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*Title Page

The challenge of establishing preclinical models for segmental bone defect research

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Running title: bone defect research

Abstract

Abstract:

A considerable number of international research groups as well as commercial entities work on the development of new bone grafting materials, carriers, growth factors and specifically tissue engineered constructs for bone regeneration. They are strongly interested in evaluating their concepts in highly reproducible large segmental defects in preclinical and large animal models. To allow comparison between different studies and their outcomes, it is essential that animal models, fixation devices, surgical procedures and methods of taking measurements are well standardized to produce reliable data pools and act as a base for further directions to orthopaedic and tissue engineering developments, specifically translation into the clinic. In this leading opinion paper, we aim to review and critically discuss the different large animal bone defect models reported in the literature. We conclude that most publications provide only rudimentary information on how to establish relevant preclinical segmental bone defects in large animals. Hence, we express our opinion on methodologies to establish preclinical critically-sized, segmental bone defect models used in past research with reference to surgical techniques, fixation methods and post-operative management focusing on tibial fracture and segmental defect models.

1. Clinical Background

In general, bone possesses a good healing capacity and the vast majority of bone defects, when stimulated by well balanced biological and micro-environmental conditions, heal spontaneously. Refinements in surgical techniques, implant design and peri-operative management have significantly improved the treatment of complex fractures and other skeletal defects caused by high energy trauma, disease, developmental deformity, revision surgery, and tumour resection [1-6]. However, an unfavourable wound environment, sub-optimal surgical technique or biomechanical instability can lead to formation of large defects with limited intrinsic regeneration potential [7]. Such defects pose a major surgical, socio-economical and research challenge and can highly influence the patient's quality of life due to limb length discrepancy and prolonged, postoperative treatment courses [8, 9].

Even though cancellous bone fractures of the proximal humerus, distal radius or the tibia plateau often lead to impaction of bone and consequently a defect after reduction [4], the tibia shaft represents the most common anatomic site for segmental bone defects. This is because it is devoid of muscle coverage on its anteromedial surface [8]. The poor soft tissue coverage both increases the risk of bone loss and complicates treatment [8]. Historically, limb amputation was the principal treatment option when facing segmental, non-healing defect sites [10].

Over the years, bone grafts have advanced as the "gold standard" treatment to augment or accelerate bone regeneration [1, 2, 11-17]. However, significant drawbacks are associated with this approach. Additional anaesthetic time and personnel are needed for graft harvesting [13, 15, 18]. In many cases, insufficient grafts are obtained and the access to donor sites is limited [13, 14, 19, 20]. Donor site pain or haemorrhage can occur and the donor bone is predispositioned for failure [4, 13, 14, 21]. Moreover, the risk of infection is significantly increased. Graft failures usually result from incomplete transplant integration, particularly in large defect sites [15]. In addition, graft devitalisation and subsequent resorption processes can lead to decreased mechanical stability [22]. Vascularised autografts are technically demanding and allografts and xenografts carry the risk of immune-mediated rejection, graft sequestration and transmission of infectious disease [9, 23-29]. The dense nature of cortical bone allografts impedes revascularization and cellular invasion from the host following implantation [19]. This limited ability to revascularize and remodel is believed to be responsible for a failure rate of 25% and a 30-60% complication rate associated with allografts [19, 30]. In addition, the maintenance of bone banks is rather costly. A technique introduced to avoid graft integration-related difficulties is commonly referred to as the "Ilizarov technique" which involves osteotomy and distraction to stimulate bone formation. It is used as a treatment modality for large bone defects, infected non-unions, and limb length discrepancy [31]. However, the Ilizarov technique is a long-lasting procedure, highly inconvenient for the patient [32, 33] with recurrent pin track infections as a frequent complication [25, 34].

In order to avoid the limitations associated with the current standard treatment modalities for segmental bone deficiencies, there has been a continuous interest in the use of naturally derived and synthetic bone graft substitutes during the past decades.

More recently, the concept of tissue engineering has emerged as an important approach to bone regeneration research. Tissue engineering unites aspects of cellular biology, biomechanical engineering, biomaterial sciences and trauma and orthopaedic surgery. Its general principle involves the association of cells with a natural or synthetic supporting scaffold to produce a three-dimensional, implantable construct. To biomechanically simulate human *in vivo* conditions as closely as possible, and to assess the effects of implanted bone grafts and tissue engineered constructs on segmental long bone defect regeneration, a number of large animal models have been developed. However, reviewing the current literature most of the preclinical models reported in the literature are not well described, defined and standardized. This year, the Journal of Bone and Joint Surgery published a number of review papers on preclinical models in fracture healing and on non-unions [49]. However, these articles provide only rudimentary information on how to establish relevant preclinical segmental bone defects in a large animal model. Hence, the aim of this leading opinion article is to provide both detailed information on the advantages and disadvantages of the different animal models and to comprehensively share the expertise and knowledge of three research groups that successfully established a preclinical animal model for critically sized segmental bone defects.

2. Definition of a Critical-Size Bone Defect

An experimental osseous injury inflicted to study bone repair mechanisms should be of dimensions to preclude spontaneous healing [35]. Therefore, the non-regenerative threshold of bone was determined in research animal models inducing so-called critical-sized defects. Critical sized defects are defined as "the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal" [30, 36, 37] or as a defect which shows less than 10 percent bony regeneration during the lifetime of the animal [37].

Although the minimum size that renders a defect ''critical'' is not well understood, it has been defined as a segmental bone deficiency of a length exceeding 2-2.5 times the diameter of the affected bone [25, 34]. Results of various animal studies suggest that critical sized defects in sheep, however, could be approximately three times the diameter of the corresponding diaphysis [34]. Nevertheless, a critical defect in long bone cannot simply be defined by its size but may also be dependent on the species phylogenetic scale, anatomic defect location, associated soft tissue and biomechanical conditions in the affected limb as well as age, metabolic and systemic conditions, and related morbidities affecting defect healing [25, 36].

3. Large animal models in bone defect research

Animal models in bone repair research include representations of normal fracture-healing, segmental bone defects, and fracture non-unions in which regular healing processes are compromised without presence of a critical-sized defect site [38]. In critical-sized segmental defect models bridging of the respective defect does not occur despite a sufficient biological microenvironment due to the removal of critical amounts of bone substance. In contrast, in a true non-union deficient signalling mechanisms, biomechanical stimuli or cellular responses may prevent defect healing rather than the defect size.

When selecting a specific animal species as a model system, a number of factors need to be considered. In comparison to humans, the chosen animal model should clearly demonstrate both significant physiological and pathophysiological analogies in respect to the scientific question under investigation prior to animal selection. Moreover, it must be manageable to operate and observe a multiplicity of study objects post surgery over a relatively short period of time [39-41]. Further selection criteria include costs for acquisition and care, animal availability, acceptability to society, tolerance to captivity and ease of housing [42].

Several publications over the last decades have described dogs as a suitable model for research related to human orthopaedic conditions [43]. It was found that in regard to bone weight, density and bone material constituents such as hydroxyproline, extractable proteins, IGF-1, organic, inorganic and water fraction, dogs were the closest to humans although clear differences in bone microstructure and remodelling have been described [44, 45]. While the secondary structure of human bone is predominantly organized in osteones, osteonal bone structure in dogs is limited to the core of cortical bone, whereas in areas adjoining the periosteum and endosteum mainly laminar bone is found. This is characteristic for large, fast-growing animals [46]. It has been reported that higher rates of trabecular and cortical bone turnover can be generally observed in dogs compared to humans [47] and differences in loads acting on the bone as a result of the dog's quadrupedal gait must be taken into consideration as well. A review article by Neyt states that between 1991 and 1995 11% of musculoskeletal research was undertaken in dogs. This is confirmed by Martini et al. who find that between 1970 and 2001, 9% of orthopaedic and trauma related research used dogs as animal models for orthopaedic and trauma-related research [43, 48]. Recently, the use of dogs as experimental models has significantly decreased mainly due to ethical issues, although between 1998 and 2008 approximately 9% of articles published in leading orthopaedic and musculoskeletal journals described dogs as animal models for fracture healing research [49].

Mature sheep and goats possess a bodyweight comparable to adult humans and long bone dimensions enabling the use of human implants [50]. The mechanical loading environment occurring in sheep is well understood [51, 52]. The loading of the hind limb bones, forces and moments, is roughly half of that determined for humans during walking (Fig. 1). Since no major differences in mineral composition [53] are evident and both metabolic and bone remodelling rates are akin to humans [54], sheep are considered a valuable model for human bone turnover and remodelling activity [55]. Bone histology however reveals some differences in bone structure between sheep and humans. In sheep, bone consists principally of primary bone structure [56] in comparison with the largely secondary, haversian bone composition of humans [57]; furthermore the secondary, osteonal remodelling in sheep does not take place until an average age of 7-9 years (Fig. 2)[50]. Although a significantly higher trabecular bone density and greater bone strength was described for mature sheep when compared to humans, the trabecular bone in immature sheep is weaker, has a lower stiffness and density, a higher flexibility due to higher collagen content [58], and shows comparable bone healing potential and tibial blood supply [59].

