain, age, observation time and observation methods, implantation site, defect form and size, fixation methods or other criteria. Standardization thereby enables comparison between the studies and capturing of well-established results by creating a uniform language (Reichert, Saifzadeh et al. 2009, Reichert, Epari et al. 2010). Laboratory animal science concentrates on the appropriate application of animals in research with regard to ethical, scientific, and legal aspects. It is hence necessary to require profound knowledge about animal welfare, animal biology, animal breeding and housing and accurate surgical techniques when working with animal models (Auer, Goodship et al. 2007, Conn 2008). The ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) as well as the GSPC (Gold Standard Publication Checklist) thereby intend to enhance the description of research with animal models and to prevent the conduct of unnecessary research trials (https://www.nc3rs.org.uk/arrive-guidelines, 28 November, 2018) (Hooijmans, de Vries et al. 2011). Their implementation into study designs with preclinical animal models could accelerate the provision of reliable standardization, which was described as necessary in the surveys and the papers from the literature search. Ultimately, such guidelines would facilitate the still missing clinical translation of bone tissue engineering and reduce the number of unnecessary animal experiments. The following chapters provide

hence information about general classifications of laboratory animals and their application in the evaluated studies.

4.2.1 Animal species

The philosopher Bernard Rollin once mentioned that no matter how brilliant a study design and its implementation on the applied animal might be, no matter how much money available and how thorough the investigators knowledge and talent turns out, it would all be wasted if the animal choice is not correct (Rollin 1990, Conn 2008). Throughout history, animals served as experimental models for scientists to obtain insight of biomedical functions and anatomical structures (Conn 2008). Naturally, it would be favorable to determine one species for research that approximately reflects the biological characteristics of a human being. However, the recreation of authentic human conditions in animal models that reliably represent pathogenic processes is difficult (Auer, Goodship et al. 2007). It should be remembered that animal models remain simplified imitations of the actual system in question (Liebschner 2004). One species cannot be compared to another and therefore needs to be assessed individually (Conn 2008). Moreover, it has been annotated that conflicting outcome may occur when using the same study design in different animals (Nunamaker 1998). The term animal modeling was meant to describe the modeling of humans and hence should be considered as "analogy" between the raised research question and the animal substitute that is necessary to understand the targeted question (normally with prospects for further applicability in humans) (Conn 2008). The choice of an appropriate animal species depends hence on the targeted inquiry and is influenced by a multitude of factors (e.g. costs, housing, availability, animal size, animal resistance, osseous characteristics etc.) (Nunamaker 1998, Bigham-Sadegh and Oryan 2015).

The preclinical studies described in the survey and in the papers from the literature search were conducted on various animals (dog, goat, horse, monkey, mouse, pig, rabbit, rat, sheep) but no common guideline for choosing one specific animal species could be determined. It was rather the more unspecific choice

between small and large animal models that was distinguished when evaluating the data of the survey and the literature search. The use of small animal models was thereby described more often and advantages in housing, costs and availability might be associated with this choice. Large animal models occurred less often and primarily when load bearing tests were part of the examinations, which will be described more in detail in the following chapter. Moreover, the evaluated studies showed a broad range of different surgical designs in different animal models and no standardized procedure could be determined. The provision of reliable regulations in order to design a preclinical animal model able to provide the extrapolation of study data to other species would hence be desirable, but remains challenging due to the individuality of each study design (Conn 2008).

4.2.1.1 Choosing between small and large animal models

The animal choice automatically influences the study design and its conduct, whereby it was mentioned that the chosen animal model should ideally mimic the surgery method applied in the clinics and enable the evaluation of the study outcome (Akar, Tatara et al. 2018). As already indicated, choosing the animal species for testing bone tissue engineered constructs meant primarily choosing between a small or a large animal model in the evaluated studies.

Studies conducted on rabbits and rats, as small animal models, and sheep, as large animal model, represent the most commonly described experiments in the surveys and in the paper obtained from the literature research. A comment written from a participant of the survey described small animal models to be an appropriate research model but used too often for trying to directly translate their outcome into clinical situation. Large animal models, on the other hand, were mentioned in the survey to have quite a variability, which is not described or taken into account appropriately. Indeed, important differences in bone anatomy, development, and physiology need to be taken into account when working with different animals as research models for bone tissue engineering (Reichert, Saifzadeh et al. 2009). Parameters affecting the variability like breed variety

(dogs), different bone remodeling rates (dog, sheep), growth rates (pig) or mechanical strength (dog, sheep) (Gothard, Smith et al. 2014) need to be weighed up.

More than half of the studies revealing satisfying results were conducted in small animal models when querying the satisfaction with the experimental outcome of the models reported in the survey. As already described in chapter 1.5.1, small animal models, which appeared in 85% of the reviewed paper and 69% of the studies received with the survey, provide advantages in handling, housing, availability, and cost-effectiveness compared to large animal models (Shanbhag, Pandis et al. 2017). Also, small animals achieve skeletal maturity and bone healing in less time (Liebschner 2004). Such advantages could explain why researchers might tend to use rather small animals for their study design. Then again, 10 out of 12 studies categorized as “least satisfying” described studies involving small animals, whereas only 2 of the studies in this category referred to large animal models. Even though small animal models happen to facilitate the study conduct, researchers might be unsatisfied with the results when searching to imitate human like conditions since large animal models were mentioned to provide more reliable outcome compared to small animal models (Liebschner 2004). Pellegrini et al. reported that rats are applicable for evaluating immunological and age-related aspects for tissue regeneration and for testing effects due to systemic disorders like osteoporosis (Pellegrini, Seol et al. 2009) but they cannot be considered as human miniatures (Sengupta 2013). Moreover, surgery in small animal models was mentioned to be more challenging to do and results in lower quantities of newly formed tissues (Nunamaker 1998, Akar, Tatara et al. 2018). Large animal models, on the other side, which were used in 16% of the reviewed studies and in 31% of the described studies in the survey, generally provide a bigger size and a bony anatomy that is closer to the one of humans, making surgical methods and biomechanical testing easier to handle and the outcome more effective (Liebschner 2004). Moreover, their size was mentioned to allow the upsizing of tissue engineered constructs to a relevant defect dimension in order to enable the application in humans (Pobloth, Johnson et al. 2016). The increased work with large animal models would hence be

favorable when testing bone tissue engineered constructs. Thereby, the size and shape of ovine long bones was discussed to enable the application of implants that were originally constructed for the use in human patients (Newman, Turner et al. 1995, Reichert, Epari et al. 2010). In this context, it is important to have in mind that the larger and hence clinically more relevant the defect volumes gets, an adequate supply of vascularization is required (see chapter 1.4). This may have major implication for the choice of the animal model and its success since the creation of a functioning vascularization system remains extremely demanding. The animal model should therefore ideally allow the recreation and evaluation of vascularized bone (Akar, Tatara et al. 2018) which mainly depends on the creation of an extensive blood vessel network that surrounds the defect site and enables the transport of vital components for bone repair (Auer, Goodship et al. 2007, Frohlich, Grayson et al. 2008, Laschke and Menger 2012, Roux, Cheng et al. 2015). The within lying complications and the relating high expenses could explain why the number of the evaluated studies with large animal models is considerably lower than the one with small animal models and why there remains a considerable number of studies resulting in unsatisfying outcome. As already mentioned, it would be important to expand further knowledge about vascular aspects by choosing the large animal as appropriate test model. Additionally, large animals show a closer imitation of the human bone healing process than rodents, notably in terms of biomechanical testing and fixation methods (O'Loughlin, Morr et al. 2008) which will be described in more detail below. Consequently, their anatomical shape such as their physiological and pathological analogies to humans should encourage the choice of large animal models over small animal models (Pellegrini, Seol et al. 2009).

It is not surprising that already the beginning of establishing a preclinical animal model for bone tissue engineering represents a major challenge, since even the first step, consisting of only choosing between a small and a large animal, depends on several considerations which will be described in the following chapters.

4.2.1.2 Impact of different bone structures on fracture healing

For research in bone tissue engineering it is indispensable to observe differences and analogies in the bone structure of the animal species used for the study and the human being since it may have great impact on the surgical design and the healing process. Most animals exhibit an osseous anatomy, cell biology, immunology and histology distinct from human individuals, which influences the process of fracture healing and therefore needs to be factored into the evaluation of the study outcome (Nunamaker 1998, Auer, Goodship et al. 2007).

Thereby, the bone structure of small animal models showed variations between different species and different stages of their skeletal growth (Liebschner 2004). Li et al. and Liebschner et al. critically discussed the structure of the Haversian systems in the bone cortex of rodent animal models. They reported that the Haversian-type bone structure of skeletal mature rodents showed limited remodeling, whereby rats were mentioned to show only trabecular remodeling and no intracortical remodeling. Li et al. described the complete absence of Haversian-type remodeling in rodent animals (Liebschner 2004, Li, Chen et al. 2015). Moreover, it was annotated in the survey that rabbits showed different bone marrow environment compared to humans, whereby differences in the bone macro-and microstructure need to be kept in mind when analyzing bone healing. Rabbits, even though classified as small animal models, provide long bones and a human-like lumbar spinal structure (Liebschner 2004). They were mentioned to achieve skeletal maturity shortly after sexual maturity (approximately at the age of 6 months), whereby the bone tissue comprises a high rate of vascular canals of osteons that surround the bone marrow canal and the periosteum. Thereby, a dense Haversian system occurs in between the layers (Pearce, Richards et al. 2007) resulting in intracortical bone remodeling and faster bone turnover rates compared to rodents (Castaneda, Largo et al. 2006). Differences of osseous phenotypes and other physiological variations, like the fact that rodents show significantly higher rates in their metabolic system and their capillary density (Conn 2008), indeed do affect the outcome of bone tissue engineering research constructs adversely and need to be taken into account when working with

preclinical animal models (Liebschner 2004), especially since studies with rodents make up more than three quarters of the evaluated studies from literature search and the survey. Clinical translation depends on reliable study results which eventually should mimic human conditions as closely as possible. Therefore, the large proportion of studies with rodents should be questioned. Sheep, in contrast, are considered to be an important animal for research in bone tissue engineering (Li, Chen et al. 2015) since they were mentioned to possess bone mineral compositions, bone remodeling rates, body weight and metabolic processes similar to humans (Reichert, Epari et al. 2010) and their biomechanics are well understood (Reichert, Epari et al. 2010, Li, Chen et al. 2015). They were used in 22% of the studies received with the survey (thereof only one study with unsatisfying outcome) and in 5% of the reviewed paper. However, histological differences in the bone structure of sheep were observed, showing a large amount of primary bone structure compared to the secondary, Haversian bone structure of humans (Reichert, Epari et al. 2010). In sheep, secondary remodeling occurs rather late (7-9 years), leaving a bone histology different to the one of humans by showing higher trabecular bone density and strength (Li, Chen et al. 2015).

The exact imitation of human like conditions in preclinical animal studies is hence unrealizable. However, research should pay special attention to the implementation of large animal models in order to come as close as possible to human comparison.

4.2.1.3 Impact of different animal species on signaling pathways

It was mentioned in the survey that the remodeling environment of rats would not be human-like and that working with rats could be unfavorable regarding their exuberant response to BMP-2 compared to humans. As described in chapter 1.3.4, BMPs are members of transforming growth factor-β (TGF-β) super family of growth factors and attracted high attention for their capability to promote bone regeneration (Urist 1965, Sampath and Reddi 1981, Campana, Milano et al.

2014, Kirby, White et al. 2016). Therefore, in particular BMP-2, has been intensively studied for its osteoinductive activities (Wozney 1992). Different preclinical studies in rats involving testing of BMPs were evaluated for this thesis and their outcome reflected the aforementioned capacities of BMPs. The studies demonstrated that they showed to be valuable candidates for new bone formation and bone repair (Johnson, Boerckel et al. 2011, Keibl, Fugl et al. 2011, Zhang, Tsurushima et al. 2011, Boerckel, Kolambkar et al. 2012, Foo, Reagan et al. 2013, Willett, Li et al. 2013, Priddy, Chaudhuri et al. 2014, Corre, Merceron et al. 2015). However, in line with the comment made in the survey, Osyczka et al. reported that BMPs promoted osteogenesis in MSCs of rodents, but mostly failed to effect the bone forming capacity of human MSCs when using equal dosages (Osyczka, Diefenderfer et al. 2004). In addition, working with BMPs has led to concerns when comparing the required high and cost-intensive dosage for experimental trials on bone formation compared to the concentration observed in physiological bone fusion (Valentin-Opran, Wozney et al. 2002, Hu, Wang et al. 2016). Severe side effects such as osteolysis, inflammation, systemic and local toxicity, malignant bone degeneration and bony hypertrophy occurred when working with recombinant human BMP-2 (rhBMP-2) on both animals and humans (Poynton and Lane 2002, Carragee, Hurwitz et al. 2011). This might be circumvented when working with biomaterial carriers also capable of regenerating bone using lower quantities of growth factors (Priddy, Chaudhuri et al. 2014).

The challenge when working with BMPs and preclinical animal models will hence be to determine the optimal mix and dosage in order to justify their (cost-intensive) implementation in research and to allow further standardization.

4.2.2 Animal strain

The next step for establishing a preclinical animal model, after determining its species, is to decide for one particular strain among many others (see chapter 1.5.1). More than 700 rat inbred strains and 70 rat outbred stocks can currently be found in the Rat Genome Database (RGD, https://rgd.mcw.edu, September

12, 2018). The International Mouse Strain Resource (IMSR, http://www.findmice.org, September 12, 2018) provides more than 600 mouse inbred lines and 16 outbred stocks.

Thereby, the creation of rodent inbred models was meant to produce strains that simulate specific human conditions in health and disease (Conn 2008) and to enable studies on genetic variability which are possible due to the limitless amount of genetically identical individuals (Beamer, Donahue et al. 1996). Furthermore, such genetically delineated lines were described as more uniform, more available, more reliable and more standardized compared to the genetically undefined outbred stocks (Festing 2010), which, on the other hand, were thought to show larger phenotypic variations (Jensen, Porsgaard et al. 2016). A considerable number of varieties was hence described for a lot of different animal species but predominantly in mice and rats in which a multitude of different lines exist (Conn 2008).

The evaluation of the studies from the literature search and the survey revealed that different inbred lines were used in 31% of the experiments when working with mice and rats and outbred stocks in 54%. Thereby, femoral defects were conducted more often in Sprague Dawley rats, namely in almost half of all reported femoral defects in rats, whereas Wistar rats were utilized in less than 10% when operating on the femur. This might be related to the fact that Sprague Dawley rats are considered to be an ideal surgical model and provide advantages in terms of ease of handling, high disease withstanding, little mortality after birth, early procreative capacity, low neonatal mortality and fast growth with the achievement of a considerable full-grown size (Parker, Chen et al. 2014). For bone tissue engineering, murine outbred strains, which were rarely used in the evaluated studies (or their lineage not precisely indicated), were mentioned to be an appropriate animal model for testing the level to which genetically different populations may vary when examining skeletal biomechanics (Wallace, Judex et al. 2015). However, diversity in gene functions in murine outbred lines might lead to more scattered results of the studies and inbred strains are hence needed for precise genotypic standardization.

When using the rabbit as animal model for research, the outbred New Zealand White rabbit was used in 85% of the cases. It was mentioned that these strains happened to show less aggressiveness and develop less diseases compared to other breeds (Mapara, Thomas et al. 2012). Moreover, an advantage of using outbred strains would be that they supposedly mimic more accurately human conditions (Shultz, Badowski et al. 2013) since human populations are genetically diverse. In this context, a main issue discussed in literature is the fact that a lot of studies conducted on animals generally show a lack of concordance between the outcome of animal research and its clinical application in humans (Ioannidis 2012) and that it would not be possible to apply obtained study outcome reliably on humans, also referred to as a poor extrapolation (Conn 2008). A wider adoption of outbred lines, especially when using large animal models, would hence be favorable for clinical translation.

Furthermore, the process of fracture healing was mentioned to depend on the different genotypes of the animal strains since genetic variability was mentioned to influence material properties as well as the reaction of newly formed bone to mechanical stimuli (Judex, Donahue et al. 2002, Auer, Goodship et al. 2007). Beamer et al. demonstrated that there exist considerable genetic impacts on the regulation of peak bone density among different female mice inbred strains (Beamer, Donahue et al. 1996). This has been further elaborated by Manigrasso et al., who demonstrated that femoral fracture healing in different inbred mice strains showed variations with respect to the quantity of bone and cartilage created in the fracture callus resulting in different structural and material properties of the newly formed bone (Manigrasso and O'Connor 2008). The considerable variety of different inbred and outbred strains surely offers a broad range of possible study designs but also complicates, once again, the standardization of the studies with preclinical animal models, which recurrently turns out as inconsistent in the analysis of the studies evaluated for this thesis (survey outcome and literature research). Conclusions from one study cannot be simply applied on study designs with different animal strains but should remain strain specific. Consequently, comparisons within one strain demands a considerable number of suitable study outcome and hence a higher number of

studies involving one particular animal strain. Moreover, standardization of the study designs is needed in order to allow such comparison and eventually the translational success to other studies.

Moreover, one participant of the survey commented that the immunological aspect of tissue regeneration in humans differs entirely from what we observe in animal models (Zhang, Li et al. 2015) and that we get only an idea of how bone tissue engineering will eventually work in humans. Indeed, variations of immunological aspects (Zhang, Li et al. 2015) and in the bone morphology of trabecular and cortical bone were shown to exist not only between different species, but also across and in between mice strains (Judex, Garman et al. 2004, Wallace, Judex et al. 2015). Once again, findings from research with one animal strain cannot be automatically applied on further study designs using different strains since the process of fracture healing in one study might entirely differ from the healing process observed in a similar study with other genetically different strains (Judex, Garman et al. 2004).

4.2.2.1 Impact of immune modulation on fracture healing

The comparison of study results obtained in immunodeficient strains and the outcome obtained in immunocompetent strains was stated to be crucial for the understanding of the process of bone healing which is influenced by the presence of immunological cells and inflammation (Zhang, Li et al. 2015). A high number of genetically engineered immunodeficient mice and rats is available for experimental studies involving human cells with the object of avoiding possible rejection reactions (Belizário 2009). Study results then need to be critically compared to the outcome obtained by studies using immunocompetent animals since fracture healing considerably depends on underlying immunological aspects (see chapter 1.2.3).

Generally, the motivation for the use of animals with manipulated immune status lies in the gain of further knowledge about the immune system and its role within the process of bone repair. For bone tissue engineering the use of such animals relies on the need for immunodeficient strains for testing xenogeneic constructs

containing human cells. Such strains, which were used in 21% of the evaluated studies in rodents (survey outcome and literature search), show defects in B-cells and T-cells as well as knockdown of toll-like receptors (TLR), transcription factors and genes for cytokines (Belizário 2009). Among them, nude mice and rats represent hairless rodents characterized by an insufficient immune system due to genetic mutation and the absence of a thymus which leads to a decreased inflammatory response (Hougen 1991, Belizário 2009). This may have great impact on bone tissue regeneration, considering the influence of inflammatory processes on the behavior of osteoblasts, osteoclasts, T-cells and transplanted cells which are all, inter alia, responsible for bone healing (Frohlich, Grayson et al. 2008, Mori, D'Amelio et al. 2013, Zhang, Li et al. 2015). As described in chapter 1.2.3, the process of fracture healing exhibits a sequence of well-organized phases including an inflammatory response to the injury which ensures cell organization, blood clot formation, angiogenesis, tissue granulation and finally bone remodeling. The depletion of T-cells, which represent cells of the lymphoid lineage and coordinate the adaptive immune response, thereby enhances the decrease of bone repair (Einhorn and Gerstenfeld 2015, Oryan, Monazzah et al. 2015, Baht, Vi et al. 2018). There exists hence a strict interplay between the bone healing process and the immune system which, however, still needs to be fully elucidated (Schaffer and Barbul 1998, Konnecke, Serra et al. 2014, Schmidt-Bleek, Petersen et al. 2014, Baht, Vi et al. 2018). Therefore, several studies have been published, trying to show evidence for the connection between bone fracture healing and immune cell involvement: El Khassawna et al. demonstrated that studies in mice lacking T-cells entail more rigid bone tissues which are unable to provide satisfactory quality and hence are prone to injury (El Khassawna, Serra et al. 2017). In contrast, studies in mice exhibiting a manipulated adaptive immune system by the way of decreasing the number of CD8+ T cells resulted in improved bone repair, whereas the increase of these cells adversely affected the healing process (Barbul, Breslin et al. 1989, Reinke, Geissler et al. 2013). Liu et al. showed that the use of pro-inflammatory T-cells resulted in the decrease of bone formation by enhancing bone marrow mesenchymal stem cell apoptosis through down regulation of runt-related

transcription factor 2 (RUNX2) and TNF-α caused by interferon-γ (IFN-γ). The other way round, the application of regulatory T-cells in murine cranial defects induced the decrease of IFN-γ and TNF-α which increased bone marrow mesenchymal stem cell triggered bone formation (Liu, Wang et al. 2011). Toben et al. demonstrated that the use of recombination activating gene 1 knockout (RAG1−/−) mice, exhibiting a defect which entails a lack of lymphocytes, remarkably entailed accelerated fracture repair (Toben, Schroeder et al. 2011). The impact of the adaptive immunity on fracture healing is hence rather controversially debated since studies regarding the role of T-cells happen to demonstrate both better healing after bone fracture as well as negative impact on osseous repair after injury (Park and Barbul 2004, Schlundt, Schell et al. 2015, El Khassawna, Serra et al. 2017).