In a variety of study designs, pigs are considered the animal of choice and were - despite their denser trabecular network [60] - described as a highly representative model of human bone regeneration processes in respect to anatomical and morphological features, healing capacity and remodelling, bone mineral density and concentration [44, 61]. However, pigs are often neglected in favour of sheep and goats given that the handling of pigs has been described as rather intricate [50]. Furthermore, the length of the tibiae and femora in the pig is relatively small, which causes the need for special implants, as one cannot use implants designed human use.

3.1. Tibial fracture models

Animal fracture models have been widely investigated to identify and further characterize physiological and pathophysiological processes of fracture healing of long bones. One of the most important elements in the study of fracture healing or fixation is the establishment of standardized methods to create reproducible fractures. Although a substantial number of articles on fracture models in animals and treatment options have been described over the last decades, only few publications describe the actual infliction of a fracture by trauma rather than the creation of a bony defect < 3 mm size by osteotomy, which is generally accepted as an alternative since it is less problematic to standardize. In 1988, Macdonald et al. [62, 63] reported a device for the reproducible creation of transverse fractures in canine tibiae utilizing a three-point bending technique.

Similarly, to compare the effects of reamed versus unreamed locked intramedullary nailing on cortical bone blood flow Schemitsch et al. created a standardized spiral fracture by three-point bending with torsion in a fractured sheep tibia model [64, 65], a method also described by Tepic [66] to establish a standardized oblique fracture in sheep tibiae in order to compare healing in fractures stabilized with either a conventional dynamic compression plate (DCP) or an internal point contact fixator (PC-Fix). A minimally invasive approach to create a multifragmental fracture in the sheep femur (classification by the Association for the Study of Internal Fixation, AO type 32-C), in which the bone was weakened by two short, transverse anterior osteotomies and bi-cortical drill holes created through small incisions, has recently been described by Wullschleger et al. (unpublished data, Fig. 3). The insertion of two chisels and one blade bar were then used to initiate cracks connecting both the osteotomies and the drill holes, thereby creating a standardized multifragmental fracture. This technique could easily be adopted when establishing standardized tibial fractures as well.

Fracture models of osteotomized long bones have been well characterized over the years in different large animal species. A number of publications have described fracture models in dogs since the dog, beside pigs, is considered the most closely related model for research of human orthopaedic conditions. The effect of bending stiffness of external fixators on the early healing of transverse tibial osteotomies was described in a canine model by Gilbert [67]. Tiedemann et al. assessed densitometric approaches to measure fracture healing in 6-mm tibial segmental defects and single-cut osteotomy defects in adult mongrel dogs [68]. Bilateral tibial transverse osteotomies were performed with a 2-mm gap by Markel et al. to quantify local material properties of fracture callus during gap healing [69]. To compare the dosage-dependent efficacy of recombinant human bone morphogenetic protein-2 (rhBMP-2) on tibial osteotomy healing, adult female dogs underwent right midshaft tibial osteotomies with a 1-mm gap. The operated bones were stabilized using type I external fixators [70]. In a similar study by Edwards, bilateral tibial osteotomies were performed to evaluate the capacity of a single percutaneous injection of rhBMP-2 delivered in a rapidly resorbable calcium phosphate paste (alpha-BSM) to accelerate bone-healing [71]. The effect of shock wave therapy on acute tibial fractures was studied by Wang et al. in adult dogs after creation of bilateral tibial osteotomies with a 3-mm defined fracture gap [72]. Similar models were also described by Hupel to compare the effects of unreamed and reamed nail insertion [73], Jain et al. [74] to investigate whether or not the limited contact design of the low-contact dynamic compression plate (LC-DCP) provides advantages over the dynamic compression plate (DCP) in the context of cortical bone blood flow, biomechanical properties, and remodelling of bone in segmental tibial fractures and Nakamura [75] to evaluate effects of recombinant human basic fibroblast growth factor (bFGF) on fracture healing in beagle dogs.

As previously mentioned, mature sheep and goats possess a bodyweight similar to adult humans, show no major differences in bone mineral composition with similar metabolic and bone remodelling rates, and therefore are considered a valuable model for human bone turnover and remodelling activity often used in fracture research. In the period between 1990 and 2001, sheep as an animal model were used in 9-12% of orthopaedic research, compared to only 5% between 1980 and 1989 [43]. Over the last ten years numbers of studies utilizing sheep and goats as animal models have even increased to 11-15% [49].

The significance of postoperative mechanical stability for bony repair of a comminuted fracture was investigated in a sheep study comparing four commonly applied operative methods of stabilizing fractures. In this study, a triple-wedge osteotomy of the right sheep tibia was used as a fracture model [76]. Using a standard osteotomy of the ovine tibia stabilised by an external skeletal fixator, Goodship et al. elucidated the influence of fixator frame stiffness on bone healing rates [77]. Wallace et al. [78] used a similar model to investigate serum angiogenic factor levels after tibial fracture. Likewise, transverse mid-diaphyseal osteotomies with an interfragmentary gap of 3 mm, as an experimental fracture model in sheep, were used to assess fracture repair processes [79-82]. To validate the principle of external fixation dynamization in order to accelerate mineralized callus formation by *in vivo* measurements of callus stiffness, transverse fractures with an interfragmentary gap of 3 mm width were created in the mid third of the tibial diaphysis [83]. Hantes et al. investigated the effect of transosseous application of low-intensity ultrasound on fracture-healing in a midshaft osteotomy sheep model [84]. Epari et al. were the first authors to reported on the pressure, oxygen tension and temperature in the early phase of callus tissue formation of six Merino-mix sheep that underwent a tibial osteotomy to model fracture conditions [85]. In this study, the tibia was stabilized with a standard mono-lateral external fixator. It was found that the maximum pressure during gait increased from three to seven days. During the same interval, there was no change in the peak ground reaction force or in the interfragmentary movement.

Oxygen tension in the haematoma was initially high post-op and decreased steadily over the first five days. The temperature increased over the first four days before reaching a plateau on day four.

Mechanical strain during callus distraction is known to stimulate osteogenesis and it is so far unclear whether this stimulus can enhance the healing of a fracture without affecting bone length. Just recently, Claes et al., reported for the first time that a slow temporary distraction and compression of a diaphyseal osteotomy accelerates fracture healing [86] in a mid-diaphyseal osteotomy fracture model of the right tibia in sheep, stabilized by external fixation (Fig. 4).

3.2. Tibial segmental defect models

In order to ascertain whether newly developed bone graft substitutes or tissue engineered constructs (TEC) comply with the requirements of biocompatibility, mechanical stability and safety, the materials must be subject to rigorous testing both *in vitro* and *in vivo*. To extrapolate results from *in vitro* studies to *in vivo* patient situations however is often difficult. Therefore, the application and systematic evaluation of new concepts in animal models is often an essential step in the process of assessing newly developed bone grafts prior to clinical use in humans. To simulate human *in vivo* conditions as closely as possible, a variety of large critical sized tibial defect models - mainly in sheep - have been developed over the past decade in order to investigate the influence of different types of bone grafts on bone repair and regeneration. Critical sized segmental defects in long bones are usually defined by multiplying the shaft diameter by 2.0-2.5 [25, 34]. Interestingly, the method of producing the gap may influence the outcome of those studies. Kuttenberger et al.

could show that by using a CO_2-laser, the osteotomy ends were not as impaired in structure as when using an oscillating saw [87].

To evaluate the effects of different bioceramics on bone regeneration during repair of segmental bone defects Gao et al. [88] implanted biocoral and tricalcium phosphate cylinders (TCP) in sheep tibial defects of 16 mm length. The defects were stabilized medially using two overlapping contoured auto-compression plates of 4 mm thickness (8 and 6 holes) and cortical screws. When compared to TCP, with the biocoral implants, a significant increase in external callus and density was seen after three weeks and an increase of torque capacity, maximal angle of deformation and energy absorption could be measured after 12 weeks while microscopically osseointegration appeared better. However, in his study, Gao used both male and female animals with a relatively large variation in body weight. Both factors, gender and body weight are known to have an influence on bone regeneration due to effects both on the biomechanical environment and hormonal feed-back control mechanisms. Hence, variations in sex and body weight should be avoided by all means. The defect fixation method used in this study can most likely be interpreted as a means to countervail bending forces on the implant after earlier failures. However, defect fixation by overlapping plates is not necessarily *lege artis* and has never been introduced and applied clinically to our knowledge. Therefore, a thicker and hence, stiffer plate should be chosen instead.

Den Boer et al. reported a new segmental bone defect model where a 30 mm segmental defect was inflicted on sheep tibiae and stabilized by an interlocking intramedullary nail (custom made AO unreamed humeral nail). X-ray absorptiometry was applied to quantify healing [55]. Groups of this pilot study included untreated controls and autograft. After 12 weeks, despite higher bone mineral density in the autograft group, no significant difference in torsional strength and stiffness could be revealed. Since 33% of the control animals showed sufficient bridging of the defect, it needs to be questioned if the authors succeeded in establishing a reliable non-union model. Removal of the periosteum or a larger defect site might have been beneficial. In a subsequent study, the authors described the fabrication of biosynthetic bone grafts and their application in the very same animal model [4]. The five treatment groups included empty controls, autografts, hydroxyapatite alone, hydroxyapatite combined with rhOP-1, and hydroxyapatite with autologous bone marrow. At 12 weeks, healing of the defect was evaluated radiographically, biomechanically and histologically and revealed that torsional strength and stiffness were two fold higher for animals treated with autograft and hydroxyapatite plus rhOP-1 or bone marrow. Since healing was only evaluated after 12 weeks, no conclusions could be drawn regarding the process of healing. The mean values of both combination groups were comparable to those of autografts. A higher number of defect unions was described when hydroxyapatite plus rhOP-1 was applied rather than hydroxyapatite alone. Analysing this study, it has to be taken into account that animals treated with hydroxyapatite and bone marrow were of a different breed with a higher average body weight. Animals were held at a different holding facility and accustomed to unequal forage all of which could possibly influence study outcomes.

Bone healing in critical sized segmental diaphyseal defects in sheep tibiae was also investigated by Gugala et al. [3, 37]. Defects were bridged with a single porous tubular membrane or with anatomically shaped porous double tube-in-tube membranes. Membranes with different pore structures were applied alone and/or in combination with autologous bone graft. The diaphyseal defects were 40 mm in length and stabilized with a bilateral AO external fixator. Operated animals were 6 to

7 years of age. Of the six treatment groups however, only in groups where the defect was filled with autogenous cancellous bone graft and covered with a single perforated membrane or where the bone graft was administered in a space between a perforated internal and external membrane, could defect healing be observed. The authors partly contribute the healing effect to their membrane system; however a control group, where autologous bone graft is administered without any membrane was not described. It could also be criticized that post surgery animals were suspended in slings over the entire experimental period preventing the animals from sitting and therefore getting up, thus not reflecting the normal physiological load bearing conditions.

Wefer et al. [89] conducted a study to develop and test a scoring system based on real-time ultrasonography to predict the healing of a bone defect filled with a porous hydroxyapatite bone graft substitute or cancellous bone graft from the iliac crest and stabilized by anterolateral plate osteosynthesis. After sacrifice tibiae were tested in torsion to failure. The results were correlated with radiographic and ultrasound scores obtained. Sheep with ceramic implants that developed non-unions showed a significantly lower score than sheep with sufficient implant integration. A significant correlation between these scores and the biomechanical results was found. However, although the authors describe their 20 mm defect as a critical sized model, no control group was included for comparison. Hence, based on the clinical experience in sheep the contributing authors from three different research groups have gathered over the years, and the definition of a critical defect length requiring at least 2-2.5 times the diameter of the bone, the critical nature of the defect in this study can be questioned.

The effects of new resorbable calcium phosphate particles and paste forms, which harden in situ after application, on bone healing were investigated by Bloemers et al. [90]. They used a 30 mm segmental tibial defect fixed by a custom made AO unreamed interlocking titanium tibial nail. Twelve weeks after defect reconstruction, radiological, biomechanical, and histological examinations were performed. Radiographically, the resorbable paste group performed better than all other groups. Biomechanical tests revealed a significantly higher torsional stiffness for the resorbable calcium-phosphate paste group in comparison with autologous bone. The study indicated that new calcium phosphate based materials might be a potential alternative for autologous bone grafts in humans. As with several other studies critically reviewed in this article, animals of a minimum age of 2 years with a significant variation in body weight were used in this study. As mentioned before, it must be considered that secondery osteonal bone remodelling in sheep does not occur until an age of 7-9 years. Therefore, it might be difficult to extrapolate results from this study for applications in adult human patients as human bone primarily undergoes secondery osteonal bone remodelling. Insulin-like growth factor I (IGF-1) exerts an important role during skeletal growth and bone formation. Therefore, its localized delivery appears attractive for the treatment of bone defects. To prolong IGF -1 delivery, Meinel et al. entrapped the protein into biodegradable poly(lactide-co-glycolide) microspheres and evaluated the potential of this delivery system for new bone formation in a non-critical 10 mm segmental tibia defect [91]. The defect was stabilized using a 3.5 mm 11 hole DCP. Administration of 100 µg of IGF-1 in the microspheres resulted in bridging of the segmental defect within 8 weeks. To avoid excessive load on the operated limbs and fracturing of the freshly operated tibial defects, the animals were accommodated in a suspension system for a period of 4 weeks postoperatively thus preventing physiologic-like biomechanical conditions. When interpreting data published in this study, it must be taken into account that the close position of the screws to the defect proximally and distally, and the obvious fact that the screws at the defect site had not been inserted at a defined angle might have influenced and biased the outcomes.

In a 48 mm tibial defect model in sheep ceramic implants of 100% synthetic calcium phosphate multiphase biomaterial were evaluated [92]. The defect was stabilized with a 4.5 mm neutralizing plate. Although not reported by the authors, one can observe bent plates and axial deviations in presented x-ray and CT images, hence, from a clinical point of view, it must be concluded that the chosen fixation in that model seemed not to be sufficient (Fig. 5). The x-ray series of the 2 year animal suggests that the internal fixation device had been explanted 12-14 weeks post surgery, a fact not described and explained by the authors. Assuming recovery and bone regeneration without any complications, in human patients, internal fixation devices would usually not be removed until 12-18 months post implantation. Good integration between the ceramic implants and the adjoining proximal and distal bone ends was observed. A progressive increase in new bone formation was seen over time, along with progressive resorption of the ceramic scaffold. Based on x-ray analysis, at the one-year time-point, approximately 10% to 20% of the initial scaffold substance was still present, and after two years it was almost completely resorbed. The authors state that approximately 10-20% of the periosteum was deliberately left in situ as a source of osteogenic cells. However, one might conclude that this procedure appears to be rather difficult to standardize in order to develop a reproducible model.

Another study using an ovine segmental defect model investigated the influence of rhTGFβ-3 on mechanical and radiological parameters of a healing bone defect [93]. In 4-5 year old sheep, an 18 mm long osteoperiosteal defect in the tibia fixed with a unilateral external fixator was treated by rhTGFβ-3 delivered by a poly(L/DL-lactide) carrier, with the carrier only, with autologous cancellous bone graft, or remained untreated. Weekly *in vivo* stiffness measurements and radiological assessments were undertaken as well as quantitative computed tomographic assessments of bone mineral density in 4 week intervals. The follow up of the experiment was 12 weeks under partial weight bearing since animals were kept in a support system to prevent critical loads on the fixator and its interface to bone thus not reflecting physiological loading conditions. The 18 mm defect size described as spontaneously non-healing, might not have been sufficient to establish a non-union model in a fully weight bearing biomechanical environment. In the bone graft group, a significantly higher increase in stiffness was observed than in the PLA/rhTGFβ-3 group and a significantly higher increase than in the PLA-only group. The radiographic as well as the computer tomographic evaluation yielded significant differences between the groups, indicating the bone graft treatment performed better than the PLA/rhTGFβ-3 and the PLA-only treatment.

Sarkar et al. assessed the effect of platelet rich plasma (PRP) on new bone formation in a 25 mm diaphyseal tibial defect in sheep [94]. The defect was stabilized with a custom-made intramedullary nail (stainless steel, diameter proximal 12 mm, distal 10 mm) with two locking screws each proximal and distal. To reduce stress at the screw/bone interface, a custom made stainless steel plate was additionally applied medially representing an unconventional fixation method not found in the clinic. However, no reasoning for the additional medial plating was provided in the publication by the authors. Defects were treated with autogenous PRP in a collagen carrier or with collagen alone. A control group to establish the critical nature of the defect was not included and has therefore to be questioned. After 12 weeks, the explanted bone specimens were quantitatively assessed by X-ray, computed tomography (CT), biomechanical testing and histological evaluation. Bone volume, mineral density, mechanical rigidity and histology of the newly formed bone in the defect did not differ significantly between the PRP treated and the control group, and no effect of PRP upon bone formation was observed.

In 2007 Tyllianakis [95] determined the size of a bone defect that can be restored with one-stage lengthening over a reamed intramedullary nail in sheep tibiae. Sixteen adult female sheep were divided into four main groups: a simple osteotomy group (group I) and three segmental defect groups (10, 20, and 30 mm gaps, groups II-IV). One intact left tibia from each group was also used as the non-osteotomized intact control group (group V). In all cases, the osteotomy was fixed with an interlocked Universal Humeral Nail (UHN-Protek-Synthes). Healing of the osteotomies was evaluated after 16 weeks by biomechanical testing. The examined parameters were torsional stiffness, shear stress, and angle of torsion at the time of fracture. The regenerate bone obvious in x-rays in the groups with 10 and 20 mm gaps had considerable mechanical properties. Torsional stiffness in these two groups was nearly equal and its value was about 60% of the stiffness of the simple osteotomy group. Gradually decreasing stiffness was observed as the osteotomy gap increased. No significant differences were found among the angles of torsion at fracture for the various osteotomies or the intact bone. These results showed that the group with the 10 cm gap had 65% of the shear stress at failure compared to the simple osteotomy group.