Since the use of immunodeficient laboratory animal models was reported to affect the results of research for bone tissue engineering considerably and since even the understanding of bone healing mechanism in animals with healthy immune systems remains incomplete, it was suggested to treat their application with caution (Zhang, Li et al. 2015). Corre et al. proposed that, when choosing immunocompromised animal models for research, the outcome obtained due to the choice of the strain should be clearly distinguished from the outcome gathered due to the experimental conditions (Corre, Merceron et al. 2015). The increased use of immunodeficient models, which according to the analysis of the studies is the case, might have led to a higher number of studies with satisfying outcome and hence entail the success of the preclinical animal model. However, study results need to be compared to the outcome gathered by equivalent immunocompetent models.

4.2.3 Animal age

The animal’s age in a study design represents a crucial parameter to consider since the process of bone healing happens to reveal age sensitive conduct, whereby younger organisms exhibit faster and more reproducible bone healing rates during their growth phase compared to full-grown and mature organisms

(Nunamaker 1998, McGovern, Griffin et al. 2018). With relation to the age of experimental animals, skeletal and sexual maturity as well as the body weight represent important indicators that could affect study results and therefore need to be considered for research in bone tissue engineering (Pearce, Richards et al. 2007).

For every bone, stages of bone development start from narrow calcification centers to a series of modifications in form and shape in order to finally reach a skeletally adult, mature appearance (Gilbert 2000). Thereby, intramembranous ossification occurs in flat bones of the skull, the majority of the cranial bones and the medial clavicles of the human body, whereas long bones, some facial bones, lateral medial clavicles and vertebrae are formed by endochondral ossification (see chapter 1.2) (Ornitz and Marie 2002). Growth plate closure then indicates skeletal maturity and can be assessed by radiographs (Hughes and Tanner 1970). Furthermore, sexual maturity, defined for female rodents as vaginal opening and for male as balanopreputial partition (Sengupta 2013), can be considered as an important process since bone growth ceases in most mammals after achieving sexual maturity (Kilborn, Trudel et al. 2002). Jilka et al. examined the relevance of the murine model for research on bone tissue more closely and indicated that longitudinal bone growth in mice continued after sexual maturity (6-8 weeks), whereas human long bones do not continue to grow afterwards (Jilka 2013). This is an important factor to consider when working with mice for testing bone tissue engineered constructs. The evaluation of the studies from the survey outcome and the literature search revealed that the calculated mean age of preclinical mice models, which were used in 13% of the studies, amounted to only 1,9 months. Regarding the data given by Jilka et. al, longitudinal bone growth in the utilized mice might not had been finished and study results need hence to be critically looked at. Moreover, the comparison to outcome obtained from studies with older animals should be interpreted with care. Additionally, Kilborn et al. reviewed literature for time and age of growth plate closure and sexual maturity in different animal species, whereby mice were found to complete growth plate closure at the age of 5 months and findings indicated that bone growth in rats continues after achieving sexual maturity (which was listed to be reached at the

age of 1,8 to 2,1 months in Sprague Dawley rats) and physeal plates, until then, do not close compared to other species (Kilborn, Trudel et al. 2002). For rats, no reliable information for the age at growth plate closure could be gathered, but they referred to more ancient reports that mentioned 29 months to be the age for growth plate closure in tibias of rats (Strong 1925). The analysis of the studies from the literature search and the survey outcome revealed a mean age of 2,7 months for the utilized rats. Moreover, male rabbits were mentioned to show closed growth plates at the age of 6,8 months and female rabbits at the age of 5,3 months, whereas the calculated mean age of the applied rabbits in the studies described in the survey and the papers from literature search was 3,5 months. From this follows that a large number of preclinical models were examined before growth plate closure, which may contribute to an overestimation of the study outcome. As already described for the mice models, study results need to be carefully interpreted when using immature animal models since bone formation considerably depends on the growth stage of the bone. For standardization of studies with preclinical animal models, the use of animals revealing an appropriate age regarding skeletal maturity to test bone formation would be favorable and regarding the fact that small animals achieve maturity relatively fast compared to large animals, working with small animal models of suitable age is feasible.

In contrast, growth plate closure in sheep, as representative of large animal models, was described at the age of 17 months and sexual maturity was listed to be reached at the age of 5,5 months (Kilborn, Trudel et al. 2002). However, it is crucial to mention that the process of reaching sexual maturity was described to show considerable time ranges between the two genders and even between individuals of the same sex. Also, it was proposed that sexual maturity should not be equated with adulthood but rather with the commencement of an intermediate step, the adolescence (Sengupta 2013). Moreover, Malhotra et al. examined differences in bone growth of skeletally mature sheep compared to skeletally immature sheep after the implementation of defects in the femur and the tibia, whereby the age of their skeletally immature sheep was even 18 months (Malhotra, Pelletier et al. 2014). In a paper written by Reichert et al., they even

favorized the usage of 7 to 9 year old sheep since secondary remodeling rates then occur and enable the better comparison to humans (see chapter 4.2.1) (Reichert, Epari et al. 2010). The evaluation of the studies from the literature search and the survey outcome resulted in a mean age of 3,7 years, which, according to the data given by Reichert et al., would be too young as that skeletal maturity could have been achieved. Compared to small animal models, large animal models revealing an appropriate age are difficult to apply regarding increased costs for animal care and housing. However, clinical success depends on reliable and transferable study outcome and it is therefore indispensable to use preclinical animal models with sufficient age.

Furthermore, the animal's body weight correlates with its age and is therefore used for age estimation in animals, although it does not represent a precise indicator (Nafei, Danielsen et al. 2000). Fig.37 represents the changes of body weight during aging in male Wistar rats, published by Sengupta et al. (Sengupta 2013). The graph demonstrates that the growth curve clearly flattens at the age of about 4 months, indicating that stable growth then gradually comes to an end. From the age of 15 months, body weight starts to even out and does no longer change remarkably. No rat older than 7 months was used in the evaluated studies, which might be due to the increased costs when working with older animals. However, considering the flattening of the curve at the age of 4 months, it might not be necessary to wait until the age of 15 months before starting with research. Nevertheless, the mean age of Wistar rats used in the evaluated studies was 2,3 months and thus, according to graph shown in Fig.37, too young to ensure stable growth rates. The obtained data need hence be interpreted with caution.

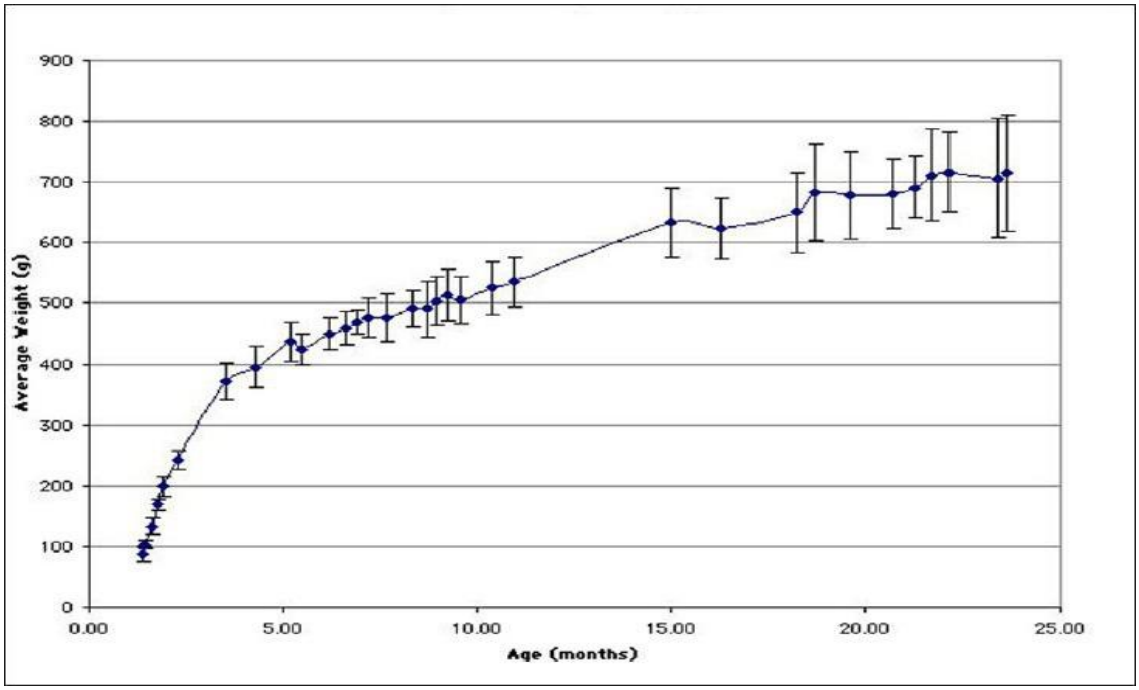

Fig. 37 Development and diversity of body weight in the lifespan of male Wistar rats. Reprint from International Journal of Preventive Medicine, 4(6),624-630; Figure 2, with permission from author and International Journal of Preventive Medicine.

Furthermore, research for literature reporting growth rates in ovine models was conducted and is outlined in Fig.38. Van Niekerk and Casey published average daily growth rates (g/day) for Merino sheep that were shown to amount to 149 g/day, whereby the rate was 107 g/day from birth to 10 kg, 189 g/day from 10 to 23 kg, 256 g/day from 23 to 32 kg and 163 g/day from 32 to 41 kg (Van Niekerk and Casey 1988). The evaluated studies from literature research indicated the use of sheep with an average body weight between 39 kg and 76 kg. According to the graph shown below, this would mean that their growth rate is not yet but already about to descend. Consequently, the used preclinical animal models would still be growing, which would hence influence the study results in a hitherto unknown extend. Once more, time and costs might play an important factor when choosing animals of lower body weight since feeding and housing of the animals are expensive co factors in research with large animals.

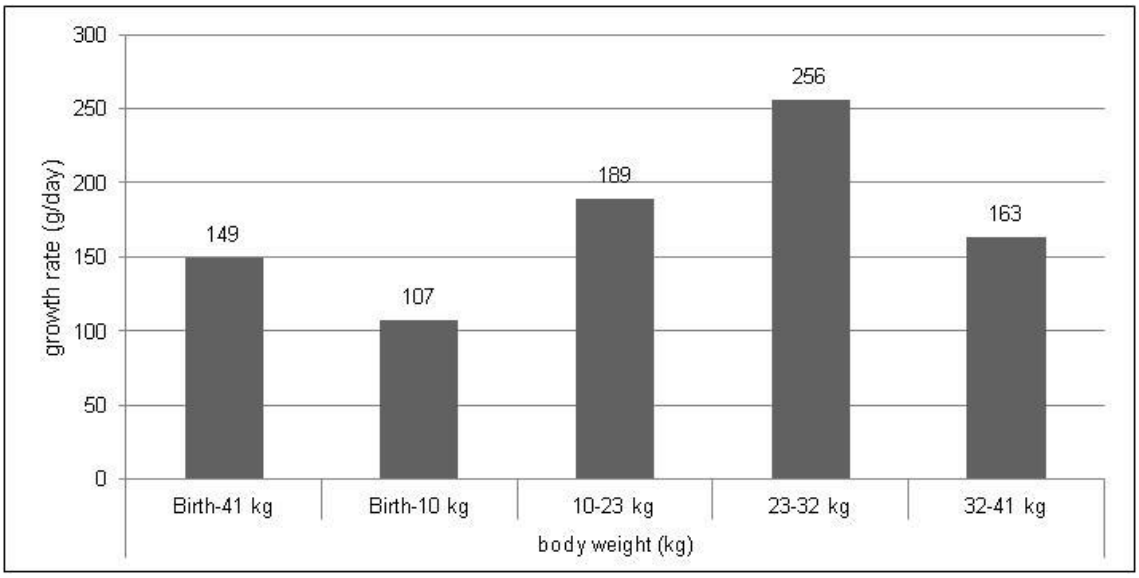

Fig. 38 Growth rate (g/day) of merino sheep from birth to 41 kg. Data obtained from Van Niekerk and Casey 1988.

4.2.3.1 Impact of age on bone biology and fracture healing

Significant differences have been reported to exist in the bone biology of young and adult animals and humans, namely in its mechanical properties, its form and function and in the molecular and cellular structure of bone (Boskey and Coleman 2010, Jackson, Andrews et al. 2017). Thereby, the age-related transformation of the bone physiology and the underlying decline of osteogenic and angiogenic ability consequently result in altered fracture healing (Meyer, Meyer et al. 2003, Lu, Hansen et al. 2008, Histing, Kuntz et al. 2013), whereby the mechanotransduction of bone in elderly individuals has been discussed to change due to modifications of hormone levels and signaling pathways resulting in delayed fracture healing and graduate loss of bone mass (Haffner-Luntzer, Liedert et al. 2015). Moreover, the aging process of the immune system of an organism leads to changes in its cellular composition and was reported to reduce the ability of bone to regenerate (Schlundt, Schell et al. 2015), whereas Xing et al. studied the enhanced healing capacity of aged mice after rejuvenation of immunological cells and thereby, once again, demonstrated the crucial role of the immune system on the process of fracture healing (see chapter 4.2.2.1). Moreover, Liebschner et al. reported that skeletal immature animals were able to

repair bone defects in a less complicated way, namely faster and more consistently compared to aged individuals (Liebschner 2004), which consequently affects the results of studies analyzing fracture healing in younger animals and thereby considerably complicates the comparison of different study designs. Another aspect that has to be considered is the fact that human individuals generally have a longer life expectancy than the animal models for research and that clinical translation therefore might be impeded since the long-term side effects of fracture healing in aged individuals cannot be evaluated adequately (Auer, Goodship et al. 2007). However, age constitutes an important factor to be observed since elderly patients make up an important target group for the concept of bone tissue engineering. In a recent paper, Jackson et al. even proposed that the disregard of the animal's age, predominantly with regard to rodents, and hence the use of inappropriately aged models could have impact on the missing translation of preclinical animal research into clinical trials (Jackson, Andrews et al. 2017). They outlined age and bone related physiological changes in rodent animals, whereby the development of the rat's brain, for instance, was described to proceed over the first 9 weeks, which has to be considered when creating cranial defects (Jackson, Andrews et al. 2017).

The rat, as rodent animal model, was examined at the age of 1,5 to 2,5 months (48%), at the age of 3 to 4 months (44%) and at the age of 6 months (8%). Sprague Dawley rats were used in 42% of all evaluated studies and their age ranged between 7 weeks and 6 months, indicating that sexual maturity has already been reached. The mean age of rats used for the creation of cranial defects was 3,1 months. According to the data given by Jackson et al., the cranial development was already completed and study designs should hence present suitable results for further standardization. As already mentioned, the age of mice, if indicated, varied mainly between the age of 1,5 months and 3,5 months. Rabbits were hardly applied under the age of 3 months (4%), but rather between the age of 3 to 5 months (57%) or 6 months up to 12 months (39%). Sheep and goats younger than 12 months were not described in the studies. They were examined starting at the age of 12 months up to 3 years (goat) and 9 years (sheep). The evaluated studies show hence a rather inconsistent choice of age

among different animal species with a relatively large number of poorly indicated time specifications. Regarding the average time of growth plate closure as it was reviewed by Kilborn et al. (Kilborn, Trudel et al. 2002), it can be concluded that primarily the evaluated studies using sheep provided animals of an appropriate maturity.

Noteworthy, when evaluating the studies of the literature research and those obtained by the survey run, data concerning the animal's age was often not indicated or vaguely defined. Thereby, the indication "adult" was summarized under the term "not defined" since it does encompass a broad range of possible ages. For instance, half of the studies using rabbits for research did not define the age of the animal such as 32% of the research work on rats and 24% of the described studies on murine models. Furthermore, the animals' age indicated in the studies often referred to the time of acquisition and not the actual study conduct. Age, however, represents an important factor when establishing a preclinical animal model for testing bone related questions and in some cases, its disregard could result in the diminishment of scientific validity and in the expansion of experimental variability (Jackson, Andrews et al. 2017). Although different opinions and age specifications about the appropriate time frame for one animal species have been reported (Kilborn, Trudel et al. 2002, Jilka 2013, Jackson, Andrews et al. 2017), it must be outlined that, in order to obtain clinical translation of preclinically tested bone tissue engineered constructs, research on animals need to more rigorously apply current knowledge on age related processes for the utilized animal strain. This is even more important as the target population for bone surgery apart from trauma surgery is mainly an elderly population that may suffer from regeneration and healing deficits like age-related osteoporosis and other age-related conditions.

4.2.4 Observation methods

The application of different assessment methods represents an integral step in scientific research which is motivated by the interest to achieve answers to the targeted question. In the context of bone tissue engineering, histological methods

help visualizing the nature of newly formed tissue and its cellularity; radiographs allow outcome measures of bone formation and bone union, whereas only mechanical tests can be applied for both functional and structural evaluation of the bone (Spicer, Kretlow et al. 2012, Martine, Holzapfel et al. 2017, McGovern, Griffin et al. 2018, Schindeler, Mills et al. 2018). Testing mechanical properties through biomechanical testing, namely compression, tension, torsion and flexion tests, thereby helps analyzing and confirming criteria like solid bone fusion, termed bone bridging and mechanical strength (Liebschner 2004) such as stress fracture resistance, ductility and elastic moduli (Muschler, Raut et al. 2010, Schindeler, Mills et al. 2018). However, two-dimensional radiographic techniques and three-dimensional imaging processes also allow assessment of bone quality regarding bridging, volume extension and bone density.

A wide range of different examination techniques have been applied in the evaluated studies in order to observe the process of bone healing, namely the arrangement, volume and structure of the newly formed bone. Observation methods thereby varied between radiology, tomography, histology, electron microscopy, molecular biology, biomechanical testing and other methods (see Tab.4). In rodent animal models (mice and rats), the combination of µ-ct, x-rays and histological observation occurred predominantly as primary outcome measure. Biomechanical tests were applied in only 32% of all studies with rodents, whereby proportionally more often on rats than on mice. This might be due to the bigger size of rats compared to mice, which facilitates the implementation of biomechanical tests. Almost the same applies to the work with rabbits, which mainly implied the performance of histological, radiological and µ-ct examinations, including biomechanical tests in only 33% of the cases, mostly when examining the radius (21%). However, as already mentioned above, only the application of biomechanical tests help visualizing functional characteristics of the newly created bone in animal models and their implementation remains crucial for the obtainment of reliable study results which might ultimately allow further steps to the still missing clinical translation of previous research.