Teixeira et al. treated tibial segmental defects of 35 mm size in both male and female sheep aged four to five months. Considering the age of the animals and the preservation of the periosteum, the critical size of this defect can be questioned and results cannot necessarily be extrapolated to adult humans, as described correctly by the authors. An empty control group was not included in the experiment. The bone defects in the diaphysis of the right hind limb were stabilized with a titanium bone plate (103 mm in length, 2 mm thickness, and 10 mm width) combined with a titanium cage. As reported by the authors, plate bending occurred in 42% of the animals and was partly attributed to the connection of the titanium cage to the plate. However, it appears that the bending of the plate was rather a result of insufficient thickness of the fixation device. The titanium cages were either filled with autologous cortical bone graft or with a composite biomaterial consistent of inorganic bovine bone, demineralised bovine bone, a pool of bovine bone morphogenetic proteins bound to absorbable ultra-thin powdered hydroxyapatite and bone-derived denaturized collagen. Bone defect healing was assessed clinically, radiographically and histologically. Titanium cages might keep implanted scaffolds and biomaterials in place initially and biomechanically support defect fixation, however, it must be taken into consideration that – since titanium is not resorbable – the cages might hinder complete bone remodelling in the long run.

Radiographic examination showed initial formation of periosteal callus in both groups at osteotomy sites, over the plate or cage 15 days postoperatively. At 60 and 90 days callus remodelling occurred. Histological and morphometric analysis 90 days post surgery showed that the quantity of implanted materials still present were similar for both groups while the quantity of newly formed bone was less ($p=0.0048$) in the cortical bone graft group occupying 51 +/- 3.46% and 62 +/- 6.26% of the cage space, respectively [96].

Recently, Liu et al. reported on the use of highly porous beta-TCP scaffolds to repair goat tibial defects [13]. In this study, fifteen goats were randomly assigned to one of three groups, and a 26 mm-long defect at the middle part of the right tibia in each goat was created and stabilized using a circular external fixator. In Group A, a porous beta-TCP ceramic cylinder that had been loaded with osteogenically induced autologous bone marrow stromal cells was implanted in the defect of each animal. In Group B, the same beta-TCP ceramic cylinder without any cells was placed in the defect. In Group C, the defect was left untreated. In Group A, bony union could be observed by gross view, X-ray and micro-computed tomography (μCT) detection, and histological observation at 32 weeks post-implantation. The implanted beta-TCP scaffolds were almost completely replaced by host bone. Bone mineral density in the repaired area of Group A was significantly higher than in Group B, in which scant new bone was formed in each defect and the beta-TCP hadn't been completely resorbed after 32 weeks. Moreover, the tissue-engineered bone of Group A had similar biomechanical properties as the contralateral tibia in terms of bending strength and Young's modulus. In Group C, little or no new bone was formed and non-union occurred, demonstrating the critical nature of the defect.

To investigate the effect of chondroitine sulphate on bone remodelling and regeneration, Schneiders et al. [97] created a 30 mm tibial mid-diaphyseal defect site and reconstructed it using hydroxyapatite/collagen cement cylinders. Defect stabilization was achieved by insertion of a universal tibial nail (UTN, Synthes, Bochum). However, to place the scaffold into the defect, the authors had to use a second operative aditus mid-diaphyseally. The published data suggest problems with defect fixation not only due to reported implant failures but also to clearly evident signs of locking bolt loosening, poor contact between bone and nail, and the proximal nail end extending into the articular space (Fig. 6), facts not reported by the authors. Moreover, it can be supposed that either the insertion of the nail or undesired movement of the loosened nail has caused damage to the cylindrical biomaterials at testing. When interpreting the acquired data, it also has to be taken into account that obviously no fabrication method has been described to reliably reproduce implants of corresponding geometrical shape.

The rapid progression of bone graft research and the great number of novel developments must be supported by systematic assessment based on clinical practicability and experience, the knowledge of basic biological principles, medical necessity, and commercial practicality. From our literature review, it can be concluded, that in a majority of the mentioned studies, follow up periods, which in most cases don't exceed 6 months, are not suitable to evaluate long term effects of bone substitutes and scaffolds on bone regeneration and remodelling, and to determine in vivo resorption kinetics of the respective biomaterial. Variations in defect sizes and methods of defect fixation as well as postoperative treatment and management concepts make it difficult to compare studies and draw reliable conclusions. The modifications of commercially available fixation devices and supporting systems to prevent peak loads from acting on implants suggest the occurrence of implant failures usually expected early after surgery. As a result, most experimental settings do not reflect the actual clinical conditions faced and impede the extrapolation of results.

4. *Conclusions and Opinions*

The reconstruction of large bone segments remains a significant clinical problem. Large bone defects occur mainly as a result of extensive bone loss due to pathological events such as trauma, inflammation, and surgical treatment of tumours. Present therapeutic approaches include the application of bone graft transplants (autologous, allogenic, xenografts), as well as implants made of different synthetic and natural biomaterials or segmental bone transport. However, no existing therapy has been proven to be fully satisfactory. A large number of research groups worldwide work on the development of new bone grafting materials, carriers, growth factors, and tissue engineered constructs for bone regeneration and are therefore interested to evaluate their concepts in reproducible large segmental defect models. The optimization of cell-scaffold combinations and locally or systemically active stimuli will remain a complex process characterized by a highly interdependent set of variables with a large range of possible variations. Consequently, these developments must be nurtured and evaluated by clinical experience, knowledge of basic biological principles, medical necessity, and commercial practicality. The area of bone tissue engineering which has its main focus on the development of bioactive materials depends on the use of animal models to evaluate both experimental and clinical hypotheses. To tackle major bone tissue engineering problems, researchers must rely on the functional assessment of biological and biomechanical parameters of generated constructs. However, to allow comparison between different studies and their outcomes, it is essential that animal models, fixation devices, surgical procedures and methods of taking measurements are standardized to achieve the accumulation of a reliable data pool as a base for further directions to orthopaedic and tissue engineering developments.

Table captions

Table 1: The table lists a selection of publications on segmental bone defect studies in sheep tibiae and summarizes animal age, selected defect size, defect fixation, animal housing as well as supportive devices. The majority must be considered short term studies where no complete bone remodelling can be expected during the experimental period. In many cases, authors fail to report important information concerning animal age, housing and supportive devices.

Table 2: Summary of advantages and disadvantages of common defect fixation methods

Table 3: Comparison of animal models for fracture and segmental bone defect research

Table 4: Summary of human and large animal bone properties

Table 5: Factors influencing the quality and quantity of bone healing in long bone critical-sized defects (CSD)

Table 6: Bone biomechanical properties of different animal species and humans

Figure captions

Fig. 1: The sheep has become a standard model for understanding the mechanical conditions that occur after injury and investigating surgical treatments such as segmental defect healing. However, limited work has been published on modelling this process. Hence, reconstruction of the musculoskeletal model of the sheep lower limb across the complete gait cycle was performed. The figure shows mechanical loading on mid-shaft level of the tibia (A). The bones are mainly compressed with minor shear forces. Compared to humans, the loading in sheep tibias is in pattern similar but in magnitude roughly half of the one in humans (B) [52].

Fig. 2: Ground and polished sections of MMA embedded sheep tibia stained with Toluidine blue. a) Plexiform/laminar appearance of cortical bone with longitudinally arranged vessels (arrows) between the bone lamellae. Absence of a clearly visible cement line between adjacent lamellae. Scale bar = 200µm. b) Remodelling of an area with originally plexiform bone which has been replaced by secondary osteons (*). Scale bar = 50µm. c) Remodelling of plexiform bone in the immediate neighbourhood of an implant. During healing and subsequent remodelling new bone is deposited in form of secondary osteons, seen in the upper part of the image (*). Scale bar = 50µm. d) Transversely cut secondary bone with numerous osteons that can be clearly distinguished. The cement lines that separate neighbouring osteons (arrows). Scale bar = 50µm.

Fig. 3: Ovine femoral multifragmentory fracture (AO type 32-C) right after (A) and 8 weeks post surgery (B).

Fig. 4: Histological pictures of the fracture callus (Paragon stain) of right tibiae, 8 weeks after surgery. Left side: temporary distraction animal with larger callus and bridging of the osteotomy gap at one site by calcified bone. The connective tissue has a light blue color. Right side: control animal with smaller callus (red) and remaining interfragmentary zone of fibrocartilage (violet).

Fig. 5: Implant bending 1.5 months after surgery (A) and tibial axial deviation 7.5 months post treatment (B).

Fig. 6: 3 cm tibial mid-diaphyseal defect site reconstructed with hydroxyapatite/collagen cement cylinders. Signs of locking bolt loosening (arrow) and the proximal nail end extending into the articular space are evident 12 weeks after implantation.

Fig. 7: Schematic representation of commonly applied methods for the fixation of segmental defects in large animal models. A) Plate fixation, B) External fixator, C) Intramedullary nail.

Fig. 8: 2-cm segmental bone defect in a sheep tibia stabilized with a narrow 4.5 mm LC-LCP plate (A); implant failure occurring 2 days post surgery (B).

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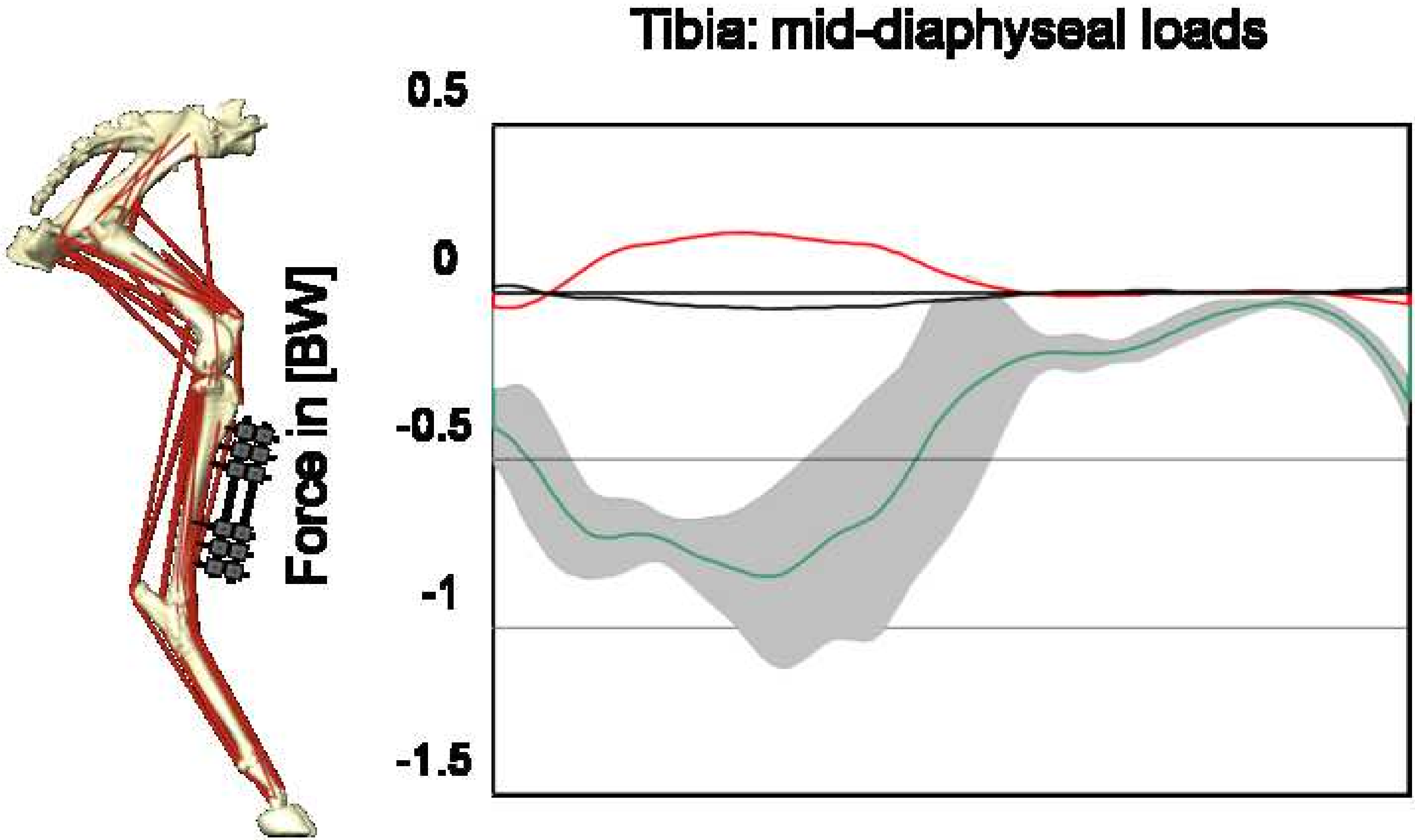

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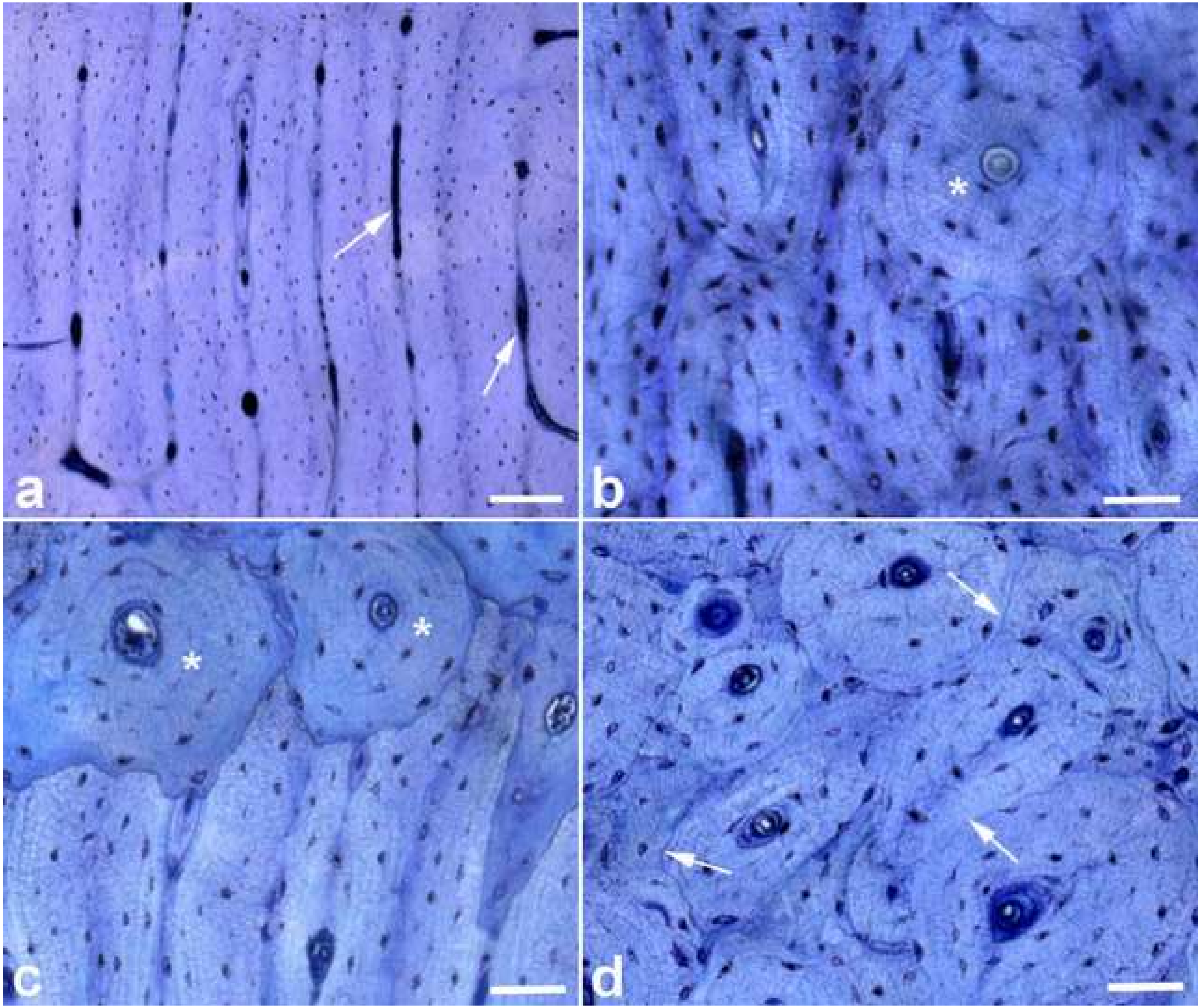

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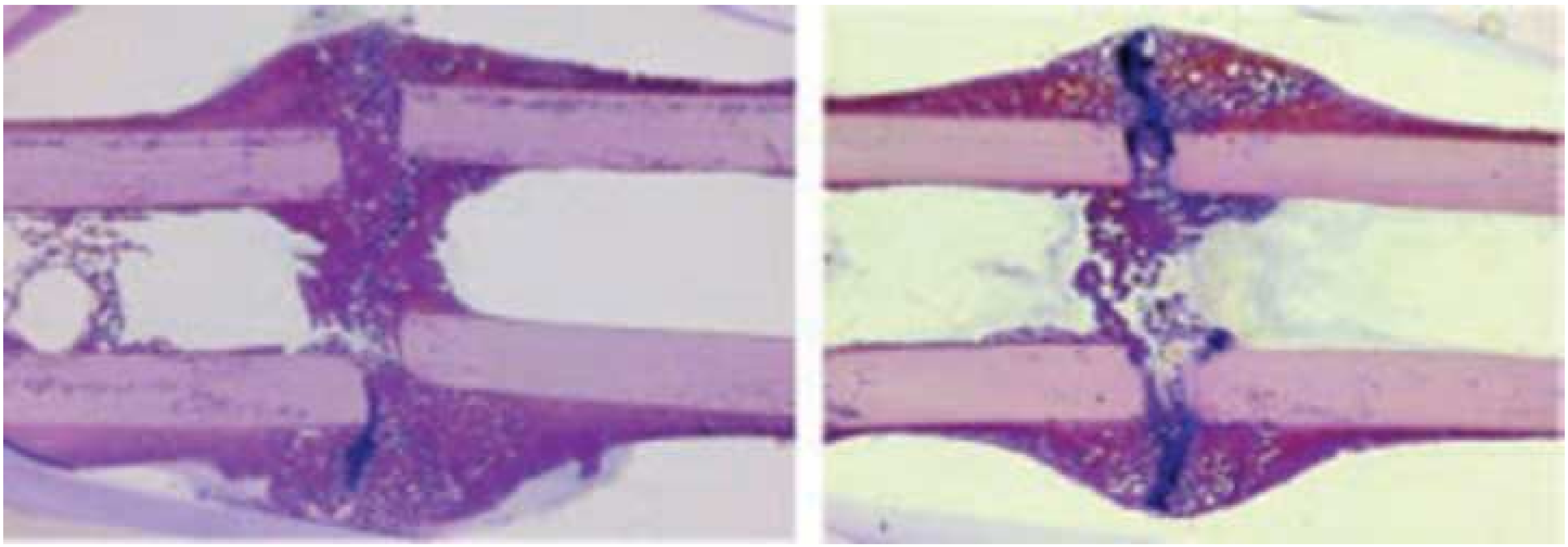