Moreover, the further evaluation of research in large animal models revealed that almost two thirds of all evaluated studies did not ascertain mechanical properties

of their constructs, which in turn could reduce the reliability and quality of the study results and hence the possibility of their successful clinical translation. In one third of the studies obtained by the survey, researches were unsatisfied with their animal model, whereby most study designs applied radiographs and histological methods to analyze their outcome, whereas mechanical tests occurred in only one case. Researches might not have been satisfied with the models because the choice of the observation methods was not adapted to their research question and the limited use of biomechanical tests would not allow reliable comparison between their study results and the outcome of other preclinical animal models. Even though two-dimensional radiographic techniques and three-dimensional imaging processes allow the assessment of bone quality and have become the gold standard for the analysis of bone formation (Schindeler, Mills et al. 2018), biomechanical tests remain crucial when examining functional aspects. The use of µ-ct examinations might provide information about bone mineralization and allows the evaluation of aforementioned qualities of newly formed bone, which then might also reflect the quality of the mechanical properties (Muschler, Raut et al. 2010). Nevertheless, biomechanics still represent the substantial parameter to analyze the bone quality which is relevant for the appropriate mechanical integration (Muschler, Raut et al. 2010). It was furthermore proposed that assessment of mechanical properties might additionally require the implementation of histological evaluation in order to confirm bone strength in the microarchitecture of the bone (Schindeler, Mills et al. 2018).

Moreover, as described in the chapter 1.4, vascularity plays a crucial role and represents a vital parameter for the success and survival of an engineered construct (Muschler, Nakamoto et al. 2004, Laschke and Menger 2012). Blocking angiogenesis with therefore applied inhibitors was shown to lead to suppressed fracture healing and non-union like ectopic osseous formation (Hausman, Schaffler et al. 2001). Concerning this matter, it was clearly annotated in the comments of the survey that the moment a graft is put inside a human body, there would be a race between tissue regeneration and apoptotic signals influencing the final outcome of a tissue engineered bone. Indeed, a review focusing on

vascularization in the field of bone tissue engineering was published by Santos and Reis, outlining current limitations in the context of bone tissue engineering. Thereby, slow ingrowths and anastomoses of newly developed blood vessels with the surrounding circulation as well as constraint and late provision of vital molecules by diffusion was discussed to lead to cell death after implantation and eventually to the pitfall of a bone tissue engineered construct (Santos and Reis 2010). Amini et al. thereby referred to a need for matrix structures that provide cell survival of the seeded cells even before vascularization, which usually only appears within days to weeks (Amini, Xu et al. 2016). Management of post implantation angiogenesis therefore needs to be controlled using highly elaborated imaging techniques that are able to combine aspects of histological, immunohistochemical, microtomographic and microscopic examinations (e.g. perfusion weighted MRI, vessel wall labeling by promoters like Cadherin 5 or the injection of fluorescent molecules) (Lafage-Proust, Roche et al. 2015, Akar, Tatara et al. 2018). The combination and assessment of such methods would certainly augment the quality of the animal models and hence allow the obtainment of more sound results which are necessary for the clinical translation of bone tissue engineering. However, the application of methods devised with the motive to depict vascular processes was described in less than 10% of the evaluated studies. Reasons therefore might be the higher costs and the high time requirement for their implementation as well as the need for skilled scientists providing necessary knowledge in order to correctly operate the elaborated technology and to interpret its outcome.

Moreover, methods like 3D imaging, SPECT, or fluorochrome analysis with calcein were conducted rarely but evenly in large and small animals. Once again, higher costs for the implementation as well as the requirement of specific knowledge about technical procedures and the assessment of the outcome certainly limit the use of such methods. The use of fluorochrome in preclinical animal models was mentioned to be challenging and valid standardization still complicated since many different kinds exist and the application of the adequate type and its concentration demands experience and knowledge (van Gaalen, Kruyt et al. 2010). However, the use of fluorochrome for bone tissue engineering

was stated to enable the evaluation of the exact spot of osteogenesis and the onset time as well as the analysis of the newly formed bone type and its formation rate (van Gaalen, Kruyt et al. 2010). It might hence be favorable to implement the advantages of fluorochrome labeling more often in the study designs with preclinical animal models. Furthermore, the use of SPECT was brought up for its provision of high resolution images without artefacts, which also allows the examination of smaller animals such as mice (Wirrwar, Schramm et al. 2001, Lienemann, Metzger et al. 2015). In addition, it follows the guideline of reducing animal sacrifice since the in vivo monitoring of bone healing processes is possible without affecting the examined animal (Wirrwar, Schramm et al. 2001). In order to actually implement the aforementioned principle of “3Rs” and to standardize animal research, studies need to rely more often on new methods. This might be cost-intensive and complex but surely provides promising results for the clinical translation of bone tissue engineered constructs.

In conclusion, many parameters are necessary to assess the properties of newly formed bone. A wide range of techniques designed to provide quantitative and qualitative description of newly formed bone is currently available and studies on preclinical animal models should therefore focus on the implementation of research question related outcome measures and analysis. However, the evaluation of the studies described in the survey and in literature revealed that the use of a combination of clinically significant observation methods (e.g. biomechanical testing, fluorochrome labeling, SPECT, imaging techniques able to assess osseous vascularization) was often not described and that research often focused on histological and radiological observation methods. This might be one of the reasons why clinical translation of bone tissue engineering still fails to appear.

4.2.5 Observation time

Significant discrepancies in observation time were indicated in the evaluated studies for this thesis. Thereby, the final observation time of preclinical animal models reported in the survey varied from 3 to 16 weeks in small animals and

from 12 to 48 weeks in large animal models. The evaluated publications reported times ranging from 2 to 25 weeks in small animals and 5 weeks to 48 weeks in large animals. As described in the previous chapter, each scientific issue demands different observation methods as well as different observation times depending on the research question to be answered. It has been proposed that if the objective of a study was to observe long-term effects in bone healing and remodeling driven by bone tissue engineered constructs, follow-up periods should be chosen generously to allow reliable outcome (Reichert, Saifzadeh et al. 2009). If, however, the research question seeks to analyze earlier procedures of bone healing processes, observation time naturally follows a different time schedule. The targeted outcome parameter hence indicates the specific time frame for one particular observation among many. For instance, Garcia et al. defined the term bone union as the first observable signs of osseous bridging, whereby 8 weeks have been described to be necessary for bone healing in human individuals, 5 weeks in rats and 4 weeks in mice (Garcia, Histing et al. 2013). The term non-union was furthermore defined as an enduring failure of fracture healing and a healing duration exceeding 6 months for humans, 15 weeks for rats and 12 weeks for mice was mentioned to define long term failure of osseous union. However, only 8% of the evaluated studies from the survey and literature search using rats as animal model adhered to a duration of 15 weeks for observation time and only 26% of the studies using mice exceeded their study duration to 12 weeks. Regarding the applied observation time, there remains hence only a small number of studies that would be suitable to demonstrate reliable outcome for the assessment of definite fracture healing or bone remodeling rates, which demand even longer observation times. However, if the purpose of a study was to monitor bone bridging in rodent animal models, it was proposed that it should be assessed within the first visible signs of osseous union. Observation time points between first and complete bone continuity would be harder to set reasonably and would not provide any more significant results for defining the already ongoing process of bone union (Garcia, Histing et al. 2013). Therefore, Garcia et al. proposed observation time points at 2 weeks and 1, 2 and 3 months and in rats at 2, 5, 10 and 15 weeks (Garcia, Histing et al.

2013). However, with respect to the fact that the animal's strain and age influence the temporal course of fracture healing (see chapter 4.2.2 and 4.2.3), the observation time should be adapted. Moreover, the use of different fixation methods and surgical designs may also influence the "normal" healing period and sequence (see chapter 1.5.2). One of the evaluated studies aimed to examine early vascularization and bone repair in a critical-sized defect under the effect of MSCs and endothelial progenitor cells (EPCs) and therefore applied adequate observation methods (vWF staining) in the appropriate time frames, i.e. 1, 4 and 8 weeks (Seebach, Henrich et al. 2010). The histological assessment of early vascularization and the beginning of bone formation was performed after 1 and 4 weeks, whereby only bone formation was assessed at 8 weeks using histology, µ-ct and mechanical tests.

It would be favorable if study designs applied appropriate periods of observation times based on the purpose of their research, even if this entails a high time requirement in the case of long-term studies and consequently more costs.

4.2.6 Implantation site

Whereas the use of ectopic animal models reaches its limits for evaluating bone regeneration, orthotopic models enable more appropriate assessment of ongoing processes during osseous healing (Black, Goriainov et al. 2015). Both literature research and survey evaluation revealed that femur, tibia, radius and cranium represent commonly used anatomical sites for receiving bone tissue engineered constructs (see Fig.21C and Fig.32). Whether a defect is tested in the femur, the tibia, the radius, the humerus, the ulna or the maxillofacial zone and the cranium, thereby mostly depends on what kind of tissue is targeted with the tissue engineered construct under examination. Studies need to credibly represent animal bone defects that are comparable to clinically significant scenarios of human bone injury (Pearce, Richards et al. 2007, Black, Goriainov et al. 2015). Moreover, quite apart from finding the potentially ideal defect location, the intactness or fraction of the periosteum and therein presented MSCs constitutes an important surgical aspect when creating an osseous injury since cellular and

molecular processes in fracture healing considerably depend on periosteal participation and its angiogenic contribution (Zhang, Xie et al. 2005, Colnot 2009, Chang and Knothe Tate 2012, Lin, Fateh et al. 2014, Neagu, Tiglis et al. 2016). Thereby, the provision of an intact periosteum after injury might not always be granted in clinical cases and studies with preclinical animal models involving defects with ruptured periosteum might therefore represent clinical conditions more closely. However, only 40% of the evaluated studies precisely indicated the condition of the periosteum when creating osseous defects of which 15% described its preservation during surgical operation. It would be favorable if more studies indicated whether the created defects healed with or without the help of an intact periosteum in order to reliably compare study results and hence allow better clinical application.

Another aspect to keep in mind when creating a defect, before choosing its precise implantation site, would be the distinction between the different bone formation types. As described in chapter 1.2.2, bone formation of the cranium occurs mainly through intramembranous ossification and underlying cell differences due to distinct embryonic tissue origin (neural crest and mesoderm) have been described to influence the fracture healing of the cranial bone (Quarto, Wan et al. 2010). This is an important aspect to consider if conducted research is focused on finding strategies for endochondral bone formation (Spicer, Kretlow et al. 2012). Thereby, cranial bones origin from both neural crest derived cells (frontal bone) and mesoderm derived cells (parietal bone) and it has been demonstrated that the former evince higher osteogenic rates and healing capacities (Quarto, Wan et al. 2010). Indeed, no unsatisfying outcome was described for cranial defects when evaluating the outcome of the survey, which might affirm an increased healing potential in defects created in the cranium of animal models. Moreover, osteoinductive cells of the facial bones descend from the neural crest and form bones through intramembranous ossification, whereas long bones are formed by osteoblasts that originate from the mesoderm and develop bone by endochondral formation (Couly, Coltey et al. 1993, Reichert, Gohlke et al. 2013). Even though both bone types have been shown to exhibit the same osseous structure, the functional and molecular differences between

the osteoblasts were hypothesized to influence the process of fracture healing (Reichert, Gohlke et al. 2013). Therefore, Aghaloo et al. examined differences between bone marrow stromal cells of the mandibular and the tibia of rats regarding the time needed for bone regeneration after injury, whereby findings indicated that the osteogenic performance of the mandible cells preponderated (Aghaloo, Chaichanasakul et al. 2010). Similar findings were described by Reichert et al. who described the enhanced osteogenic potential of ovine mandible osteoblasts compared to tibial counterparts (Reichert, Gohlke et al. 2013). Again, researchers that participated in the survey did not describe study outcomes as unsatisfactory when classifying animal models with mandibular defects, whereas two studies indicating tibial defects in the goat and the mouse were mentioned in this category. However, the tibia was mentioned to represent the most common localization for bone defects, which was related to its poor stabilization by only few surrounding muscle coverage (Reichert, Saifzadeh et al. 2009). Moreover, distal femoral defects in rabbits were suggested to be an important implantation site for recreating bone defects since they represent a commonly affected area after bone tumors or total knee replacements (Li, Chen et al. 2015).

The evaluation of the studies (survey and literature) revealed that femur and tibia, classically exposed to high weight-bearing loads, represented the most commonly used implantation sites (71%) for testing the macrostructures of the newly formed bone and its behavior under realistic biomechanical conditions. A large number of the studies seek hence to imitate clinical conditions more realistically by choosing commonly affected defect sites, which is important for accelerating the necessary steps towards clinical translation. Thereby, femoral defects occurred recurrently in both small (42%) and large (32%) animal models, whereby long bones in large animals were discussed to provide more reliable information about osseous rigidity and load- bearing aspects under biomechanical conditions than long bones in small animal models (Pearce, Richards et al. 2007, O'Loughlin, Morr et al. 2008). Defects in the tibia were rather reported in large animals (39%) and results might hence emerge more informative regarding their clinical translation. The animal model designs detailed

in the surveys additionally indicated when study outcome was not satisfying revealing that 8 out of 12 unsatisfying results occurred when choosing the femur of small animals. Again, the missing clinical references in small animal models might be a reason why animal models turned out unsatisfactory.

Moreover, the bone structure and size of the radius was mentioned to provide convenience regarding the operation of segmental defects and their histological and radiographic observation (An and Freidman 1998). Additionally, due to the stabilizing effect of the adjacent ulna, no supplementary fixation device is needed (An and Freidman 1998). The same internal fixation effect applies to the ulna, leaving additional fixation devices unnecessary (Horner, Kirkham et al. 2010). It is therefore not surprising that a large majority of all defects created in rabbits (85%) were described in the radius as forearm bone, whereas the ulna as defect model was tested less often. This might also be due to its comparatively lower rounded anatomical shape that was considered as less favorable for surgical operation as well as histological, radiographic and biomechanical observation (An and Freidman 1998).

If, however, the focus of a study did not involve the imitation of load-bearing conditions as observed in humans but predominantly structural properties, Liebschner et al. discussed smaller animals as more favorable regarding their quicker healing rates, larger amounts of data collection and lower cost expenditures (Liebschner 2004). Defects in the cranium of small animals thereby represented a popular model to test engineered constructs (McGovern, Griffin et al. 2018). The evaluation of both survey and literature outcome revealed that surgical procedures in the cranium were mainly conducted in rats (62%), whereas only one cranial defect was described in a large animal model (Jensen, Tvedesoe et al. 2016). Such defects provide advantages regarding the cranial bone structure similar to a plate, which was proposed to simplify the approach to uniform defects and to allow reproducibility and standardization without the need for stabilization (An and Freidman 1998). Moreover, the comparison of the outcome provided by study designs testing cranial defects in animal models is possible since there exists already a large amount of them (An and Freidman 1998). Additionally, the anatomical location was mentioned to simplify access for

surgical interventions and observation methods and the surrounding Dura mater stated to be capable of providing nutritional supply for the implant (Gomes and Fernandes 2011, McGovern, Griffin et al. 2018) and hence to have an important influence on the outcome of osseous reconstruction in cranial defects (Cooper, Mooney et al. 2010). However, it is questionable whether the provision of an intact Dura mater after injury can be assumed. Research driven by the incentive to imitate clinical conditions in order to improve clinical translation should hence carefully weigh up the decision of leaving the Dura mater intact or not. Thereby, 36% of the studies describing cranial defects and evaluated for the thesis indicated the protection of the Dura mater during the surgical procedure. It would, however, be interesting to compare study results describing defects with and without an intact Dura mater and eventually necessary in order to allow standardization of the defect designs and ultimately clinical application of the results.

Moreover, even though rats represent a popular model for the creation of cranial defects (62% of all evaluated studies describing defects in the cranium), their fast bone healing rates were discussed to pose major issues (An and Freidman 1998) when used for scientific purpose in the context of bone tissue engineering. Study results could hence be hard to apply for clinical translation. Furthermore, surgery can be technically challenging regarding the small size of the animals and complicated if research demands special surgical finesse like the aforementioned maintenance of an intact Dura mater, if intended (Cooper, Mooney et al. 2010).

As mentioned earlier in this chapter, studies that seek to create reliable designs for bone tissue engineered constructs, of which the outcome might soon-to-be applicable for the treatment of comparable human bone defects, are crucial and imply the combination of results obtained from different animal species as well as different anatomical sites.

4.2.7 Defect dimension

Another parameter that shows broad range of data is the defect size. The evaluation of all study designs revealed that most of the authors and participants

of the survey indicated a defect size for their model but did not specify the explicit defect dimension (length, width, depth, height). Such information often failed to occur in the description or showed significant variations. Especially the description of defects created in the mandible or the maxilla showed extreme heterogeneity which complicated the provision of a well-structured summary of the alveolar defects and, most importantly, impedes the standardization of the defect design in general.

As described in chapter 1.3.1 and 1.5.2, critical-size defects represent an important fracture model to test bone tissue engineered constructs since they cannot heal spontaneously and hence depend on interventions that affect the bone healing process. However, the definition of a critical-size defect across various anatomical locations, animal species, animal strains and different age groups is difficult to determine and to standardize and therefore hard to apply. Fig.39 indicates the wide size range of defects, which were referred to as critical-size defects, in the different implantation sites of rats. Segmental defects in long bones thereby varied from 1 mm to 10 mm. Drill holes tested in rats and described as critical ranged between 5 mm and 8 mm, yet 4 mm drill hole defects described as non-critically sized were also reported. Similarly, segmental defects in rabbits ranged from 5 mm to 20 mm, all described as critical. Drill hole defects in rabbits showing the size of 6 mm were reported in papers and described as both critically sized and non-critically sized. Segmental femoral defects created in sheep, which were likewise described as critical, varied from 20 mm to 30 mm, whereas 8 mm large femoral defects and 8 mm normal healing femoral defects were also described by participants of the survey. This huge variation underlines the fact that there exists no exact definition for the creation of a critical-size defect. Two defects of the same size might likewise occur as critical in one study and as non-critical in another study. Each study applies different defect designs, which complicates reliable comparison between the study results and hence does not allow any standardization for the preclinical animal models and even less the clinical translation of their outcome.

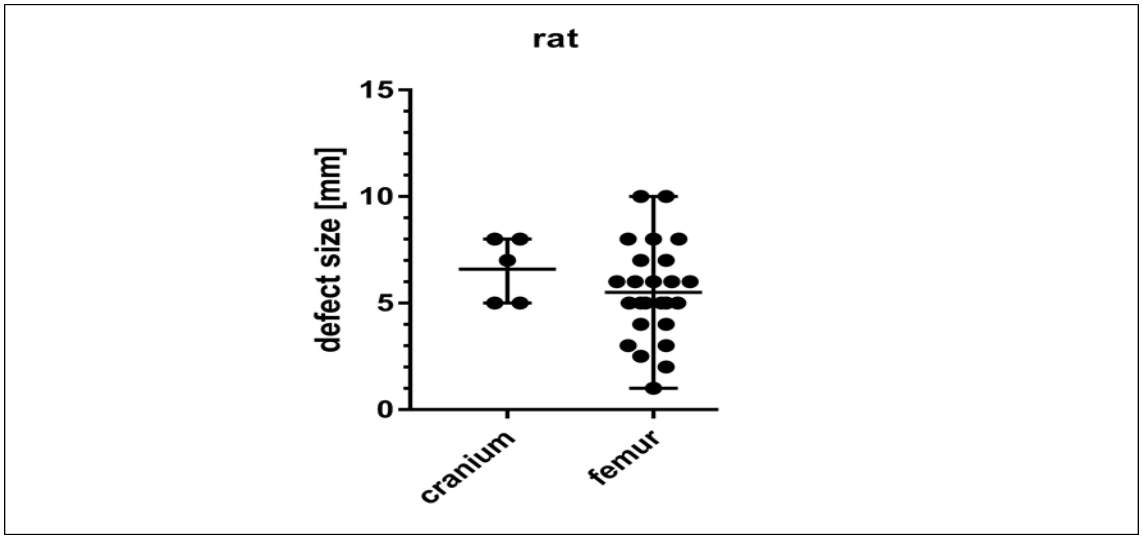

Fig. 39 Critical size defects in rats. The graph shows the range of defect sizes (mm) for critical size defects for different implantation sites in rats that have been reported in the reviewed papers and in the survey. The indicated sizes in the diagram are the diameter size for drill hole defects created in cranium and the distance between the two bone fragments for segmental defects in the femur. A wide range of sizes, varying mainly between 5 mm and 8 mm for cranial defects and between 1 mm and 10 mm for femoral defects, was applied and therefore complicates the standardization of critically sized defects.