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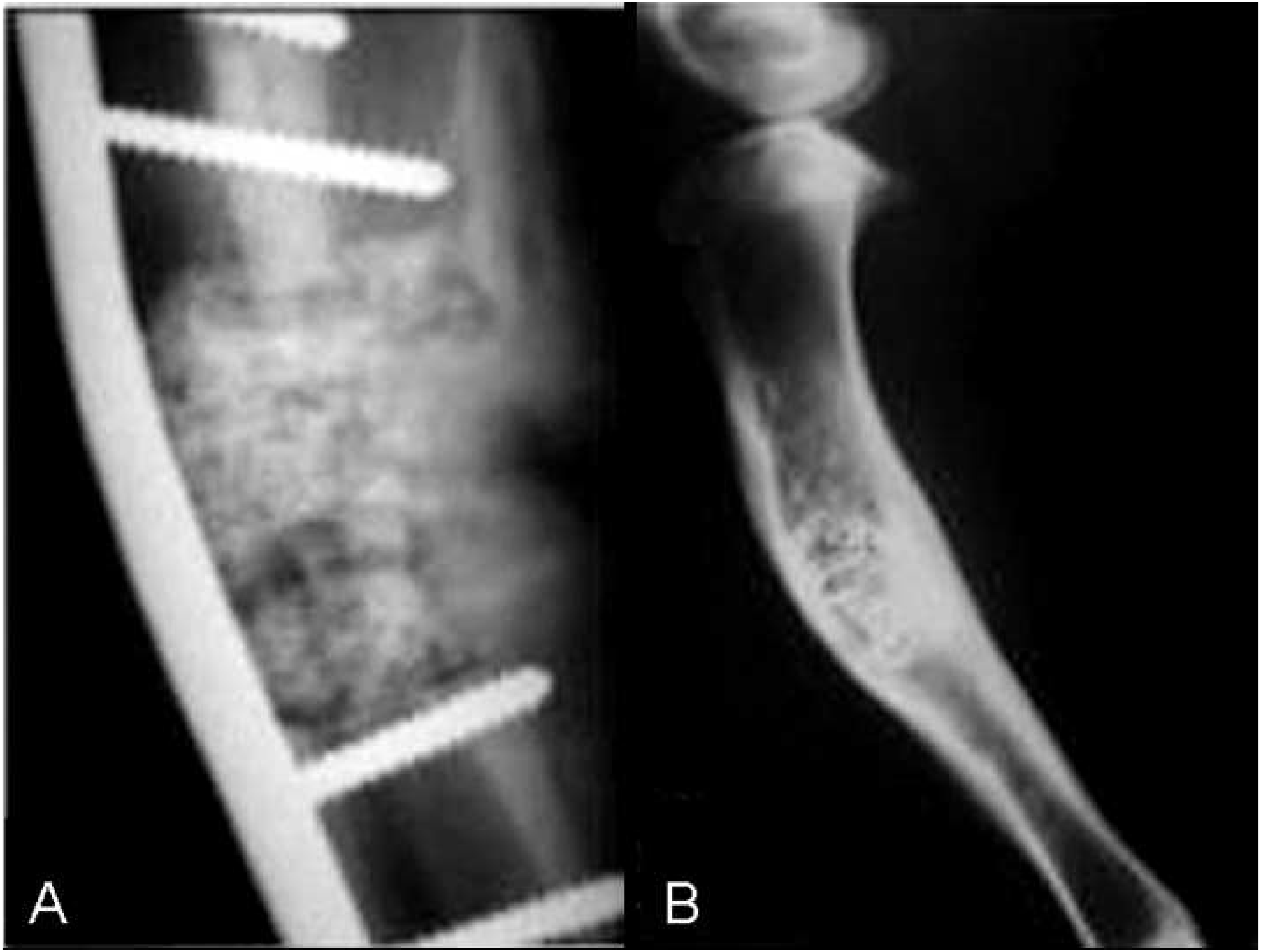

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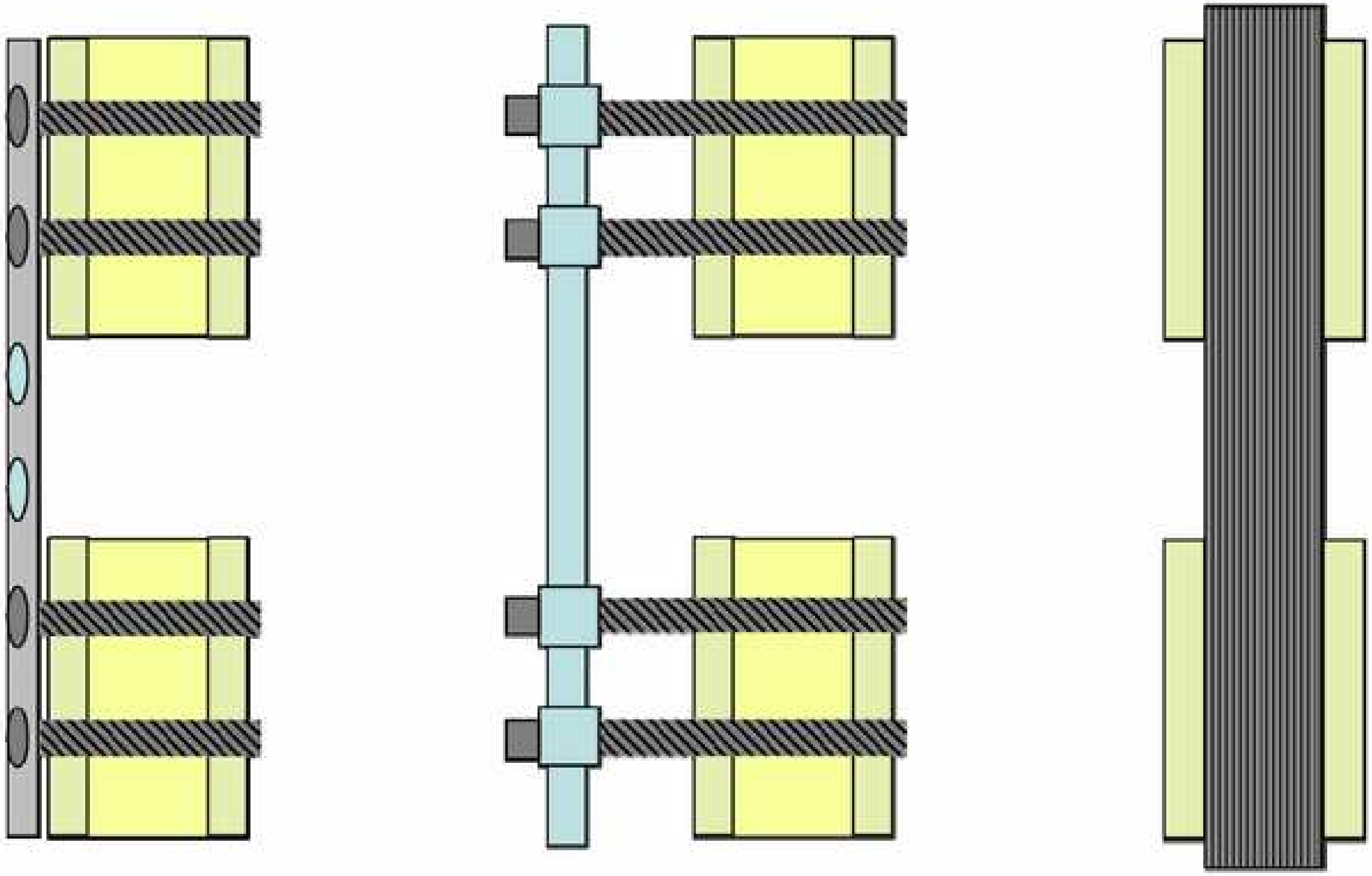