However, there have been attempts of determining precise indications for the size of such defects. For instance, a circular defect with a diameter of 8 mm was considered to represent a critical-size defects in the cranium of Sprague Dawley rats (An and Freidman 1998, Spicer, Kretlow et al. 2012), whereas 15 mm were mentioned to be necessary for cranial critical defects in adult New Zealand White rabbits (An and Freidman 1998). However, no cranial defect of 8 mm was described in the evaluated studies when working with Sprague Dawley rats and only one study occurred indicating a 15 mm critical-size defect in the cranium of New Zealand White rabbits with an average age of 9 months (Kim, Sharma et al. 2012). Once again, it might be the vast definition for critical-size defects that renders study designs inconsistent and impedes the drawing of conclusions from one study to another.

Long bone defects were widely defined as critical when exceeding two times the diameter of the bone in question resulting in a necessary defect size of at least 12 mm or even 15 mm in the radius of New Zealand White rabbits (An and Freidman 1998). Such defects occurred in 86% of all evaluated radial defects in rabbits of which even 92% showed the size of 15 mm or more. Such study

designs would hence rather fit into the framework of detailed regulation. On the other side, as already mentioned, it was proposed that critically sized defects should be hardly defined by exact size indications, but rather to be circumscribed as defects that do not heal spontaneously (which underlines the need for empty defects as described in chapter 4.2.9) or have less than 10% regeneration during the lifetime of an animal (Schmitz and Hollinger 1986). A rather abstract definition that was mentioned to complicate the standardization for defect sizes in preclinical animal models but also to avoid the risk of incorrectly defining only subcritical defect as critical and hence necessary since a non-critical-size would heal by itself, without intervention (Spicer, Kretlow et al. 2012). It is thereby important to note that almost all study results in rodent animal models, which researchers from the survey mentioned to have turned out as unsatisfactory, revealed a defect size of only 5 mm or smaller. This could lead to the conclusion that defining an exact defect size as guideline for research would after all limit the number of studies resulting in unsatisfactory outcome.

Critical-size defects might furthermore lead to non-unions which reflect the long-term failure of osseous healing and exhibit the appearance of scar tissue in between the fracture endings (see chapter 1.2.3) (Garcia, Histing et al. 2013). Clearly, non-unions and delayed healing processes constitute a large clinical and economic burden for causing significant loss of function and life quality, not forgetting the serious pain caused by such defects (Victoria, Petrisor et al. 2009). Preclinical study models representing non-unions are hence important in order to recreate clinical conditions more closely and to investigate possibilities to cure such defects. However, non-union models were reported in less than 10% of all evaluated studies (survey and literature), whereby mice and rats were predominantly used. Thereby, segmental defects of only 2-3 mm in mice and 5-8 mm in rats were described as non-unions, which might not be big enough for examining the behavior of non-union defects adequately. Additionally, one of the participants in the survey commentated that there is still a major need for better non-union models which are difficult to treat and lead to ischemic bone wounds. Indeed, whereas the performance of critical-sized defects has been widely investigated over the last years, the step towards non-union model studies was

reported to increase only slowly (Garcia, Histing et al. 2013). The establishment of such models might be challenging in small animal models since their bone structure was mentioned to possess increased bone healing potential (Manigrasso and O'Connor 2004) and therefore complicates the standardization of non-union models for research. Moreover, high-resolution imaging methods are needed in order to allow adequate assessment of the ongoing processes of tissue formation in non-unions as well as well-defined time points for such observation.

In the end, the exact definition and hence standardization for both critical-size defects and non-unions in preclinical animal models is hard to determine and therefore complicates scientific research on the models. Studies do not follow any guidelines since they do not exist and therefore reveal various defect designs which hardly allow comparison to each other.

4.2.8 Fixation methods

Different fixation methods have found their way into surgical operation techniques allowing adequate stabilization of the created defects in preclinical animal models (see chapter 1.5). Thereby, fixation can be obtained by different osteosyntheses techniques including external fixation, internal plates or intramedullary nails and wires.

Papers describing segmental defects in load-bearing sites reported the use of different fixation devices. Cranial defects, drill holes and defects at implantation sites that are sufficiently stabilized by adjacent bones do not need any supplementary fixation device and are therefore not discussed in this chapter. External fixators, reported in 19% of all cases, were discussed as easily applicable and hardly disruptive for the soft tissue environment, whereas pin loosening and pin associated infections as well as extended healing periods were mentioned to have detrimental impact on this temporary fixation method (Reichert, Epari et al. 2010). Moreover, the external manipulation of the device and self-inflicted injuries are hard to control when operated on animal models (Drosse, Volkmer et al. 2008). The mentioned drawbacks might explain the low

frequency of external devices in the evaluated studies, whereby studies using external fixators in human individuals have likewise reported complications such as pin loosening, inadequate bone alignment and osseous union as well as the risk of soft tissue necrosis and osteomyelitis (Green 1983, Milenkovic, Mitkovic et al. 2018). Intramedullary nails, although commonly chosen for stabilization in human bodies (Schneider, Michel et al. 2001), were associated with disadvantages considering the diminishment of blood circulation due to drilling and nailing in animal models, leading to temperature-associated osseous necrosis and therefore, albeit reversible, resulting in delayed healing processes (Reichert, Epari et al. 2010). On the other hand, Histing et al. reported positive outcome regarding axial and rotational rigidity of intramedullary nails that have been applied to stabilize femoral defects in mice (Histing, Menger et al. 2016). The implementation of such fixation devices, however, was reported in less than 20% of the evaluated studies, whereas the application of internal plates was described in more than 52%. However, almost half of the studies from the survey that reported unsatisfactory outcome applied plates as internal fixation method. Even though plates were mentioned to ensure sufficient fixation, their application involves disadvantages regarding false alignment, decreased vascularity, and osseous damage due to augmented pressure caused by the plate (Reichert, Epari et al. 2010). The emergence of plate-associated complications stands in contrast to the still relatively high number of evaluated studies reporting to apply such devices, whereby such disadvantages might lead to the persisting limitations of preclinical animal models and lower the translational success of the study outcomes. However, the last-named drawback could be avoided when using new plate systems which have been discussed to allow the circumvention of direct contact between bone and plate (Reichert, Epari et al. 2010). Moreover, the use of compliant plates allowing moderate movements in between the bone fragments and hence the transmission of only axial loadings to the newly formed bone has been reported to enhance bone repair compared to stiff plates (Boerckel, Kolambkar et al. 2012).

Regardless of the disadvantages, it is important to have in mind that fixation methods ensure stabilization and therefore minimize the risk of hypertrophic non-

unions characterized by endochondral ossification and the development of a callus which results in the formation of non-mineralized cartilage at the fracture site (Garcia, Histing et al. 2013). Leaving defects without any fixation or applying fixation devices that cannot ensure axial and rotational stabilization in segmental defects (single pins, intramedullary pins and rods) can lead to unmanageable biomechanical situation that do not represent standardized defect designs (Thompson, Miclau et al. 2002).

4.2.9 Empty defects

Concurrent control groups have already been mentioned to represent a crucial part of a valid experimental set-up. Further steps towards clinical translation can only take place if study designs allow the comparison of the created defect with defects of the same size and shape but left empty or filled with autologous bone. Drosse et al. underlined that the understanding of osseous reconstruction in connection with bone tissue engineering demands the observation of critical-size defects (Drosse, Volkmer et al. 2008), whereby problems concerning the creation of a critical-size defect following precise definitions have already been discussed in this thesis (see chapter 4.2.7). Some defect designs referred to as critical may heal without any intervention at all. Therefore, it is necessary to provide an empty defect that confirms the created defect as critical since healing in the control group did not take place (Liebschner 2004). Moreover, the involvement of additional defects containing autologous bone would be favorable when testing bone tissue engineered constructs in preclinical animal models. Autologous bone still represents today`s gold standard in the clinics and the direct comparison of defects containing tissue engineered constructs to defects filled with autologous graft would show their different effects on bone healing best.

Out of all evaluated studies from literature research (the question of whether studies included an empty defect or a control group with autologous bone was not part the survey), 55% described the usage of empty defects, whereas less than 10% of the studies described the implementation of a defect filled with autologous bone. Thereby, murine models were not part of the evaluation of

studies containing autologous fillings as control group since the animals were considered too small to allow the creation of second injury sites for the bone harvesting. The results reported in the studies showed that most of the empty defects consistently developed only small amounts of fibrous tissue formation and hence did not show complete bone bridging. The outcome demonstrates that most of the studies examining critical-size defects in preclinical animal models imitated clinically relevant injuries relating to the fact that the created defects would not heal when left empty. The study results can therefore be considered as potentially relevant regarding their implementation for clinical translation. However, there remains a large number of studies that did not involve the creation of a control group in their preclinical animal model, which, as a result, lowers their informative value for clinical translation.

4.3 Scientific and clinical perspectives

The evaluation of the survey and the literature search demonstrated that intense research is being conducted with the aim of finding solutions that would fill the still existing lack of suitable preclinical animal models for bone tissue engineering and of which the outcome would allow further steps towards clinical translation. Thereby, the survey data revealed accordance and differences given by both scientists and surgeons concerning their opinion in the assessment of bone tissue engineering and the therefore conducted research with preclinical animal models, whereby the latter group would, if clinical translation takes place, ultimately take advantage of the novel constructs.

Regarding the assessment of the preclinical animal models currently used to test bone tissue engineered constructs, the evaluation of the surveys generally revealed a rather optimistic attitude and models were mostly considered as relevant (see Fig.28). Moreover, most of the participants considered them to be well developed, although not yet clinically useful and still reliant on further optimization (see Fig.27). Thereby, clinicians showed slightly more optimism concerning the models compared to scientists. This might be due to their closer relation to clinical cases and hence their experience with present treatments of

bone injuries. It is thereby important to keep in mind that clinical translation depends on both theory and practice and therefore clinicians represent an interesting target group. They might contemplate clinical translation in a more balanced way concerning practical application and aspects of pure theory, applied theory and the interpretation of study results. Conversely, especially scientists with more professional experience, who are possibly holding a more theoretic point of view, considered the models as poor (12%), dependent on optimization (47%) and the outcome of the studies less often as well translated in the clinics (35%) (see Fig.20). As already mentioned, the idea of bone tissue engineering exists since the early 90s, which represents a large amount of time and could explain the more pessimistic assessment of scientists with higher experience level and hence more experience concerning the failure of the study results in terms of clinical application.

Even though some few surgeons queried in the survey envisaged the outcome of preclinical studies as already transferable into the clinics (see Fig.18), it has to be emphasized that, as described in the previous chapters, the need for better standardization of the study design as well as the disregard or lack of knowledge concerning factors that affect bone healing (e.g. animal species, strain, age, surgical design, vascularization etc.) represent essential limiting factors. It is therefore not surprising that the implementation of advanced therapy medicinal products (ATMPs), which represent genetic, cell or tissue level based medical treatment solutions for human diseases, is confined to only few market licenses in Europe (Ten Ham, Hoekman et al. 2018, Yu, Gupta et al. 2018).

Regarding the assessment of bone tissue engineering, there remains a considerable number of surgeons (38%) who demonstrated a rather skeptical attitude towards novel tissue engineered constructs and would apply them on only few or even none of their cases (see Fig.12), predominantly when treating non-unions. Aspects concerning further costs and supplementary time necessary to investigate patient specific needs could be one of the reasons why some surgeons might hesitate to use new tissue engineered strategies in the clinics. Also, new designs, even if already clinically accepted by the FDA, generally demand a notable amount of time before long term success can be identified.

Understandably, if during their professional career surgeons have made positive experiences with currently applied clinical strategies for bone augmentation, they might not be willing to take risks when learning the implementation of novel strategies. The evaluation showed that most of the participating surgeons who use autologous bone as bone graft material for the repair of non-unions would not agree to take such risks since the so-called gold standard has provided satisfactory outcome over the years. Nevertheless, as described in chapter 1.3.2, even the gold standard for bone augmentation reveals severe negative side effects that need to be considered when used in the clinics.

Moreover, despite the fact that one surgeon indicated that bone tissue engineering would always be a domain of academic institutions and not for daily practice, both surgeons and scientists preponderantly marked that clinical application of bone tissue engineered constructs would take place one day and that research on bone tissue engineering is important. Therefore, novel methods are needed in order to surmount current limitations of preclinical animal models in bone tissue engineering such as the lack of standardization which hinders their way from academic institution into clinical practice. Indeed, when asking about the time needed for clinical application, opinions on temporal classifications differed slightly. The majority of the participants indicated that future implementation in the clinics could be realistic in the next ten years (see Fig.29). However, scientists showed slightly more confidence for the nearer future, namely five years, although their assessment of preclinical animal models turned out less enthusiastic regarding the progress of the models and the transferability of the study results into the clinics. There is hence a slightly contradictory view and given the fact that researchers still pore over elementary issues of current bone tissue engineered constructs, in particular the maintenance of sufficient oxygen and nutrient supply (Laschke and Menger 2012) as well as the regulation of approved and standardized procedures for the entire study design (Frohlich, Grayson et al. 2008, Reichert, Saifzadeh et al. 2009, Schindeler, Mills et al. 2018), it will be interesting to see if the clinical breakthrough could be plausible in only five years. For sure, novel strategies continue to arise, trying to solve aforementioned problems, particularly the missing standardization of research

with preclinical animal models and the resulting lack of study results suitable for clinical translation.

4.4 Conclusion

Even though the participants of the survey assessed the currently available preclinical animal models to test bone tissue engineered constructs as well developed and reproducible (see Fig.27), the further need for optimization in order to achieve clinical translation was emphasized. The studies described in the survey and the papers from literature search clearly reveal that the establishment of a preclinical animal model demands the attention to a wide range of interdependent considerations starting with the choice of the animal species, the animal strain, its age and gender. Apart from this, choosing the right defect location, surgical design as well as the adequate fixation method and whether the creation of control groups should take place, requires theoretical and practical reflections. Moreover, the implemented observation methods and the time spend to observe bone healing make important contributions to the interpretation of the study results.

The evaluation of the preclinical animal models described in the survey and the studies from literature search thereby revealed a rather inconsistent choice of study designs, whereby the number of studies involving small animals was significantly higher than the one with large animal models. Information regarding the animal's age and the created defect dimensions was often not or vaguely indicated. Current knowledge about adequate age groups and defect sizes, which would allow reliable interpretation of the study results, was often not applied or ignored. Consequently, the used animals were often too young and the created defects too small to enable the provision of clinical relevant scenarios. In addition, studies recurrently undercut the necessary time for observing bone healing properly and adequate observation methods were not always applied. Moreover, studies including preclinical animal models often lack the necessary standardization as for considering their outcome relevant for clinical translation.

Naturally, the heeding of established guidelines indicating the appropriate animal model, its age, adequate defect sizes, fixation methods and surgical designs

would be necessary when working with preclinical animal models in the context of bone tissue engineering. However, it is important to stress that currently no common guideline, which offers clear indications for adequate study designs, exists and that the compliance with such generally valid guideline for the design of preclinical animal models has hence so far faced difficulties in implementation. This probably represents one of the main reasons why, until now, the establishment of reproducible and translatable animal models, which would each allow answers to targeted research questions and hence suitable outcome for clinical translation, has failed to be demonstrated. Respecting simultaneously all factors that affect the study outcome of preclinical animal models presents an almost impossible thing to do and even though no fully satisfactory model allowing the introduction into clinical trials currently exists, well-versed researchers from all over the world constantly establish novel models with new approaches (see chapter 4.5) in order to finally reach the primary objective of clinical translation.

However, even though promising results have been reported, the large discrepancy between research efforts and the very limited translational success stands out. This thesis shows that no consensus and no standardization on the use of preclinical animal models for bone tissue engineering currently exists resulting in a lack of well-defined, reproducible and accepted preclinical models. There is a major need for better defined and optimized preclinical models in order to improve translational aspects of the models.

4.5 Future perspectives

The beginning of bone tissue engineering dates back over 30 years and until today its clinical application has failed to be demonstrated. The previous chapters provided information about current study designs including preclinical animal models and their limitations regarding further steps into the clinics. Thereby, limitations in the establishment of well standardized research when working with preclinical animal models was detected, resulting in an inconsistence of the study

results, which hinders the clinical translation of bone tissue engineered constructs.

This thesis clearly exposed the critical impact of the experimental study design (chosen age of the preclinical animals, the breed or strain, the defect size and localization as well as fixation methods) on the process of bone healing and eventually the translational success of study results. However, the evaluation of the survey and the literature revealed that there still exists a large number of studies that do not respect appropriate criteria for preclinical animal models or show a lack of reporting. Consequently, the study outcome becomes difficult to interpret and to compare and the study design unreproducible. Moreover, the missing clinical translation of current preclinical models is a crucial concern since it questions the actual use of such animal models for the envisaged future clinical application in humans. In this respect, it would be necessary to weigh up the further implementation of preclinical animal models that fail to enable clinical translation. Furthermore, it would be favorable to monitor complete integrity and validity of stated preclinical information by preclinical experts.

Moreover, the evaluation of the survey revealed that scientists seem to be in general more satisfied with large animal models than with small animal models. The lower number of studies in large animals could be explained by the higher expenses and demanding housing, but also shows a potential reason for the low number of studies with translational success. A higher number of studies involving large animal models would hence be favorable since they allow a closer comparison to human like conditions.

Despite the fact that the majority of the survey participants considered a clinical availability of bone tissue engineered constructs as conceivable within the next 10 years, the major need for optimization of preclinical animal models was underlined. Novel, future-orientated strategies need hence to arise, whereby it was mentioned in the comments that personalized three-dimensional bioprinting with incorporated bone represents an interesting research field regarding large sized bone defects. It describes a novel manufacturing technology allowing the fabrication of well-defined constructs nearby identical to biological tissues and able to function as scaffolds on which cells can adhere and grow for the following

transplantation into defects (Derby 2012, Rose and De Laporte 2018). This in turn will require suitable preclinical animal models as well as further understanding of biological procedures in order to allow standardization. Clinical translation could therefore be difficult to reach in the near future.

For now, clinical translation of bone tissue engineered constructs has not taken place and could actually remain a domain of academic institutions if research does not start to establish valid study protocols that serve as binding guidelines. The standardization of study designs at every level is indispensable in order to prevent unnecessary mistakes at the expense of animals, redundant costs, effort and time. Moreover, further clarification on cell communication and interaction would be necessary in order to correctly apply the novel constructs and to surmount ongoing complications such as adequate tissue vascularization (Stevens 2008, Schindeler, Mills et al. 2018). Therefore, additional preclinical in vivo studies are required, starting in smaller animals which are necessary to assess the proof of concept and proceeding to larger animals which allow further biomechanical evaluation of an implant in more human like conditions (Salgado, Coutinho et al. 2004) and finally, maybe one day, reaching clinical studies, which would improve the quality of life for many patients in today's aging population. Moreover, financial support is required in order to promote further knowledge and navigate the transparency of the studies. For the future, it would hence be favorable to focus on both clinical and scientific considerations, whereby the importance of intensive cross-disciplinary collaboration between scientists at the bench and surgeons at the bedside needs to be emphasized again.

5 Summary

Autologous bone still represents today's gold standard for the treatment of critical size bone defects and fracture non-unions despite associated disadvantages regarding limitations in availability, donor site morbidity, costs and efficacy. Bone tissue engineered constructs would present a promising alternative to currently available treatments. However, research on preclinical animal studies still fails to provide clinical applicable results able to allow the replacement of currently applied methods. It seems that the idea of bone tissue engineering, which has now been integral part of academic studies for over 30 years, got somehow stuck at an intermediate level, in between intense preclinical research and striven stages of initial clinical trial phases. A clear discrepancy exists between the number of studies with preclinical animal models for bone tissue engineering and the number of clinically approved bone tissue engineered constructs available to patients.