A. Plate fixation

B. External fixator

C. Intramedullary nail

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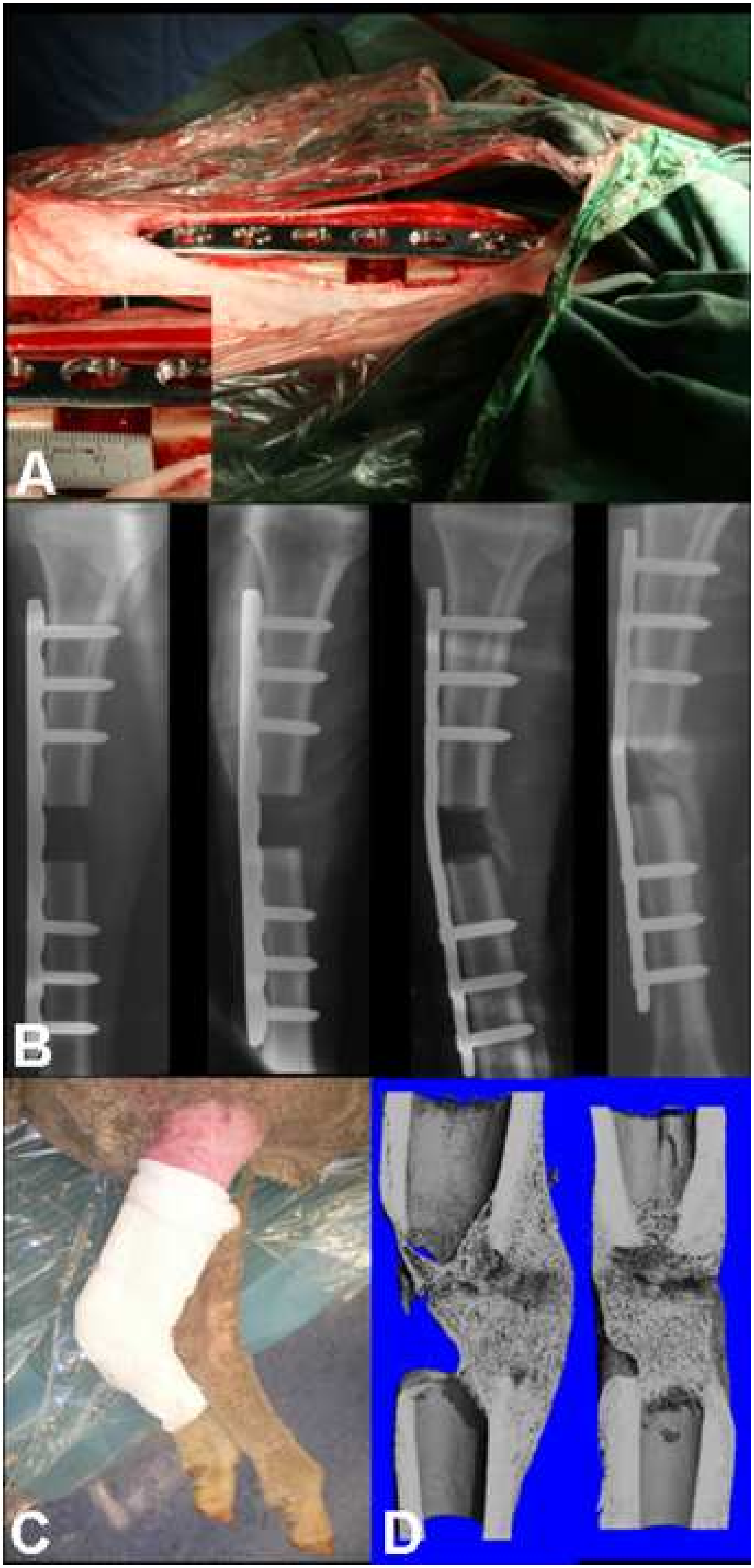


Table

Table 1:

Author	***Animal age (years)***	***Defect size (mm)***	***Follow-up (months)***	***Fixation***	***Animal housing***	*Support*
Gao et al., 1997	a	16	4	Overlapping autocompresson plates, 8 and 6 holes, 4 mm thickness	a	a
DenBoer et al., 1999/03	a	30	3	Custom-made AO unreamed nail (Synthes)	a	a
Gugala et al. 1999/02	6-7	40	4	AO bilateral external fixator	Single boxes	Suspending slings
Wefer et al., 2000	≥ 2	20	12	Anteerolat. plate osteosynthesis (not specified)	a	a
Bloemers et al., 2003	≥ 2	30	3	AO unreamed tibial nail (Synthes)	6-8 animals in a 60 m^2 cage	a
Meinel et al., 2003	a	10	5	3.5 mmm DCP, 11 holes	a	a
Mastrogiacomo et al, 2006	2	48	12	4.5 mm plate (not specified), 10-12 holes	Single boxes	Fibre glass cast
Maissen et al., 2006	4-5	18	3	Unilateral external fixator	Single boxes	Custom-made support system
Sarkar et al., 2006	5.5-7	25	3	Custom-made intramedullary nail plus medial stainless steel plate	Single boxes	a
Tyllianakis et al., 2007	1-2	10,20,30	4	Universal Humeral Nail (UHN, Synthes)	Single boxes for 3 days post surgery	a
Liu et al., 2007	a	26	8	Circular external fixator	Single boxes	a

[a]*information not provided by the authors*

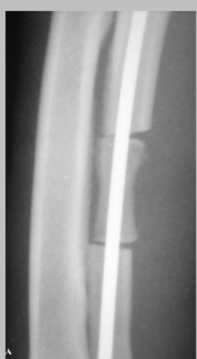
Table

Table 2:

	Advantages	***Disadvantages***
External fixator	- Versatility - Ease of application - Marginal effect on surrounding soft tissue - Minimal intraoperative trauma - Open space for implantation of biomedical construct	- Clinically only applied as temporary fixation device - Schantz screw loosening - Pin track infections
Intramedullary nail	- Standard treatment for diaphyseal fractures of lower extremity - Availability of UTN to avoid reaming related problems - Central load carrier - High tolerance of maximum applied forces - Low axial deviations - Reaming debris as possible source of multipotent stem cells	- Impairment of bone blood circulation by reaming - Thermal necrosis after reaming - Air or fat embolism - Failure of locking bolts - Limited application of load-bearing scaffolds - Prolonged healing period
Plate fixation	- Standard treatment for metaphyseal fractures - Optimal reduction - Minimal influence on defect (LC-LCP) - Open space for implantation of biomedical construct	- Eccentrical load carrier - Impairment of periosteal blood flow (non LCP) - Bone loss through stress protection (non LCP) - Unclear role of plate fixation in tibial shaft fractures - Prone to axial deviations and implant failure

Table

Table 3:

	Animal species and defect location	Defect formation	Defect fixation	Application
Animal Models of Fracture (Osteotomy) 	**Dog**[a] ○ Femur [1] [2] ○ Tibia [3] [4] [5] [6] [7] [8] [9] [10] ○ Radius [11] [12] [13] [14] **Sheep**[a] ○ Tibia [15] [16] [17] [18, 19] [20] [21] [22] [23] [24] **Goat** ○ Tibia [25] [26] [27] **Pig** ○ Pelvis [28] ○ Femur [29] ○ Tibia [30] [31] ○ Spine [32] [33]	○ Manually created fractures[b] ○ Three-point bending[b] ○ Guillotine-like apparatus[b] ○ Oscillating saw, high speed dental burr, or scissors[b] ○ Ballistic injury	**Internal** ○ Intramedullary rod/pin ○ Plate and screws ○ Screws ○ Cerclage wires **External** ○ External fixators ○ Casts ○ Splints **No fixation** Mice and rats[c]	○ Study of normal fracture healing ○ Drug delivery to fracture sites ○ Effects of - Drugs - Growth hormones - Angiogenic factors - LASER on fracture healing ○ Effect of fixation methods on periosteal, cortical and soft tissue blood flow [34] ○ Effect of type and rigidity of fixation on fracture healing and rate of remodelling [35] ○ Creation of non-unions [36] ○ Creation of an infected ballistic wound model ○ Evaluation of various assessments (e.g. x-ray) of fracture healing ○ In vitro testing of spinal fracture fixation systems
Animal Models of Segmental Bone Defect (Ostectomy) 	**Dog** ○ Calvaria [37] [38] [39] ○ Mandible [40] [41] [42] ○ Radius [43] ○ Ulna [44] ○ Femur [45] [2] [46] ○ Tibia [5] ○ Fibula [47], **Sheep** ○ Calvaria [48] ○ Mandible [49] [50] ○ Femur [51] [52] [53] ○ Tibia [54, 55] [56] [57, 58] [55] [59] [60] ○ Metatarsus [61] [62] [63] [64] **Goat** ○ Calvaria [65] ○ Mandible [66] ○ Iliac wing [67] ○ Femur [68] [69] [70] ○ Tibia [71] [72] ○ Radius [73] **Pig** ○ Calvaria [74] ○ Tibia [75] [76] [76] ○ Fibula [77] ○ Radius [78] ○ Spine [79]	○ Oscillating saw ○ Gigli's wire	**Internal** ○ Intramedullary nail/pin ○ Plate and screws **External** ○ External fixators	○ Study of integration, degradation and remodelling of bone substitutes – Bone grafting ▪ Autograft ▪ Allograft ▪ Xenograft – Biomaterials of natural and synthetic origin ▪ Demineralised bone matrix ▪ Biomaterials [80] 1. Hydroxyapatite/tricalcium-phosphate ceramics 2. Polymers 3. Metals 4. Composites ○ Bone substitutes plus autogenous bone marrow ○ Evaluation of osteogenic potential of cell-seeded composite implants ○ Assessment of osteoinductive properties of growth factors