The aim of this thesis was hence to evaluate preclinical animal models for bone tissue engineering as well as the perception of scientists and clinicians towards these models. Moreover, the general role of bone tissue engineering and its clinical need assessed by scientists and surgeons was investigated. A survey was conducted questioning both scientific and clinical opinions on currently available study designs and researchers' satisfaction with preclinical animal models. Additionally, a literature research was conducted, resulting in 167 papers from the last 10 years that report current designs of preclinical orthotopic animal studies in bone tissue engineering. Thereby, the focus lied on the description of the models regarding animal species, strain, age, gender and defect design. The outcome of the literature search was evaluated and compared to the outcome obtained from the survey.

The survey data revealed that both scientists and surgeons generally remain positive about the future role of bone tissue engineering and its step to clinical translation, at least in the distant future, where it then might replace the current gold standard, autologous bone. Moreover, most of the participants considered preclinical animal models as relevant and well developed but the results as not yet realizable in the clinics. Surgeons thereby demonstrated a slightly more

optimistic perception of currently conducted research with animal models compared to scientists. However, a rather inconsistent description of present preclinical study designs could be discerned when evaluating the reported study designs in the survey and the papers of the literature search.

Indeed, defining an appropriate animal species, strain, age, gender, observation time, observation method and surgical design often depends on different indications and research questions and represents a highly challenging task for the establishment of a preclinical animal model. The existing lack of valid guidelines for preclinical testing of bone tissue engineering leads hence to a lack of well standardized preclinical animal models. Moreover, still existing knowledge gaps regarding aspects that affect the process of fracture healing, such as vascularization or immunological aspects, were found to hinder clinical translation of bone tissue engineered constructs.

Using literature review and survey, this thesis points out critical issues that need to be addressed to allow clinical translation of bone tissue engineered constructs. It can be concluded that currently existing study designs with preclinical animal models cannot live up to the claim of providing suitable results for clinical implementation. The here presented comprehensive summary of currently used preclinical animal models for bone tissue engineering reveals a missing consensus on the usage of models such as an apparent lack of reporting and standardization regarding the study designs described in both papers from the literature review and the survey. It thereby indicates a crucial need to improve preclinical animal models in order to allow clinical translation. Despite the fact that participants of the survey generally revealed a positive perception towards the use of bone tissue engineered constructs and affirmed the clinical need for such novel designs, the missing standardization constitutes a main weak point for the provision of reliable study outcome and the translational success of the models. The optimization of reproducibility and reliability, as well as the further understanding of ongoing mechanisms in bone healing in order to develop effective tissue engineered constructs, need to form the basis of all study designs. The study outcomes might then fulfill the requirements of maybe today's and hopefully tomorrow's aging population.

6 Zusammenfassung

Über die letzten 30 Jahre hat die Rolle von Bone Tissue Engineering vielversprechenden Fortschritt gemacht und immer neue Ansätze werden etabliert. Somit stellt Bone Tissue Engineering eine aussichtsvolle Alternative zu dem heutigen Goldstandard (autogene Knochenersatzmaterialien) dar, nachdem diese häufig mit Nachteilen einhergehen: limitierte Verfügbarkeit, Morbidität durch Zweiteingriffe, ungenügend Stabilität und Kosten. Die klinische Umsetzung findet jedoch nicht so schnell statt, wie ursprünglich erhofft und es scheint, als würde die vorklinische Forschung auf der Stelle treten. Das Ausbleiben von reproduzierbaren und standardisierten vorklinischen Studien verhindert dabei eine "bench to bedside" Translation.

Ziel dieser Doktorarbeit war es, derzeitige präklinische Tiermodelle für Bone Tissue Engineering zu evaluieren und dabei zu untersuchen, woran es liegen könnte, dass die Lücke zwischen vorklinischen Studienergebnissen und klinischer Umsetzung noch immer existiert. Es wurde ein Fragebogen erstellt, anhand dessen die generelle Meinung gegenüber Bone Tissue Engineering und die Effizienz derzeitiger präklinischer Studienmodelle aus sowohl klinischer, als auch wissenschaftlicher Sicht hinterfragt wurde. Hier wurde außerdem auf die Beurteilung der Zufriedenstellung solcher Modelle seitens der Forscher eingegangen.

Darüber hinaus erfolgte eine systemische Literatursuche auf der Online-Plattform “Pubmed” mit dem Ziel Studien der letzten zehn Jahre über präklinische orthotopische Tiermodelle in Bone Tissue Engineering zusammenzufassen und die verschiedenen Studiendesigns zu evaluieren. Der Fokus lag dabei auf der Beschreibung der Tiermodelle bezüglich Tierart, Geschlecht, Alter und Defektdesign. Ergebnisse der Literatursuche wurden anschließend evaluiert und mit den Antworten aus dem Fragebogen verglichen und diskutiert.

Es hat sich anhand des Fragebogens gezeigt, dass sowohl Wissenschaftler, als auch Chirurgen positiv gestimmt sind, was die zukünftige Anwendung von Bone Tissue Engineering in den Kliniken betrifft. Jedoch beurteilten die meisten Teilnehmer des Fragebogens die präklinischen Tiermodelle zwar als relevant und gut entwickelt, deren Ergebnisse als klinisch allerdings nicht anwendbar. Dabei

fiel die Einschätzung präklinischer Forschung mit Tiermodellen unter den Chirurgen etwas optimistischer aus als unter den Forschern. Die Evaluierung der Studien aus dem Fragebogens und der Literatursuche zeigte jedoch auch, dass die darin beschriebenen Tiermodelle einen eher uneinheitlichen Studienaufbau aufweisen. Tatsächlich stellt die Etablierung eines fundierten Studiendesigns im Anbetracht der zahlreichen Möglichkeiten eine immense Herausforderung dar. Die Festlegung eines Versuchsaufbaus hängt dabei von der Wahl der Tierart, dessen Geschlecht und Alter, des chirurgischen Ablaufs, sowie der technischen und zeitlichen Beobachtungsmöglichkeit ab. Es stellte sich heraus, dass für viele Studien eine diesbezüglich notwendige Standardisierung kaum existiert und dadurch Studienergebnisse entstehen, die schwer reproduzierbar sind und somit den Ansprüchen einer klinischen Umsetzung nicht gerecht werden können. Hinzu kommen außerdem die noch immer bestehenden Wissenslücken in Bezug auf Knochenheilung beeinflussende Faktoren wie Vaskularisation und Abläufe des Immunsystems.

Abschließend lässt sich sagen, dass die durchgeführte Evaluierung von Studien mit präklinischen Tiermodellen eine fehlende Standardisierung derzeit existierender Studiendesigns darlegt und eine klinische Umsetzung der daraus resultierenden Studienergebnissen somit noch nicht möglich ist. Auch wenn die Teilnehmer des Fragebogens den Bedarf an neuen, klinisch anerkannten Methoden für Knochenaufbauten nahelegten und eine generell positive Einstellung gegenüber dem potentiellen Gebrauch von Bone Tissue Engineering Konstrukte in den Kliniken zeigten, ist die Ablösung von autologem Knochen durch solch neuartige Designs nicht realisierbar, solange die Reproduzierbarkeit der Daten aus präklinischen Tiermodellstudien fehlt. Zusammen mit wegweisenden Richtlinien und fundiertem Wissen über grundliegende Mechanismen im Knochenheilungsprozess, sollte sie die Basis eines jeden Studienaufbaus mit präklinischen Tiermodellen darstellen, um schließlich zu den Ergebnissen zu gelangen, die es für eine klinische Umsetzung von Bone Tissue Engineering bedarf.

7 References

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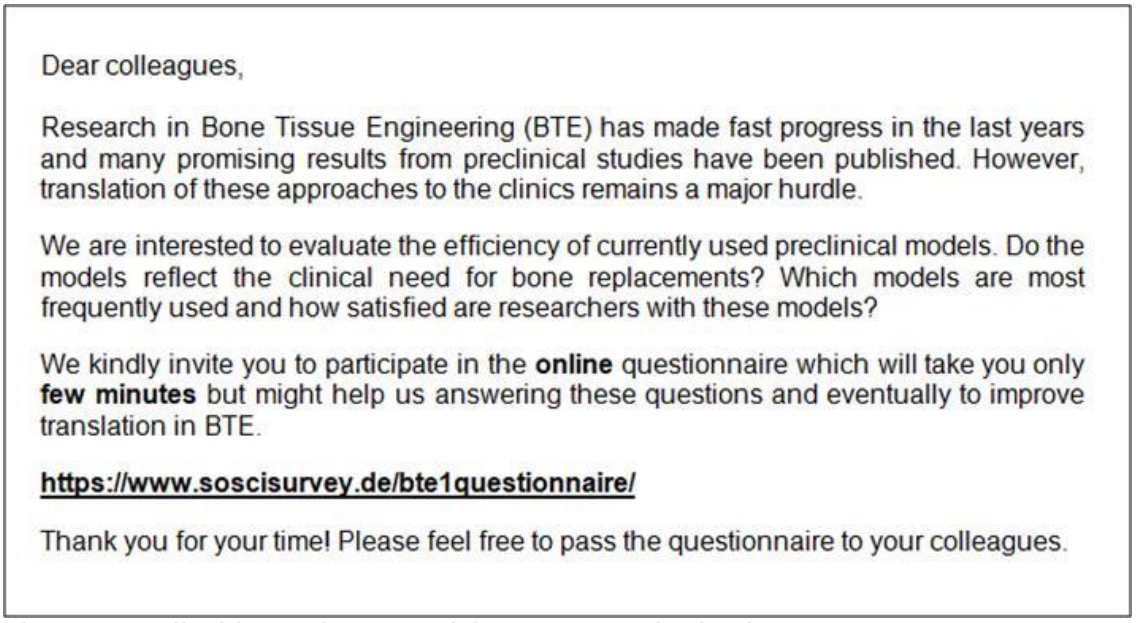
List of abbreviations

ATLA	Alternatives to Laboratory Animals
ATMP	Advanced Therapy Medicinal Products
BMP	Bone Morphogenetic Protein
EPC	Endothelial Progenitor Cell
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
FMT	Fluorescence Molecular Tomography
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon-γ
IGF-1	Insulin-like Growth Factor-1
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-11	Interleukin-11
IL-18	Interleukin-18
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
nu	nude
PDGF	Platelet-Derived Growth Factor
rhBMP-2	recombinant human Bone Morphogenetic Protein
RUNX2	runt-related transcription factor 2 (RUNX2)
scid	severe combined immunodeficiency
SPECT	Single Photon Emission Computed Tomography
TGF-β	Transforming Growth Factor beta
TLR	Toll-Like Receptor
TNF-α	Tumor Necrosis Factor alpha
VEGF	Vascular Endothelial Growth Factor
µ-ct	mico-computed tomography

List of figures

List of tables

Supplement



Dear colleagues,

Research in Bone Tissue Engineering (BTE) has made fast progress in the last years and many promising results from preclinical studies have been published. However, translation of these approaches to the clinics remains a major hurdle.

We are interested to evaluate the efficiency of currently used preclinical models. Do the models reflect the clinical need for bone replacements? Which models are most frequently used and how satisfied are researchers with these models?

We kindly invite you to participate in the **online** questionnaire which will take you only **few minutes** but might help us answering these questions and eventually to improve translation in BTE.

https://www.soscisurvey.de/bte1questionnaire/

Thank you for your time! Please feel free to pass the questionnaire to your colleagues.

Fig-S. 1 E-mail addressed to potential surgeons and scientists.

Tab-S. 1 Variables and response codes for the interpretation of the survey data provided by soscisurvey.com

VAR	LABEL	TYPE	INPUT	QUESTION	RESPONSE
CASE	Interview number (ongoing)	METRIC	SYSTEM		
TE01	1: Residual option (negative) or number of selected options	NOMINAL	SYSTEM	What is your professional background?	
TE01_01	1: Trauma surgery	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_02	1: Orthopedic surgery	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_03	1:Craniomaxillofacial surgery	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_04	1: Veterinary surgery	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_05	1: Scientist, trained	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_05a	1: Scientist, trained in (free text)	TEXT	OPEN		1: not checked 2: checked
TE01_06	1: Representative	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_07	1: Other	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE01_07a	1: Other (free text)	TEXT	OPEN		
TE02	2	NOMINAL	SELECTION	Experience:	1: 0 - 5 2: 6 – 10 3: 11 - 20 4: >20 -9: no answer
TE03	3	NOMINAL	SELECTION	I hold a PhD	1: yes 2: no -9: no answer
TE04	4	NOMINAL	SELECTION	How many cases do you treat by applying bone grafting per year?	1: none 2: 1-10 3: 10-50 4: >50 -9: no answer
TE05	5	NOMINAL	SELECTION	What kind of bone graft do you use most frequently?	1: autologous 2: allogeneic 3:substitute 4. cement 5. BMP 6. other -9: no answer
TE05_06	5: other	TEXT	OPEN		
TE06	6	NOMINAL	SELECTION	What is the most common indication where you apply bone graft?	
TE06_01	6: [No Description] 01	TEXT	OPEN		
TE07	7	NOMINAL	SELECTION	How many of those would you treat with a bone tissue engineered construct if available?	1: all 2: most 3: few 4: none -9: no answer
TE08	8	NOMINAL	SELECTION	If applicable, what fixation method do you use most frequently for these cases?	
TE08_01	8: [No Description] 01	TEXT	OPEN		
TE09_01	9: [No Description] 01	TEXT	OPEN		
TE10	10	NOMINAL	SELECTION	What indication do you target with your research?	
TE10_01	10: [No Description] 01	TEXT	OPEN		
TE11	11	NOMINAL	SELECTION	Do you think research on bone tissue engineering is important?	1: yes 2: no -9: no answer
TE11_01	11: yes Why?	TEXT	OPEN	why	

VAR	LABEL	TYPE	INPUT	QUESTION	RESPONSE
TE12	12	NOMINAL	SELECTION	Do you think bone tissue engineering constructs will ever become clinically available?	1: yes 2: no -9: no answer
TE13	13	NOMINAL	SELECTION	If yes, how long will it take?	1: 5 2: 10 3: 20 4: >20 -9: no answer
TE14	14: Residual option (negative) or number of selected options	NOMINAL	SYSTEM	What is your feeling about the preclinical models, which are currently used to test bone tissue engineered constructs?	
TE14_01	14: The models are well developed, reproducible and results translate well in the clinic	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE14_02	14: The models are well developed and reproducible but do not translate in the clinic	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE14_03	14: The models need optimization	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE14_04	14: The models are poor	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE14_05	14: I don´t have experience with preclinical models.	DICHOTOMOUS	CHECKBOX		1: not checked 2: checked
TE15_01	15a: species	TEXT	OPEN		
TE15_02	15a: age	TEXT	OPEN		
TE15_03	15a: gender	TEXT	OPEN		
TE15_04	15a: strain	TEXT	OPEN		
TE15_05	15a:observation	TEXT	OPEN		
TE15_06	15a: analysis methods	TEXT	OPEN		
TE15_07	15a: implantation site	TEXT	OPEN		
TE15_08	15a: specify site	TEXT	OPEN		
TE15_09	15a: defect	TEXT	OPEN		
TE15_10	15a: defect size	TEXT	OPEN		
TE15_11	15a: what model	TEXT	OPEN		
TE15_12	15a: fixation	TEXT	OPEN		
TE15_13	15a: satisfaction	TEXT	OPEN		
TE15_14	15a: relevance	TEXT	OPEN		
TE16_01	15b: species	TEXT	OPEN		
TE16_02	15b: age	TEXT	OPEN		
TE16_03	15b: gender	TEXT	OPEN		
TE16_04	15b: strain	TEXT	OPEN		
TE16_05	15b: observation	TEXT	OPEN		
TE16_06	15b: analysis methods	TEXT	OPEN		
TE16_07	15b: implantation site	TEXT	OPEN		
TE16_08	15b: specify site	TEXT	OPEN		
TE16_09	15b: defect	TEXT	OPEN		
TE16_10	15b: defect size	TEXT	OPEN		
TE16_11	15b: what model	TEXT	OPEN		
TE16_12	15b: fixation	TEXT	OPEN		
TE16_13	15b: satisfaction	TEXT	OPEN		
TE16_14	15b: relevance	TEXT	OPEN		
TE18_01	16: [01]	TEXT	OPEN	Comments:	

Tab-S. 2 Obtained data of the survey outcome. The table shows one by one the obtained data of each completed questionnaire, whereby the ongoing interview number begins with 156 after excluding questionnaires from the pretest and unfulfilled questionnaires**.**

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 156	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 158	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 160	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2		1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	-9		-9	-9	0	1	1	1	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 165		1	1	1	2	1		1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 169	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	Biology	1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 171	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	engineering	1	1		1	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	1	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	1	1	2	1	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 172	2	1	1	1	2	2	engineering	1	1		1	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	1	6	Hap βTCP	-9		3	-9		1	large bone defects	1	implants
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	1	2	1	1	2	2	1	rabbit	
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
			8	histology		tibia	drill hole	2-3 mm	normal healing model	none	mostly satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	rat				4	histology		femur	segmental	4 mm	normal healing	none
	TE16_13	TE16_14	TE18_01									
	not satisfied											

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 173	1	1	1	1	1	2	medicine, biology	1	1		4	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	non union fractures, cancer bone ressection	1	non union fractures
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	1	1	1	2	1	1	1	rat	10
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male	wistar	04. Dez	microtomography, histology	orthotopic	femur	segmental defect, drill hole	5mm segmental, 3mm hole	critical size model, normal healing model	internal plate	mostly satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

case 174	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	biology	1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	non-union defects, osteonecrosis of femoral head, critical size defect	1	non-union defects, osteonecrosis of femoral head, critical size defect
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	we need rapid solutions to be used in a clinical setting	1	2	1	2	1	1	1	1	sheep	2-7 years
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	female	Ripollesa-Lacauna	12	histology, Uct, Rx, vital bone markers	orthotopic	femoral head	drill hole	8mm diameter 15mm depth	a critical size model	none	very	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	sheep	2-7 years	female	Ripollesa-Lacauna	12	histology, Uct, Rx, vital bone markers	orthotopic	femoral head	segmental	30mm	a critical size model	internal plate
	TE16_13	TE16_14	TE18_01									
	not	yes										
case 175	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	-9		-9	-9	0	1	1	1	1	1	rat	adult
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male	Sprague-Dawley	4-10 weeks	uct, rx, histology, vital bone markers	orthotopic		segmental	5mm	critical size model	intramedullary nail	mostly	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 176	1	1	1	1	1	2	biology	1	1		2	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		1	peripheral nerve injury
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	1	1	1	1	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 177	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	1	1	1	1	2	medicine	2	1		2	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	broken hollow bones with gaps too big to heal properly by itself, open skullcap after fatal injury	1	I'm not a bone researcher. My focus is on cartilage repair in knee and IVD
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	1	1	1	1	1	2		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
			in my opinion, and I'm not a bone researcher, personalized 3D bioprinting with incoorporated bone (allo-or auto-)graft (aspirate or cells)is an interesting research field regarding large sized bonedefects									

case 178	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	medicine	1	1		1	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	2	1		1	trauma with bone loss	3	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	2	1	2	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 179	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	1		1	2	PhD student	1	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	trauma, infection, congenital disease	1	critical size defects, osteomyelitis, congenital pseudarthrosis of tibia
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	a number of clinical conditions result in poor bone formation and it has to be addressed with bone tissue engineering	1	1	1	1	1	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	goat	6 months	male female	Capra hircus	4 months	histology, radiographs	orthotopic	tibia	segmental	20mm	critical size	internal plate
	TE16_13	TE16_14	TE18_01									
	not satisfied	yes	the immunological aspect of tissue regeneration in vivo is entirely different from what we observe in animal models. We are able to get only an idea of how it will work but the moment the graft is put inside a human body, vascularity plays a crucial role. I believe there is race between tissue regeneration and apoptotic signals that influences the final outcome of a tissue engineered bone									

case 180

TE01	TE01_01	TE01_02	TE01_03	TE01_04		TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	1	1		1		1	2	senior research fellow	2	2
TE04	TE05	TE05_06	TE06	TE06_01		TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9			-9	-9		1	congenital pseudarthrosis tibia(CPT), Trauma, infection, cancer	1	CPT, infection
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02		TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	untreated bone defects results disability, Establishing better tissue engineered bone is much required for CPT, Trauma cases	1	1	1	2	1		1	1	1	sheep	6 months
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09		TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
		12 months	histology, x ray	orthotopic	tibia	segmental		30 mm	critical size model	external fixator	mostly satisfied	yes
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07		TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01										

case 181

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	1	1	2	biology	1	1		4	2
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9		-9	-9		-9		1	critical size defect of the mandibular
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	the available implants are not regenerating the defect	1	2	1	1	1	2	1	1	sheep	6 months
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
male		12 months	histology, CT, molecular		mandibular	segmental		critical size model	none	mostly satisfied	yes
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

case 182	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	1	1	1	2	2	biology, medicine	1	1		4	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	large bone defects, non-unions	1	large bone defects, non-unions
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	1	3	1	2	2	2	1	rat	mid-aged
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male and female	varied	8-12 weeks	histology, micro CT, mechanics, gene expressions	orthotopic	cranium, femur	segmental defect	critical	critical size model	internal plate	mostly satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	mouse	variable	male and female	variable	20 days	histology, microCT	orthotopic		drill hole	8mm	normal healing model	none
	TE16_13	TE16_14	TE18_01									
	not satisfied	no										
case 183	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	biology	1	1		4	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		1	large segmental defects
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	it offers the most promising approach to regenerating bone	1	1	1	1	2	1	1	1	rat	4 months
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male	Fischer	8 weeks	x-ray, micro CT, histology, mechanical testing	orthotopic	femur diaphysis	segmental defect	5mm	critical size model	internal plate	mostly satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
			we work with rats for all the obvious resaons, but question their clinical relevance because they respond so exuberantly to BMP-2. Humans don't. Also, it's hard to identify new and better osteogenetic agents when BMP2 is so hard to beat as a positive control									

case 184	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	medicine	1	1		3	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1	large bone defects	1	large bone defects
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	there are still many types of bone defects which do not heal sufficiently with conventional approaches	1	4	1	1	2	1	1	1	mouse	8-12 weeks
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male and female	CD1, C57BL/6	6 weeks	histology, immunohistochemistry, biomechanics, CT	orthotopic	femur	segmental defect	2-3mm	non-union model	intramedullary nail	mostly satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 185	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	2	1	1	1	1		1	1		3	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	tibial head fracture, bone defects	3	1	plate fixation	-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	1	1	2	1	1	1	sheep	6-7 years
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	male	merino	1 year	micro CT, biomechanics, histology, x-ray,	orthotopic	tibia	segmental defect	30 mm	critical size model	internal plate	very satisfied	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

case 189	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	1	2	1	1	2	Bioengineering and Stem Cell Biology	1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 191	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	1	2	1	1	2	Bioengineering, polymer chemistry, stem cell biology	1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 192	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	1	2	bioengineering, polymer chemistry, stem cell biology	1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	1	-9		-9		-9	-9		-9		-9	

case 192													
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	need new solutions to progress field, meet patient demands, and improve clinical outcomes	1	2	1	1	1	2	1	1	mouse	mostly "adult" @ 10-16 weeks
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
both	wild type (C57Bl6) or genetic mutant/reporters	4 weeks standard, some out to 8 weeks	mostly stereology/histomorphometry, qRT-PCR, immunohistochemistry, some microCT and x-ray	ectopic	tibia and femur defects	segmental defect, fracture, and drill hole (depends on questions)	critical to simple fracture	all. NO current model for hypertrophic non-union -- > major NEED	all	mostly satisfied	it is useful in answering important clinical questions, it is not always important that it replicates the clinical situation
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

case 198											
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	1	1	2	Biomechanics	1	1		4	1
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
1	-9		-9		-9	-9		-9		1	non-union
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

case 200											
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
0	1	1	1	1	1		1	1		-9	-9
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9		-9	-9		-9		-9	
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
-9		-9	-9	0	1	1	1	1	1		
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14

	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 201	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	-9		-9	-9	0	1	1	1	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 203	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	-9		-9	-9	0	1	1	1	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 207	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	3	1	2	1	2	2	biomedical engineering	1	1		3	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02

case 207	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
	TE16_13	TE16_14	TE18_01													
case 212	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
	1	1	1	1	1	1		1	2	MD specialized in General Surgery and Orthopedic and Traumatology working in a research Laboratory	4	2				
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
	-9	-9		-9		-9	-9		1	bone defects due to trauma, nonunions, bone surgical resection because of tumor, osteomyelitis, osteonecrosis; spinal fusion.	1	Bone defects of trabecular and diaphisea cortical bone; nonunions osteochondral defects (subchondral epiphyseal bone), spinal fusion in healthy and osteoporotic animals				
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02				
	1	to regenerate bone regeneration in large defects or in the presence of patient pathological local and general conditions affecting spontaneous bone regenerative capabilities	1	4	1	1	1	2	1	1	sheep	144-192				
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
	F	Outbred	24	Xray, macroscopy, microtomography, histology, histomorphometry, biomechanics	orthotopic	metatarsus	segmental	2.5 cm - 3 cm	non-union	internal plate	mostly satisfied	yes				

case 212

TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
rat	12	M	Sprague Dawlay	4	Xrays, histology, histomorphometry	orthotopic	femur	segmental defect	5 mm	non-union	external fixator, intramedullary nail

TE16_13	TE16_14	TE18_01
not satisfied	no	There is the need of preclinical in vivo model standardization to reduce/refine animal use; also post-operative management of animals during bone regeneration is to be targeted and standardized and

case 213

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
2	2	2	1	1	1		1	1		2	1

TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
3	2		1	lumbar fusion	2	1	pedicle screw and rods	1		1	

TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	looking at lumbar fusions, we need to minimize the risk for pseudarthrosis. Therefore, we need a reliable biologically enhanced tissue engineered bone graft for patients at risk to develop pseudarthrosis.	1	2	1	1	1	2	1	1		

TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14

TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12

TE16_13	TE16_14	TE18_01

case 214

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	2	1	1	1		1	1		2	1

TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
3	2		-9		2	1		-9		-9	

TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1		2	-9	1	1	2	1	1	1		

TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14

TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12

	TE16_13	TE16_14	TE18_01									
case 215	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		2	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	4	1		1	Spondylodesis	2	1	Pedicure screws	-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	Biological approach	1	2	1	1	2	1	1	1	rat	
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
					Orthotopic	Tibia	Segmental	5mm	Critical bone defect model	Internal plate	Mostly	No
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 216	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		2	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	Cervical spine	-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 217	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02

	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 218	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		3	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	2		1	Revision hip arthroplasty	2	-9		1	Revision hip arthroplasty	-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	1	1	1	1	1	2		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 219	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	3	2	2	1	1	2	Molecular Biology	1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	4	1		1	critical bone defects	2	1	press fit	-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2	1	1	1	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 220	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	2	1	1	1	1		1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	2	1		1	Pseudarthrosis	3	-9		1	Pseudarthrose	1	

case 220	**TE11**	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**
	1		1	2	1	1	2	1	1	1		
	TE15_03	**TE15_04**	**TE15_05**	**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**
	TE16_01	**TE16_02**	**TE16_03**	**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**
	TE16_13	**TE16_14**	**TE18_01**									
case 223	**TE01**	**TE01_01**	**TE01_02**	**TE01_03**	**TE01_04**	**TE01_05**	**TE01_05a**	**TE01_06**	**TE01_07**	**TE01_07a**	**TE02**	**TE03**
	1	1	1	1	1	2	Engineering	1	1		3	2
	TE04	**TE05**	**TE05_06**	**TE06**	**TE06_01**	**TE07**	**TE08**	**TE08_01**	**TE09**	**TE09_01**	**TE10**	**TE10_01**
	TE11	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**
	TE15_03	**TE15_04**	**TE15_05**	**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**
	TE16_01	**TE16_02**	**TE16_03**	**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**
	TE16_13	**TE16_14**	**TE18_01**									
case 224	**TE01**	**TE01_01**	**TE01_02**	**TE01_03**	**TE01_04**	**TE01_05**	**TE01_05a**	**TE01_06**	**TE01_07**	**TE01_07a**	**TE02**	**TE03**
	1	1	1	1	1	2	bioengineering	1	1		3	1
	TE04	**TE05**	**TE05_06**	**TE06**	**TE06_01**	**TE07**	**TE08**	**TE08_01**	**TE09**	**TE09_01**	**TE10**	**TE10_01**
	-9	-9		-9		-9	-9		1	large defects, recalcitrant wounds	1	ischemic and non-healing bone fractures and wounds
	TE11	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**
	1	there are some rare but important indications where more advanced, cell-based approaches will have value	1	3	2	1	1	2	2	1	mouse	10
	TE15_03	**TE15_04**	**TE15_05**	**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**
	M and F		12	microCT, histology	orthotopic	calvarium	punch hole	4 mm diameter	critical size	none	not satisfied	no
	TE16_01	**TE16_02**	**TE16_03**	**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**

	TE16_13	TE16_14	TE18_01												
			need better models of non-unions, difficult-to-treat, and ischemic bone wounds												
case 229	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
	1	2	1	1	1	1		1	1		1	2			
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
	4	1		1	Tibiaplateau, pseudarthroses, humeral head,	2	1	Plate + screws	-9		-9				
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
	1	Entnahmemorbidität senken	1	2	1	1	1	1	1	2	Rabbit	24 weeks			
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
	Female														
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
	TE16_13	TE16_14	TE18_01												
case 230	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
	1	1	1	1	1	2	BME Surgery DVM	1	1		3	1			
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
	TE16_13	TE16_14	TE18_01												
case 231	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
	1	1	1	1	1	1		1	2	PhD student in Orthopaedic Mechanobiology	1	2			
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
	1	-9		-9		-9	-9		-9		-9				
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			

	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 232	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	1	1	1	1		1	2		1	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		1		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06		TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04		TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 233	TE01	TE01_01	TE01_02	TE01_03		TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	2	1	1		1	1		1	1		1	2
	TE04	TE05	TE05_06	TE06		TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	2		1		traumatic bone defect	3	-9		-9		-9	
	TE11	TE11_01	TE12	TE13		TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1		1	2		1	1	1	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06		TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04		TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01										

case 236

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	2	1	1	1	1		1	1		2	1
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
2	1		1	trauma	2	-9		-9		-9	
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1		1	2	0	1	1	1	1	1		
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

case 237

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	1	1	2	Biology	1	1		4	1
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9		-9	-9		1	large bone defects due to trauma, tumor, infection	1	large bone defects
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	Might lead to acceleration of bone healing, helps to spare autologous bone material, comparatively easy to perform compared to TE of other tissues	1	2	2	2	1	2	1	1	rat	08. Okt
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
male	SD, nude rats	1, 8		orthopic	femur	segmental defect	5 mm, 10 mm	a critical size model	internal plate	mostly satisfied	yes
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									
	We use only the critical size defect model of the rat, hence we do not have a 'least satisfying model'										

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05 a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 238	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	-9		-9	-9	0	1	1	1	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 243	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 245	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	nonunion and bone defects	3	1	plate fixation	1		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	autologous bone is limited, avoid donor site morbidity	1	2	1	1	1	1	1	2		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

case 246																			
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03								
2	2	2	1	1	1		1	1		2	1								
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01								
3	4		1	Cysts	3	1	Impaction	-9		-9									
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02								
1	To handle with big defects	1	1	1	1	1	2	1	1										
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14								
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12								
TE16_13	TE16_14	TE18_01																	

case 248																			
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03								
2	2	2	1	1	1		1	1		3	1								
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01								
3	1		1	non-unions	3	1	plate osteosynthesis, nailing	1	non unions, deformity correction	1	tissue engineering								
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02								
1	avoid donor site morbidity, enhance healing capabilityby "intelligent" bone grafts	1	3	2	1	2	2	1	1										
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14								
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12								
TE16_13	TE16_14	TE18_01																	
		I do large animal models for cartiage TE, but not bone.																	

case 251														
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
1	1	1	1	1	1		1	2	Plastic and Reconstructive Surgery	3	2			
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
2	1		1	Bone defect reconstruction (hand, leg, maxilla)	2	1	titanium screws and plates	1	implant associated bone infections, avascular bone necrosis	1	avascular bone necrosis			
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
1	limited availability and donor site morbidity of autologous grafts	1	2	2	2	1	2	1	1	rabbit				
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
TE16_13	TE16_14	TE18_01												
		see Tarek Ismail et al. Engineered, axially-vascularized osteogenic grafts from human adipose-derived cells to treat avascular necrosis of bone in a rat model. Acta Biomateriala 63 (2017) 236-245												

case 253											
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	2	1	1		1	1		4	1
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
4	3		-9		-9	-9		-9		-9	
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

case 254

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
2	2	2	1	1	1		1	1		1	2
TE04	**TE05**	**TE05_06**	**TE06**	**TE06_01**	**TE07**	**TE08**	**TE08_01**	**TE09**	**TE09_01**	**TE10**	**TE10_01**
2	1		1	Pseudarthrosis	4	-9		-9		-9	
TE11	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**
1	To develop novel treatment strategies	1	2	1	1	2	1	1	1	Mouse	6 weeks
TE15_03	**TE15_04**	**TE15_05**	**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**
Female	Balb/c	12 weeks		Intra-cardiac		Bone metastases				Satisfied	We don't really know yet
TE16_01	**TE16_02**	**TE16_03**	**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**
TE16_13	**TE16_14**	**TE18_01**									

case 255

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	2	1	1	1		1	1		1	2
TE04	**TE05**	**TE05_06**	**TE06**	**TE06_01**	**TE07**	**TE08**	**TE08_01**	**TE09**	**TE09_01**	**TE10**	**TE10_01**
4	1		1	Fill cages / stimulus for arthrodesis in spine	1	1	Pedicle screw + - cages	-9		-9	
TE11	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**
1	Improve on bone substitutes properties	1	1	1	1	2	1	1	1		
TE15_03	**TE15_04**	**TE15_05**		**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**
TE16_01	**TE16_02**	**TE16_03**		**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**
TE16_13	**TE16_14**	**TE18_01**									
		Never worked with bone tissue constructs animal model directly									

case 256														
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
3	2	2	1	1	2	Biomedical Enginering	1	1		4	2			
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
3	6	allograft bone or TCP combined with aspirated bone marrow.	1	Non-Union - Tibia and clavicle	1	1	IM rod for tibia. Plate for clavicle	1	Fracture, Non-Union, Bone Defect in acetabulum or femur or tibia	1	Bone and cartilage regeneration, Stem Cell Biology			
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
1	Bone defects in the long bones and in the pelvis (acetabulum) remain an unsolved problem that limits life and threatens limbs	1	1	1	1	1	2	1	1	Goat	1-6 years			
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
Female	Spanish-Boer goats	12 weeks	microCT, histology, mechanical testing	orthotopic	Tibia, mid shaft	segmental defect	5 cm	critical size; However, we also use a PMMA spacer for 4 weeks prior to grafting, so this is a non-union model and not an acute defect model.	IM Rod w interlocking bolts	Very Satisfied. First paper recently published in CORR. Two more papers soon to follow.	Yes. 5 cm bone defect, 9 cm periosteal defect, 10 gm muscle defect, followed by a PMMA Vanco spacer for 4 weeks prior to spacer removal and grafting.			
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
Mouse (to small and our work does not involve genetic variaion), rat (remodeling environment not human-like), rabbit (Bone marrow environment not human like)	speces dependent.	either		12 weeks generally	microCT	femur			5 mm	critical defect	Plate			

	TE16_13	TE16_14	TE18_01													
case 258	no longer satisfied. Useful now only as a screen for poor performance. Too many materials work well in rat and then failr in larger animals.	No. To small, acute, non-human like marrow and remodeling.														
	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
	1	1	2	1	1	1		1	1		3	1				
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
	3	3		1	vitoss	2	-9		-9		-9					
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02				
	-9		1	2	1	1	2	1	1	1	sheep	6				
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
	TE16_13	TE16_14	TE18_01													
case 259	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
	1	1	1	2	1	1		1	1		3	1				
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
	3	1		1	reconstruction of craniofacial skeleton	3	1	scres and plates	1		-9					
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02				
	1	Limited donor sites and morbidity	1	3	1	1	2	1	1	1						
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
	TE16_13	TE16_14	TE18_01													

case 260	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	2	1	1	1	1		1	1		3	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	2		1	non union	1	1	plate or nail	1	non union, tumor, osteopenic fracture	1	non union
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	would be nice to have an alternative that is more poweerful	1	2	1	1	1	2	1	1	rat	6
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	m f			ct	femur		segmental	7mm		either	depends on the model	yes
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 261	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		2	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 263	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	2	1	1	1	1		1	1		4	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02

case 263															
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
TE16_13	TE16_14	TE18_01													
case 264															
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
2	2	2	1	1	1		1	1		3	1				
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
2	3		1	tibia plateau	2	1	locking plate	-9		-9					
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02				
1		1	2	1	1	1	1	1	2						
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
TE16_13	TE16_14	TE18_01													
case 265															
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
2	2	2	1	1	1		1	1		2	2				
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02				
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14				
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12				
TE16_13	TE16_14	TE18_01													
case 267															
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03				
1	2	1	1	1	1		1	1		4	2				
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01				
3	1		1	Non-union, post trauma bone defect	3	1	im-nail or locking plate	1	Non-union, post trauma bone defect	1					

case 269	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02					
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14					
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12					
	TE16_13	TE16_14	TE18_01														
	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03					
	2	2	2	1	1	1		1	1		4	1					
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01					
	4	2		1	hip revision surgery	2	1	none	1	acertabular bone defect, periprostetic fracture.....	1	acetabular bone defect					
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02					
	1	to improve results	1	1	1	2	1	1	1	1							
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14					
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12					
	TE16_13	TE16_14	TE18_01														
case 271	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03					
	1	2	1	1	1	1		1	1		4	2					
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01					
	2	1		1	defects in continuity (i.e. not gap defects)	2	1	plates, nails	-9		-9						
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02					
	1	Substitute solutions, that may avoid complications and maybe even be more effective	1	2	1	2	1	1	1	1	Mouse	10-12 weeks					

case 271														
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
Male and Female	Variety	4 weeks	Cellular and molecular	Injury site	Tibia	Fracture, segmental defect	2 mm	critical sized, non-union, normal healing, vascular disruption, distraction osteogenesis, aging	external fixator, nail	Very	yes			
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
rabbit	All	All		Until healing	Histological, Immunological	Local	Femur	Simple fracture		normal healing	IM nail			
TE16_13	TE16_14	TE18_01												
not satisfied	yes													

case 272														
TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03			
1	1	1	2	1	1		1	1		4	2			
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01			
4	3		1	orthognathic cases and preposthetic surgery	4	1	membran	-9		-9				
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02			
1	donor sites, costs	1	1	1	1	2	1	1	1					
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14			
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12			
TE16_13	TE16_14	TE18_01												
		Tissue engineering will always be a domain of academic institutions and not for daily practise												

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 274	0	1	1	1	1	1		1	1		-9	-9
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	-9	-9		-9		-9	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 280	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	2	2	2	1	1	1		1	1		3	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	2	5		1	nonunion, delay of consolation	3	1	plate e massive bone graft	1	nonunion, delay of consolation, arthrodesis	1	nonunion, delay of consolation
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 283	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	1	2	1		1	1		2	1
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	4	5		-9		1	-9		-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									

	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
case 284	1	2	1	1	1	1		1	1		4	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	bone defect	1	1	plate/nail	-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	medical progress	1	1	1	1	2	1	1	1	mouse	
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 286	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	2	1	1		1	1		1	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	Reconstruction of the jaw	2	1	Osteosythesis	-9		-9	
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
	1	To avoid donorside morbidity	1	4	2	1	2	2	1	1		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
	TE16_13	TE16_14	TE18_01									
case 287	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
	1	1	1	2	1	1		1	1		2	2
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
	3	1		1	Dental implantology	3	1	screw	1	Dental Implantology	1	Medication - associated osteonecrosis of the jaw

case 287	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02		
	1	Because critical size defects still cannot be reconstructed in a satisfactory manner	1	2	1	1	2	1	1	1	minipig	100		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14		
	female		24 weeks		orthotopic	jaw	periodontal defect - drilled defect	7x4x3mm	periodontitis model	none	mostly satisfied	yes		
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12		
	TE16_13	TE16_14	TE18_01											
case 290	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03		
	1	1	1	1	1	2	engineering	1	1		4	1		
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01		
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02		
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14		
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12		
	TE16_13	TE16_14	TE18_01											
case 292	TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03		
	1	1	1	2	1	1		1	1		4	1		
	TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01		
	4	1		1	tumor cases	2	1	Osteosythesis	-9		-9			
	TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02		
	1		1	4	1	1	2	1	1	1				
	TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14		
	TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12		
	TE16_13	TE16_14	TE18_01											

case 293														
TE01	**TE01_01**	**TE01_02**	**TE01_03**	**TE01_04**	**TE01_05**	**TE01_05a**	**TE01_06**	**TE01_07**	**TE01_07a**	**TE02**	**TE03**			
2	1	1	1	2	2	Medicine	1	1		3	1			
TE04	**TE05**	**TE05_06**	**TE06**	**TE06_01**	**TE07**	**TE08**	**TE08_01**	**TE09**	**TE09_01**	**TE10**	**TE10_01**			
-9	-9		-9		-9	-9		1	(Large) bone defect at initial or revision surgeries, non unions	1	Treatment of bone defects			
TE11	**TE11_01**	**TE12**	**TE13**	**TE14**	**TE14_01**	**TE14_02**	**TE14_03**	**TE14_04**	**TE14_05**	**TE15_01**	**TE15_02**			
1	due to known limitation of autologous bone graft (i.e. limited amount, donor site morbidity etc)	1	2	1	1	1	2	1	1	sheep,	skeletally mature defined as closure of growth plate, 2 to 3 years			
TE15_03	**TE15_04**	**TE15_05**	**TE15_06**	**TE15_07**	**TE15_08**	**TE15_09**	**TE15_10**	**TE15_11**	**TE15_12**	**TE15_13**	**TE15_14**			
female	WAS,	up to 16 weeks	radiographs, CT (in vivo and ex vivo), histology,	orthotopic	distal femur and Proximal tibia: cancellous bone; diaphysis tibia: cortical bone	drill hole	4 to 8 mm	Bone healing	usually not, depending on size and amount of defects in diaphysis plate for stabilization should be considered	mostly satisfied	Depending on research question, limitations: limited to no load bearing. Good screening			
TE16_01	**TE16_02**	**TE16_03**	**TE16_04**	**TE16_05**	**TE16_06**	**TE16_07**	**TE16_08**	**TE16_09**	**TE16_10**	**TE16_11**	**TE16_12**			
rat	24 weeks and older	female	different one	up to 16 weeks	radiographs, CT (in vivo and ex vivo), mechanical testing, histology	orthotopic	femur	segmental defect	1 to 5 mm	normal healing to large bone defect to CSD	plate (PEEK, ratfix)			
TE16_13	**TE16_14**	**TE18_01**												
mostly to not satisfiied	for direct translation: no, but appropriate for research question	Small animal models are appropriate research question but they are used too often for trying to directly translate into the clinical situation. Large bone defect model have quite a variability which is not described or taken into account appropriately.												

case 294

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
1	1	1	1	1	2	Biology	1	1		2	1
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9		-9	-9		1	large segmental bone defects, non-unions	1	large segmental bone defects
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	alternative treatment options are not satisfying (BMP: side effects, not applicable for tumor patients, which are one of the major causes for segmental defects; autologous bone: limited availability, donor side morbidity)	1	2	1	1	1	2	1	1		
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
rat	20-28	female	Fisher F344	14	in vivo uCT, x-ray, histology (plastic embedding), serum parameters, cell and gene expression analysis (fracture site and other tissues)	orthotopic	femur	osteotomy	2 mm	was designed as healing model but turns out to be (sub-)critical size, healing is not reproducible!	internal plate (PEEK, 6 screws)
TE16_13	TE16_14	TE18_01									
not satisfied	no										

case 297

TE01	TE01_01	TE01_02	TE01_03	TE01_04	TE01_05	TE01_05a	TE01_06	TE01_07	TE01_07a	TE02	TE03
2	2	1	1	1	2	biology	1	1		2	2
TE04	TE05	TE05_06	TE06	TE06_01	TE07	TE08	TE08_01	TE09	TE09_01	TE10	TE10_01
-9	-9		-9		-9	-9		1	critical size defects	1	trauma, tumor, infection
TE11	TE11_01	TE12	TE13	TE14	TE14_01	TE14_02	TE14_03	TE14_04	TE14_05	TE15_01	TE15_02
1	alternative to autologous bone graft	1	4	1	1	1	2	1	1	sheep	12 months
TE15_03	TE15_04	TE15_05	TE15_06	TE15_07	TE15_08	TE15_09	TE15_10	TE15_11	TE15_12	TE15_13	TE15_14
M/F		12 weeks	histology, x ray	orthotopic	femur	segmental defect	25mm	critical size	internal plate	mostly	yes
TE16_01	TE16_02	TE16_03	TE16_04	TE16_05	TE16_06	TE16_07	TE16_08	TE16_09	TE16_10	TE16_11	TE16_12
TE16_13	TE16_14	TE18_01									

Tab-S. 3 Outcome of the literature search. The tabular listing is sorted by animal species. BTE=Bone Tissue Engineering

dog													
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
/	male	/	4, 8, 16	x-ray, histology, electron microscopy	femur	drill hole	5 mm	/	/	/	/	BTE	Zou, Chen et al. 2012
not defined	male	/	30	x-ray, histology, biomechanical testing	femur condyle	drill hole	10 mm	large osteochondral defect after osteonecrosis	/	/	/	BTE	Peng, Wen et al. 2011
12-15 months	male	Beagle	3, 8	histology	mandible	alveolar defect "box-shaped defect"	9 mm height, 6 mm depth, 12 mm width	critical-size defect	/	/	/	BTE	Du, Liu et al. 2015
18 months	male	Beagle	4, 8, 16, 24	micro-ct, x-ray, histology, trichrome fluorescent labeling	mandible	alveolar defect	/	peri-implant osseous defect	/	/	/	BTE	Xu, Sun et al. 2016
not defined	male	/	8	ct, histology, biomechanical testing, molecular biology, SPECT	mandible	alveolar defect "boxlike bone defect"	10 mm height, 20 mm length	/	plate	/	/	BTE	Yao, Li et al. 2009
15,4 ± 1,2 months	/	Beagle	2, 4, 8, 12	x-ray, histology	radius	segmental defect	15 mm	/	plate	/	/	BTE	Jang, Byeon et al. 2008

goat												
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
12-14 months	/	/	4, 8, 16	ct, x-ray, histology, biomechanical testing, SPECT	femur	segmental defect	20 mm	critical-size defect	dynamic and static intramedu-llary rods	/	/	other	Hou, Li et al. 2010
/	/	/	4, 8, 16	x-ray, histology, radionuclide bone imaging	femur	segmental defect	20 mm	critical-size defect	dynamic and static intramedu-llary rods	/	/	BTE	Wu, Hou et al. 2013
not defined	male female	/	24	x-ray, biomechanical testing	tibia	segmental defect	20% of the whole tibia in length	critical-size defect	external fixation	/	/	BTE	Jian, Tian et al. 2010
not defined	female	/	1, 4, 12, 24	micro-ct, x-ray, histology	tibia	segmental defect	30 mm	/	plate	/	/	BTE	Wang, Wang et al. 2010
not defined	male female	/	4, 8, 12	ct, micro-ct, x-ray, histology, molecular biology	tibia	drill hole	10 mm	/	/	yes	no	BTE	Li, Deng et al. 2016
36 months	male	/	4, 8	x-ray, histology, biomechanical testing	tibia	segmental defect	25 mm	critical-size defect	plate	yes	no	BTE	Liu, Li et al. 2010

horse											
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
11-17 years	male	gelding horses	4, 8, 12, 16	micro-ct, x-ray, histology, flourochrome analysis	mandible	alveolar defect "full-thickness" defect	13,5 mm	delayed bone healing	/	/	/	other	Sarrafian, Garcia et al. 2015
monkey													
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
not defined	male	Macaca fascicularis	12	cone beam computed tomography, histology	tibia	segmental defect	20 mm	non-union	plate	/	/	BTE	Guo, Min et al. 2017
not defined	/	rhesus monkeys	4, 8, 12	x-ray, histology, SPECT, Perfusion weighted MRI analysis	tibia	segmental defect	20 mm	/	plate	yes	no	BTE	Fan, Zeng et al. 2014
pig													
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
537 ± 19 days	female	Danish Landrace	5	micro-ct, histology	cranium	drill hole	10 mm height, 15 mm diameter	critical-size defect	/	yes	no	BTE	Jensen, Tvedesoe et al. 2016
<6 months	/	MGH-miniature swine	12, 24, 36, 48	ct, x-ray, histology	femur	segmental defect	20 mm	non-union	plate	/	/	BTE	Schubert, Lafont et al. 2013
not defined	male female	Sus scrofa	1, 6, 12	ct, histology, fluorescent images	mandible	alveolar volume defect "subperiosteal and supraperiosteal"	3-7 cm^3, 1,5 cm vertical	spontaneaous healing, critical-size defect	/	/	/	other	Sun, Kennedy et al. 2014

sheep													
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
not defined	/	Swiss Alpine	3, 16	micro-ct, histology	femur	segmental defect	25,4 mm	critical-size defect	nail, bolt	/	/	BTE	Knothe Tate, Chang et al. 2011
>2,5 years	female	black-face sheep	3, 9	x-ray, biomechanical testing	femur/humerus	drill hole	6 mm diameter, 15 mm depth	critical-size defect	/	yes	no	other	Pobloth, Johnson et al. 2016
36 months	female	Swiss Alpine	8, 16, 48	micro-ct, x-ray, histology	femur/tibia	drill hole	5 mm diameter, 15 mm depth	/	/	yes	no	BTE	van der Pol, Mathieu et al. 2010
6 ± 3 years	female	Berga-masca	8, 16	micro-ct, histology, x-ray	femur/tibia	drill hole	8 mm diameter, 4 mm depth	large bone defect	/	yes	femur yes, tibia no	BTE	Lovati, Lopa et al. 2016
12–15 months	female	Latxa Asturian	12, 32	ct, micro-ct, histology	mandible	segmental defect	30 mm	critical-size defect	plate	/	/	BTE	Gallego, Perez-Basterrechea et al. 2015
6–7 years	/	Merino	24	micro-ct, x-ray, histological, biomechanical testing	tibia	segmental defect	30 mm	critical-size defect	plate	/	/	BTE	Berner, Henkel et al. 2017
2–6 years	female	Mountain sheep	2, 4, 6, 8 ,10, 14	micro-ct, x-ray, histology, biomechanical testing, fluorochrome analysis	tibia	segmental defect	20 mm	critical-size defect	plate, external fibreglass cast	/	/	BTE	Lohfeld, Cahill et al. 2012
7 years	/	Merino	12	micro-ct, x-ray, histology, biomechanical testing	tibia	segmental defect	20 mm, 30 mm	critical-size defect	plate, hard plaster	yes	no	other	Reichert, Epari et al. 2010

mouse												
age	**sex**	**strain**	**observation (weeks)**	**observation methods**	**implantation site**	**defect form**	**defect size**	**defect model**	**fixation**	**empty control**	**healing empty control**	**research target**	**reference**
not defined	female	BALB/c	2	micro-ct, histology, molecular biology	cranium	drill hole	3 mm	critical-size defect	/	yes	no	BTE	Shiozaki, Kitajima et al. 2013
2 months	female	nude BALB/cAnN.Cg-Foxnlnu/CrlNarl	2, 4, 8, 12	micro-ct, histology	cranium	drill hole	4 mm	critical-size defect	/	/	/	BTE	Liao, Chang et al. 2014
3-3,5 months	male	C57bl/6J	4, 8	micro-ct, x-ray, histology	cranium	drill hole	2,5 mm	/	/	yes	no	BTE	Samsonraj, Dudakovic et al. 2017
/	/	BALB/c	4, 8	micro-ct, histology	cranium	drill hole	5 mm	/	/	yes	no	BTE	Nair, Tsai et al. 2013
/	/	BALB/c-nu immunodeficient	12	micro-ct, histology	cranium	drill hole	4 mm	critical-size defect	/	yes	no	BTE	Nakajima, Kunimatsu et al. 2018
2 months	/	C57BL/6J	4, 6	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	4 mm	/	gauge metal pin	/	/	BTE	Long, Zhu et al. 2014
95 ± 2,6 days	male	nu/nu nude	12	micro-ct, x-ray, histology	femur	segmental defect	1 mm 2 mm 3 mm	critical-size defect	external fixation, pin	/	/	other	Zwingenberger, Niederlohmann et al. 2013
3-3,5 months	male female	C57BL/6	0, 2, 5, 12	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	1,5 mm 2 mm	critical-size defect	plate-bolts system	/	/	other	Liu, Li et al. 2013
2,5 months	female	GFP+ transgenic	1, 4, 8	micro-ct, x-ray, histology, molecular biology	femur	segmental defect	2 mm	/	plate	/	/	BTE	Xing, Jin et al. 2014

mouse (continued)

/	female	MF-1 nu/nu immunodeficient	4	x-ray, histology	femur	segmental defect	5 mm	critical-size defect	gauge needle	yes	no	BTE	Kanczler, Mirmalek-Sani et al. 2009
2 months	/	athymic nude-Foxn1nu	2, 4, 6, 8	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	2 mm	critical-size defect	external fixation	yes	no	other	Srouji, Ben-David et al. 2011
/	/	immunodeficient nude	0, 2, 4, 6	micro-ct, x-ray, histology	femur	segmental defect	3 mm	critical-size defect	pin, plate	yes	no	BTE	Yu, Wang et al. 2014
2,5 months	male	athymic nude	10	micro-ct, x-ray, histology	femur	segmental defect	3,5 mm	critical-size defect	plate	yes	no	BTE	Manassero, Decambron et al. 2016
2,5 months	male	athymic nude NUDE NMRI-nu [nu/nu];	10	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	3,5 mm	critical-size defect	plate	yes	no	other	Manassero, Viateau et al. 2013
2-2,5 months	female	C3H/HeN	2	micro-ct	radius	segmental defect	2,5 mm	non-union	/	/	/	BTE	Kimelman-Bleich, Pelled et al. 2009
1,5-2 months	female	C3H/HeN	1, 2, 3	fluorescence molecular tomography, micro-CT, histology	radius	segmental defect	2,5 mm	non-union	/	/	/	other	Zilberman, Kallai et al. 2008
1,5 months	male	BALB/c	3, 4, 6	x-ray	tibia	segmental defect	/	simple fracture model	gauge needle	/	/	BTE	Bush, Liang et al. 2016
2,5-3,5 months	male	SCID Beige	1–6	micro-ct, histology, biomechanical testing	tibia	segmental defect	2 mm	critical-size defect	external fixation	yes	no	BTE	Bahney, Hu et al. 2014

rabbit												
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
/	male female	New Zealand White	1, 4, 12, 24, 40	ct, histology, electron microscopy	cranium	drill hole	12 mm	/	/	yes	no	BTE	Tang, Gui et al. 2008
6 – 12 months	male	New Zealand White	6	micro-ct, histology	cranium	drill hole	15 mm	critical-size defect	/	/	/	BTE	Kim, Sharma et al. 2012
/	/	New Zealand White	4, 8, 12	x-ray, histology	femur condyle	drill hole	6 mm diameter, 12 mm depth	/	/	yes	no	BTE	Niu, Fan et al. 2011
3 months	female	New Zealand White	12	x-ray, histology	femur distal epiphysis	drill hole	6 mm diameter, 3 mm depth	non-critical-size defect	/	/	/	BTE	Coletta, Lozano et al. 2014
/	male female	New Zealand White	60 days	x-ray, histology, biomechanical testing, fluorochrome analysis, electron microscopy	femur	drill hole	2 mm height, 7 mm diameter	/	/	/	/	BTE	Bhattacharjee , Naskar et al. 2016
/	male	Japanese White	2, 4, 6	micro-ct, x-ray, histology	femur	drill hole "cylindrical defect"	4x10 mm²	/	/	/	/	BTE	Togami, Sei et al. 2015
3 months	male	New Zealand White	4, 8, 12	histology	femur condyle	drill hole	5 mm	/	/	/	/	BTE	He, Lin et al. 2016
6 months	/	New Zealand White	12, 24	micro-ct, histology	femur condyle	drill hole	6 mm diameter, 10 mm depth	critical-size defect	/	/	/	BTE	Yan, Xia et al. 2018
/	female	New Zealand White	8	histology	femur	drill hole	8x5 mm²	critical-size defect	/	/	/	BTE	Shen, Yang et al. 2014

rabbit (continued)

6-8 months	male	New Zealand White	4, 8	micro-ct, histology, biomechanical testing	femur	drill hole	2,5 mm height, 5 mm diameter	/	/	yes	no	BTE	Buyuksungur, Endogan Tanir et al. 2017
not defined	/	New Zealand White	3, 6, 9	micro-ct, histology	femur condyle	drill hole	6 mm diameter, 10 mm depth	/	/	yes	no	BTE	Xia, Zhou et al. 2013
/	/	New Zealand White	4, 12	histology	femur distal epiphysis	drill hole	6 mm diameter, 10 mm length	critical-size defect	/	yes	no	BTE	Chen, Shen et al. 2015
/	male female	New Zealand White	4, 8, 12	x-ray, histology, flow cytometry, electron microscopy	femur condyle	osteonecrotic drill hole	5 mm	osteonecrosis model	/	yes	no	BTE	Sun, Li et al. 2011
6 ± 1 months	female	e Chinchilla-Bastard	4	micro-ct, histology	femur	segmental defect	12 mm	critical-size defect	plate	yes	no	BTE	Endres, Hiebl et al. 2011
not defined	male	New Zealand White	1, 4, 8, 12	micro-ct, x-ray, histology, SPECT, microfil perfusion, biomechanical testing	femur	segmental defect	15 mm	critical-size defect	plate	yes	no	BTE	Wang, Zhu et al. 2018
not defined	male	New Zealand White	4, 8, 12	x-ray, histology, biomechanical testing	femur	segmental defect	15 mm	/	plate	/	/	BTE	Duan, Zheng et al. 2008
not defined	/	New Zealand White	12	micro-ct, x-ray, histology	humerus	segmental defect	7 mm	/	plate	/	/	BTE	Kaempfen, Todorov et al. 2015
19-21 weeks	male	Japanese White	9, 21	x-ray, micro-ct, ct, histology, biomechanical testing, electron microscopy	mandible	segmental defect	10 mm	critical-size defect	plate	/	/	BTE	Hirota, Shima et al. 2016

rabbit (continued)

not defined	male	New Zealand White	0, 8, 16	micro-ct, histology	mandible	alveolar volume defect ‘‘full thickness’’ and ‘‘partial thickness’’	10 mm	non-union	plate	/	/	other	Young, Bashoura et al. 2008
/	male	New Zealand White	4, 8, 12	x-ray; histology, biomechanical testing, cone beam ct	mandible	segmental defect	20 mm	critical-size defect	screw	/	/	BTE	Alfotawei, Naudi et al. 2014
6 months	male	New Zealand White	4, 12	x-ray, histology, biomechanical testing	mandible	alveolar defect “bicortical full-thickness”	10 mm	critical-size defect	/	yes	no	BTE	Cheng, Li et al. 2015
not defined	male female	New Zealand White	4, 8, 12	x-ray, ct, histology, biomechanical testing, dual energy X-ray absorptiometry	mandible	alveolar volume defect “full-thickness”	8x8 mm²	critical-size defect	/	yes	no	BTE	Tong, Xu et al. 2016
/	female	New Zealand White	4, 8	micro-ct, histology	mandible	alveolar volume defect	10x5x3 mm3	critical-size defect	/	yes	no	BTE	Wang, Wu et al. 2017
/	/	New Zealand White	4, 12	x-ray, histology	mandible	alveolar volume defect	26x5x3 mm3	large bone defct, critical-size defect	/	yes	/	BTE	Su, Xu et al. 2013
6 months	/	New Zealand White	6	micro-ct, histology	mandible	segmental defect	2 mm	delayed bone healing	screw	yes	no	BTE	Ma, Zhong et al. 2011
6 months	/	New Zealand white	4	micro-ct, histology, biomechanical testing	mandible	segmental defect	2 mm	delayed bone healing	custommade fixation device, screws	yes	no	BTE	Ma, Yao et al. 2011

rabbit (continued)

not defined	/	New Zealand White	2, 4, 6, 8	histology, molecular biology, ELISA	radius	segmental defect	15 mm	large bone defect	/	/	/	BTE	Wang, Wei et al. 2015
not defined	/	New Zealand White	12	x-ray, histology	radius	segmental defect	10 mm	/	/	/	/	BTE	Jin, Wang et al. 2014
5 months	/	New Zealand White	4, 12	micro-ct, histology, biomechanical testing	radius	segmental defect	18 mm	critical-size defect	/	/	/	BTE	Cao, Werkmeister et al. 2014
/	female	New Zealand White	4, 8, 12	x-ray, histology, biomechanical testing, micro-angiography (ink perfusion)	radius	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Han and Li 2013
/	male	New Zealand White	1, 4, 8, 12, 16	micro-ct, x-ray, histology	radius	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Kang, Chung et al. 2014
/	male female	New Zealand White	4, 8, 12	x-ray, histology, bone mineral density	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Liu, Lu et al. 2009
/	/	New Zealand White	4, 8	micro-ct, histology	radius	segmental defect	12 mm	critical-size defect	/	/	/	BTE	Paul, Padalhin et al. 2016
5 months	male	New Zealand White	12	micro-ct, histology	radius	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Roohani-Esfahani, Dunstan et al. 2012
5 months	male	New Zealand White	12	micro-ct, histology	radius	segmental defect	13 mm	critical-size defect	/	/	/	BTE	Roohani-Esfahani, Dunstan et al. 2013

rabbit (continued)												
not defined	/	New Zealand White	4, 8, 12	micro-ct, histology, fluorochrome analysis	radius	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Zhao, Zhao et al. 2011
not defined	male female	New Zealand White	8	ink perfusion	radius	segmental defect	15 mm	/	/	/	/	BTE	Li, Liu et al. 2013
not defined	/	New Zealand White	2, 4, 8, 12	x-ray, histology, biomechanical testing, fluorochrome analysis	radius	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Fan, Lu et al. 2012
4 months	female	New Zealand White	6, 12, 16	micro-ct, x-ray, histology	radius	segmental defect	15 mm	/	/	yes	no	BTE	Lyons, Gleeson et al. 2014
3 months	/	New Zealand White	4, 8, 12	x-ray, histology, biomechanical testing	radius	segmental defect	20 mm	/	/	yes	no	BTE	Wang, Yang et al. 2010
/	male female	New Zealand White	4, 8	micro-ct, x-ray, histology; flourochrome analysis, immunofluor-escence staining	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Chen and Lv 2017
/	male	New Zealand White	2, 4, 6, 8, 10, 12	x-ray, peripheral quantitative ct, histology, biomechanical testing	radius	segmental defect	15 mm length, 3,8 mm depth, 4,5 mm width	critical-size defect	/	yes	no	BTE	Guo, Li et al. 2012
/	/	Japanese White	6, 12	x-ray, histology	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Hao, Dong et al. 2010
/	/	Japanese White	8, 16, 24	x-ray, histology, biomechanical testing	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Hao, Pang et al. 2010

rabbit (continued)

not defined	female	New Zealand White	4, 8, 12, 16	micro-ct, x-ray, histology, biomechanical testing	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Kasten, Vogel et al. 2008
/	/	New Zealand White	0, 4, 8, 12	micro-ct, x-ray, histology, biochemistry, molecular biology, fluorescence analysis	radius	segmental defect	5 mm	critical-size defect	/	yes	no	BTE	Lee, Son et al. 2016
/	/	New Zealand White	0, 12, 24, 36	micro-ct, x-ray, histology	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Pang, Hao et al. 2013
5 months	male	New Zealand White	4, 8, 12	micro-ct, x-ray, histology, flourochrome analysis, biomechanical testing	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Wang, He et al. 2013
3-4 months	male	New Zealand White	1 day; 12 weeks	x-ray, histology, biomechanical testing, fluorescence analysis	radius	segmental defect	16 mm	critical-size defect	/	yes	no	BTE	Wang, Ma et al. 2014
/	male	New Zealand White	4, 8, 12	x-ray, electron microscopy,biome chanical testing, radionuclide bone imaging	radius	segmental defect	15 mm	critical-size defect	/	yes	no	BTE	Zhu, Wang et al. 2010
3 months	male female	New Zealand White	12, 24, 48	histology, flourochrome analysis	radius	segmental defect	15 mm	large bone defect	/	yes	/	BTE	Wu, Liu et al. 2015
not defined	/	New Zealand White	4, 8, 12	x-ray, histology, biomechanical testing	radius	segmental defect	15 mm	large bone defect	/	yes	no	BTE	Zhao, Zhou et al. 2011

rabbit (continued)

6-8 months	male	New Zealand White	4, 8, 12, 24	x-ray, biomechanical testing	radius	segmental defect	15 mm	large bone defect	/	yes	no	BTE	Zhu, Shen et al. 2013
2,5 months	/	New Zealand White	12	histology	radius	segmental defect	10 mm	/	/	yes	no	BTE	Chen, Bai et al. 2014
/	/	New Zealand white	4, 8, 12	micro-ct, x-ray, histology, molecular biology, flow cytometry	radius	segmental defect	10 mm	/	/	yes	no	other	Lei, Sun et al. 2015
not defined	male	New Zealand White	3, 6	x-ray, histology	tibia	drill hole "knife blade"	5x5 mm²	/	/	/	/	BTE	Cai, Zhang et al. 2012
not defined	/	New Zealand White	4, 8, 12	x-ray, histology	tibia	drill hole	/	critical-size defect	/	/	/	BTE	Bagher, Rajaei et al. 2012
/	female	Japanese White	2, 4, 6, 8	micro-ct, x-ray, histology, biomechanical testing	tibia	segmental defect	5 mm	/	external fixation	yes	no	BTE	Goshima, Nakase et al. 2012
not defined	male	New Zealand White	4-8	histology	tibia	segmental defect	5 mm	/	ligature wire	yes	no	BTE	Ai, Ebrahimi et al. 2012
6 months	male	New Zealand White	25	micro-ct, x-ray, histology, fluorescence analysis	tibia	cubic defect	15 mm height, 2 mm depth, 3 mm width	critical-size defect	/	yes	no	BTE	Sagar, Pandey et al. 2013
/	male female	New Zealand White	4	micro-ct, x-ray, histology, molecular biology	tibia	cubic defect	10 mm length, 7 mm width	infected bone defect	/	yes	no	BTE	Wang, He et al. 2016
not defined	male	New Zealand White	3, 6	x-ray, histology	tibia	cubic defect	15 mm length, 10 mm width	large bone defect	/	yes	no	BTE	Cai, Wang et al. 2010

rabbit (continued)

/	/	New Zealand White	12	micro-ct, histology	ulna	segmental defect	15 mm	critical-size defect	/	/	/	BTE	Amini, Xu et al. 2016
4,5 months	female	New Zealand White	2, 4, 8, 12	ct, x-ray, histology, fluorescence analysis	ulna	segmental defect	20 mm	critical-size defect	/	/	/	BTE	Chen, Lau et al. 2017
not defined	/	Nihon White	4, 8	x-ray, histology, biomechanical testing	ulna	segmental defect	20 mm	critical-size defect	/	/	/	BTE	Fujita, Matsushita et al. 2012
3,5-4,5 months	male	New Zealand White	0, 4, 8	micro-ct, x-ray, histology	ulna	segmental defect	20 mm	critical-size defect	/	/	/	BTE	Kim, Kim et al. 2012
/	female	New Zealand White	4, 8	micro-ct, histology	ulna	segmental defect	10 mm	/	/	yes	no	BTE	Takahata, Okihara et al. 2015
/	/	Japanese White	12	x-ray, histology	ulna	segmental defect	10 mm	/	/	yes	no	BTE	Wang, Hu et al. 2014

rat												
age	***sex***	***strain***	***observation (weeks)***	***observation methods***	***implantation site***	***defect form***	***defect size***	***defect model***	***fixation***	***empty control***	***healing empty control***	***research target***	***reference***
not defined	male	Wistar	2, 8	histology, molecular biology	cranium	drill hole	5 mm	/	/	/	/	BTE	Zhang, Tsurushima et al. 2011
7 weeks	male	Fischer	3, 4	micro-ct, histology	cranium	drill hole	5 mm	critical-size defect	/	/	/	BTE	Kigami, Sato et al. 2013
/	/	Fisher 344	5	micro-ct, histology, molecular biology	cranium	drill hole	8 mm	critical-size defect	/	/	/	BTE	Hu, Wang et al. 2016
/	male	Sprague-Dawley	7	micro-ct, x-ray, histology	cranium	drill hole	7 mm	critical-size defect	/	yes	no	BTE	Park, Ki et al. 2010
not defined	male	Sprague-Dawley	5, 10, 20, 30 days	histology	cranium	drill hole	4 mm	non-critical-size defect	/	yes	no	BTE	Shirasu, Ueno et al. 2010
/	/	F344/NJcl-mu(immuno-compromised)	1, 4	histology	cranium	drill hole	8 mm	critical-size defect	/	yes	no	BTE	Honda, Imaizumi et al. 2011
/	male	Wistar	6	micro-ct, histology	cranium	drill hole	0,25 mm height, 5 mm diameter	/	/	/	/	BTE	Diomede, Gugliandolo et al. 2018
not defined	/	Lewis 1A-haploype RT1a	7	micro-ct, histology, electron microskopy	cranium	drill hole	5 mm	critical-size defect	/	/	/	BTE	Corre, Merceron et al. 2015
1,5-2 months	male	Holtzman	6, 10	micro-ct, x-ray, histology	cranium	drill hole	5 mm	/	/	yes	no	BTE	Jaiswal, Dhumal et al. 2013

rat (continued)

not defined	male	Wistar	1, 4	histology	cranium	drill hole	7 mm	critical-size defect	/	yes	no	BTE	Hamidabadi, Shafaroudi et al. 2018
6 months	female	Sprague-Dawley bilateral ovariectomy (OVX)	1, 4, 8, 12	micro-ct, histology	cranium/femur	drill hole "osteoporotic"	5 mm cranium; 2 mm femur	/	/	yes	yes	BTE	Liu, Rao et al. 2017
not defined	/	Sprague-Dawley	1, 5	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	/	open fracture	Kirschner-wire	/	/	BTE	Xu, Huang et al. 2015
9–10 weeks	male	Fischer 344	1, 2, 4, 6, 8	x-ray, histology	femur	segmental defect	1 mm 2 mm 3 mm 4 mm 5 mm 6 mm	critical-size defect	external fixation, Kirschner wires	/	/	other	Sato, Watanabe et al. 2014
>3 months	male	athymic RH-FOXN1rnu	1, 4, 8	micro-ct, histology, biomechanical testing	femur	segmental defect	5 mm	critical-size defect	external fixation	/	/	BTE	Seebach, Henrich et al. 2010
/	male	athymic nude	8-10	x-ray, histology	femur	segmental defect	6 mm	critical-size defect	external fixation, Kirschner-wire	/	/	BTE	Foo, Reagan et al. 2013
3 months	female	Sprague-Dawley/ athymic nude	16	microct, x-ray, biomechanical testing	femur	segmental defect	5 mm	critical-size defect	Kirschner-wire	/	/	BTE	Amorosa, Lee et al. 2013
13 weeks	female	Sprague–Dawley	2, 12	micro-ct, x-ray, histology, biomechanical testing, model development	femur	segmental defect	8 mm	critical-size defect	plate	/	/	BTE	Hettiaratchi, Rouse et al. 2017

rat (continued)

3 months	female	Sprague-Dawley	12	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	8 mm	critical-size defect	plate	/	/	BTE	Priddy, Chaudhuri et al. 2014
13 weeks	female	Sprague-Dawley/ athymic nude	2, 4, 8, 12	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	8 mm	non-union	plate	/	/	BTE	Willett, Li et al. 2013
1,5 months	male	F344	6	x-ray	femur	segmental defect	/	non-union	gauge needle	yes	no	BTE	Bush, Liang et al. 2016
not defined	male female	Fischer 344	2, 4, 8	x-ray,histology,bio-mechanical testing, ultrasono-graphy,molecul-ar biology	femur	segmental defect	/	non-union	gauge needle	yes	no	BTE	Nakamura, Akahane et al. 2010
/	male	Sprague-Dawley	0, 2, 4, 8 , 12	x-ray, histology	femur	segmental defect	6 mm	critical-size defect	external fixation	yes	no	other	Zhao, Yang et al. 2009
3 months	male	Sprague-Dawley bilateral ovariectom y (OVX)	1 day, 3 days, 7 days; 2, 4 weeks	x-ray, histology, in vivo fluorescence imaging, electron microscopy	femur	cubic defect	5 x 2,5 mm²	/	/	yes	no	BTE	Qi, Niu et al. 2015
/	/	Sprague-Dawley	4	micro-ct, histology	femur	drill hole	4 mm	/	/	yes	no	BTE	Dogan, Demirci et al. 2014
/	female	ovarectami zed	4, 8	micro-ct, histology	femur	drill hole	3 mm	critical-size defect	/	yes	no	BTE	Zhang, Chen et al. 2017
2,5 months	female	Wistar (ovariecto miz-ed)	2, 4, 8	histology	femur	drill hole	3 mm	critical-size defect	/	yes	/	BTE	Chen, Zhao et al. 2015
6 months	male	Sprague–Dawley	2	micro-ct, histology	femur	drill hole	2 mm	non-critical-size defect	/	/	/	BTE	Badieyan, Berezhanskyy et al. 2016
/	male	Sprague-Dawley	2, 4	micro-ct, histology	femur	drill hole	2 mm	non-critical-size defect	/	yes	no	BTE	Keibl, Fugl et al. 2011

rat (continued)

not defined		Sprague-Dawley	12	x-ray, histology, biomechanical testing	femur	segmental defect	10 mm	critical-size defect	orthopedic splint	yes	no	BTE	Li, Fan et al. 2013
2,5 months	female	/	1, 4, 8	micro-ct, x-ray, histology	femur	segmental defect	5 mm	critical-size defect	plate	yes	no	BTE	Jiang, Cheng et al. 2018
3 months	male	Fischer-344	4, 6, 8	micro-ct, x-ray, histology	femur	segmental defect	5mm	critical-size defect	plate	yes	not reproducible (2out of 8 bridging)	BTE	Kunkel, Wagner et al. 2017
2,5 months	male	Sprague-Dawley	4, 8	micro-ct, histology, biomechanical testing	femur	segmental defect	7 mm	critical-size defect	plate	yes	no	BTE	Nau, Henrich et al. 2016
not defined	male	Sprague-Dawley	0, 4, 12	micro-ct, x-ray, histology, biomechanical testing	femur	segmental defect	7 mm	critical-size defect	plate	yes	no	BTE	Zhang, Teoh et al. 2010
3 months	male	athymic nude	4, 8, 12, 16	micro-ct, x-ray, histology, electron microscopy	femur	segmental defect	5 mm	critical-size defect	plate	yes	no	BTE	Choi, Kim et al. 2011
13 weeks	female	Sprague-Dawley	2, 4, 8, 12	micro-ct, x-ray, histology, biomechanical testing,finite element modeling	femur	segmental defect	6 mm	critical-size defect	stiff plate, compliant plate	yes	no	BTE	Boerckel, Kolambkar et al. 2012
3 months	male	Fischer 344	4, 8, 12	micro-ct, x-ray, histological, biomechanical testing	femur	segmental defect	/	critical-size defect	Kirschner-wire	yes	no	BTE	Shimizu, Akahane et al. 2015
not defined	/	Sprague-Dawley	12 days	micro-ct, fluorochrome analysis	femur	drill hole	0,8 mm	/	/	yes	no	BTE	Tripathi, Pal et al. 2015
/	male	Lewis	10 days; 12weeks	micro-ct, ct, x-ray, histology, molecular biology	femur	segmental defect	10 mm	critical-size defect	plate	yes	no	BTE	Arkudas, Lipp et al. 2018

rat (continued)

2,5 months	female	athymic nude	2, 8	micro-ct, x-ray,	femur	segmental defect	6 mm	critical-size defect	external fixation, plate	/	/	other	Drosse, Volkmer et al. 2008
not defined	female	Sprague-Dawley	2, 4, 8, 12	micro-ct, x-ray, biomechanical testing	femur	segmental defect	8 mm	critical-size defect	plate	/	/	BTE	Johnson, Boerckel et al. 2011
4 months	female	Sprague-Dawley	3 days; 3,6 weeks	x-ray, histology, biomechanical testing	femur	cubic defect	3,5 x 4,5 mm²	/	/	yes	no	BTE	Xu, Su et al. 2010
/	male	Fischer	10	x-ray, histology, ELISA, tube formation assays	femur	segmental defect	4 mm	critical-size defect	plate	yes	no	BTE	Giles, Godbout et al. 2017
not defined	/	Sprague-Dawley	4, 6, 8	micro-ct, histology	mandible	alveolar defect ("full-thickness" defect)	5 mm	critical-size defect	/	/	/	BTE	Liu, Bao et al. 2016
7-9 weeks	male	athymic (Crl:NIH-Foxn1mu)	4, 8	flat-panel volumetric computed tomography, micro-ct, histology	mandible	drill hole (round through-and-through osseous defect)	5 mm	critical-size defect	/	yes	no	BTE	Streckbein, Jackel et al. 2013
2 months	male	Wistar	8	micro-ct, histology, electron microscopy	mandible	alveolar defect "buccal bone defect"	4 mm height, 5 mm length, 1 mm depth	critical-size defect	/	yes	not defined	BTE	Ma, Han et al. 2018
15 weeks	male	Fischer 344	8	micro-ct, histology, biomechanical testing, molecular biology	mandible	symphysis bone gap	4 x 2 mm ²	congenital non-union	/	/	/	BTE	Yagyuu, Kirita et al. 2015
9-10 weeks	male	Sprague-Dawley	2, 4, 6	micro-ct, cone beam micro-ct, histology, flourochrome analysis	maxilla	alveolar defect	/	experimental parodontitis induced bone defect	/	yes	/	BTE	Taut, Jin et al. 2013

rat (continued)

/	female	Lewis	1, 3, 6	cone-beam computed tomography, histology	maxilla	alveolar defect	3 mm	alveolar cleft osteoplasty (drill)	/	yes	no	BTE	Korn, Schulz et al. 2014
not defined	male	Wistar	2, 4, 8	molybdenum target x-ray, histology	radius	segmental defect	4 mm	/	/	yes	no	BTE	Liu, Zhou et al. 2018
2 months	male	Sprague-Dawley	2, 5, 8	micro-ct, x-ray, histology, biomechanical testing, electron microscopy	radius	segmental defect	5 mm	critical-size defect	/	yes	no	BTE	Oryan, Alidadi et al. 2017
not defined	male	Sprague-Dawley	2, 5, 8	3D-ct, x-ray, histology, biomechanical testing, electron microscopy	radius	segmental defect	5 mm	critical-size defect	/	yes	no	BTE	Oryan and Alidadi 2018
not defined	male	Wistar	1, 2	histology	tibia	drill hole	1,6 mm	/	/	yes	no	BTE	Andrade, Sa et al. 2017
9 weeks	female	Sprague-Dawley	6	micro-ct, x-ray, histology	tibia	cubic defect, drill hole(cylinder)	2 mm diameter, 2,5 mm length (cuboid), 2 mm length, 2,5 mm depth, 2 mm width (cylinder)	/	/	yes	no	BTE	Li, Ma et al. 2015
not defined	male	Wistar	2	x-ray, histology	tibia	drill hole	3 mm	critical-size defect	/	yes	no	BTE	Dhivya, Saravanan et al. 2015

Tab-S. 4 Paper excluded from the literature search outcome. The following paper were not included in the evaluation of preclinical animal models in bone tissue engineering. Main reasons therefore were differing research objectives targeted by the scientists or the use of ectopic animal models.

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