[a] Most commonly used
[b] Methods to mimic accidental fractures more closely
[c] In radial, ulnar or fibular fractures where additional bony support is present

Table

Table 4:

	Micro-structure [81] [82] [83, 84]	Macro-structure [85] [86] [87]	Application [88]	Disadvantages [81] [89] [84] [88]	Advantages [88] [90] [89]	Bone remodelling[b] [88]	Bone composition [88]	Diameter (μ) of Haversian system [83]	Diameter (μ) of Haversian canal [83]
Dog	○ Woven-fibered bone (plexiform bone) ○ Secondary osteons (with small canals) increase in number with age	*Femur:* Pronounced curvature at distal third of shaft; narrow in the middle *Tibia:* Length Similar to sheep; proximally convex medially, distally convex laterally; proximal ⅓ prismatic, remainder cylindrical	Musculoskeletal and dental research	○ Higher rate of solid bony fusion when compared to humans ○ Low non-union rates ○ Ethical issues and negative public perception ○ Significant inter-animal variations due to breed diversity	○ Tractable nature ○ Similar bone mineral density to humans	100%	Bone mineral density similar to humans	125-175	15-50
Sheep	Primary bone structure (plexiform cortical bone) in young sheep (3-4 years of age)	*Femur:* Rounded (cylindrical) shaft; convex dorsally; curved in distal ⅔; regular in diameter *Tibia:* Major weight-bearing bone of crus; long and slender; shaft curved medially and caudally at centre; round in middle, triangular proximally, flattened cranio-caudally in distal third; medial surface is subcutaneous	Orthopaedic research	○ Age-dependant bone remodelling ○ Haversian remodelling at 7-9 years of age (with medium-sized, irregular canals)	○ Docile animals ○ Similar body weight to humans ○ Dimension of long bones suitable for human implants	Larger amount of bone in-growth than humans	Higher trabecular bone density than humans (0.61g/cm^3)	75-360	18-120
Goat	Non-homogeneously distributed Haversian systems	Almost identical to sheep	Research on cartilage, menisci and ligamentous repair	Inquisitive and interactive nature	More tolerant to ambient conditions	Similar to humans	Very similar to human	50-325	18-70
Pig	○ Plexiform bone (osteonal banding) ○ Dense Haversian bone (with medium canals), increases with age	*Femur:* Relatively wide & massive diaphysis with 4 surfaces *Tibia:* Slightly curved diaphysis, convex medially	Orthopaedic and dental studies (femoral head osteonecrosis, fractures, bone in-growth, dental implants)	○ High growth rates and excessive body weight ○ Difficult handling	Bone mineral density, anatomy, morphology, and healing similar to humans	Similar to humans	Bone mineral density similar to humans		
Human	○ Mainly circumferential lamellar bone ○ Woven-fibered bone (plexiform) formed only in rapid bone repair and remodelling ○ More Haversian bone than quadrupeds (12.87 vs. 5.5[a])	○ Epiphysis- proximal and distal-spongy bone ○ Metaphysis- transition zone-spongy bone distal to epiphyseal line ○ Diaphysis - shaft of the bone – compact bone				10-15% to 40-55%	Trabecular bone density: 0.43 g/cm^3	180-325	27-170

[a] Number of Haversian systems per square millimetre
[b] Average whole body trabecular bone turn over per year

Table

Table 5:

Factors determining a CSD [1] [2, 3]
○ Age ○ Species phylogeny ○ Defect size ○ Anatomic location ○ Bone structure and vascularisation ○ Presence of periosteum ○ Adjacent soft tissue ○ Mechanical loads and stresses on the limb ○ Metabolic and systemic conditions ○ Fixation method/stiffness ○ Nutrition

Table

Table 6:

	Bone biomechanical properties	
	Cortical bone	***Trabecular bone***
Dog	**Humerus (bending)** E: 2.66 (GPA) UStress 193.23 (MPa) [93]	**Femur** E: 209 (MPa) Ustress: 7.1 (MPa) **Tibia** E: 106-426 (MPa) Ustress: 2-24 (MPa) [81]
Sheep	**Femur (compression)** E:19.3 (GPa) UStrain: 0.019 [94] [95]	**Tibia** E: 1192 (MPa) Ustress: 21.4 (MPa) [96]
Goat	**Tibia (bending)** E: 278.08 (MPa) Bending strength: 46.24 (MPa) [71]	**Femur** E: 399-429 (MPa) Ustress: 14.1-23.5 (MPa) **Tibia** E: 532-566 (MPa) Ustress: 24.7-26.1 (MPa)
Pig	**Femur** E: 14.6 (GPa) (plexiform bone) 8.3 (GPa) (Haversian bone) [97]	**Femur** E: 5900 (MPa) [81]
Human	**Femur (compression)** E: 14.7-19.7 (GPa) UStrain: 167-215 (MPa) **Tibia (compression)** E: 24.5-34.3 (GPa) UStrain: 183-213 (MPa) [81]	**Femur** E: 298 (MPa) UStrain: 5.6 (MPa) **Tibia** E: 445 (MPa) UStrain: 5.3 (MPa) [81]

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Supplementary information

Intramedullary nailing

Intramedullary nailing was first introduced by Küntscher in 1953 [1] and once established, reaming soon advanced as a novel endorsement [2]. With the enhancement of intramedullary fixation devices and development of locked nailing, the indications for intramedullary stabilisation have been further expanded over the years making it a popular option in long bone fracture management. The treatment of diaphyseal fractures of the lower extremity has advanced as a commonly accepted standard treatment [3, 4]. Despite its wide-spread application, intramedullary nailing is associated with both positive and negative side aspects. It has been reported that the process of reaming leads to a considerable impairment of bone blood circulation by largely destroying both the intramedullary arterial and venous system [5,6]. The reaming procedure as well as the insertion of the nail causes a significant increase of intramedullary pressure. This increase in pressure can lead to both air or fat embolisms by intravasation of bone marrow or fat into the vascular system and the formation of microthrombi causing pulmonary microvascular damage [7, 8]. The process of reaming may also lead to a rise in temperature in the medullary canal. Rises in temperature of 50°C and more have been described [9, 10]. As a result, thermal necrosis of bone tissue with alteration of endosteal architecture and biological failure may be induced compromising fracture or defect healing [11]. In most cases however, the damage of local tissue is reversible and compensated for within 6-8 weeks. Weak spot of various unreamed nailing systems prone to failure pose the interlocking screws and small dimension solid tibial nails do not always provide adequate stability in the proximal and/or distal defect site. The rate of failure however could be reduced over the years with advances in surgical techniques, optimization of materials and with the use of e.g. angle stable locking bolts [12], and postoperative treatment concepts. When discussing intramedullary stabilization in the context of tissue engineering and the evaluation of biomaterials and constructs for bone graft substitution, it must be considered, that, in a created defect area, an intramedullary nail impedes the placement of a solid, one-piece load-bearing scaffold (Fig. 7). However, since central load carriers are less susceptible for tilting in the frontal plane, custom made intramedullary nailing systems have been widely applied for fixation of large segmental bone defects in animal models [13-16].

Reaming mobilizes cancellous bone within the medullary canal which is likely to assemble in the area of the fracture gap. The effects of the collected material at the gap are comparable to conventional bone graft from the iliac crest suggesting that reaming debris represents a possible source of multipotent stem cells and growth factors enhancing regeneration processes [17-19].

Internal plate fixation

While intramedullary stabilisation is the treatment of choice when reconstructing closed diaphyseal shaft fractures, metaphyseal fractures are usually stabilised by extramedullary implants, preferably angle-stable plates, pre-shaped in an anatomical fashion where applicable [4]. Optimal reduction and good stability even in complicated fractures can be achieved by plate and screw osteosynthesis [20, 21]. After conventional plate osteosynthesis however, in most cases histologically and radiologically verifiable bone loss can be detected (principal of friction). This phenomenon has been attributed to stress protection according to Wolff's law. Clinical observations and recent studies however have revealed the occurrence of certain porosity within the cortical bone proximate to the plate during the early phase following plate osteosynthesis. This porosity was found to be a result of impaired perfusion underneath the plate [22]. The decrease in perfusion in proximity to the plate results from high compression forces between plate on the one hand and periosteum and bone on the other [23]. The contact pressure and the resulting circulatory disturbance prolongate fracture healing and increase the risk of infection and refracture after implant removal [24]. To overcome these drawbacks, a plate system was developed where load and torque transmission can act through the screws only. This was achieved by angle-stable, interlocking screws. As a result, to achieve a stable fracture fixation, the plate-bone contact was not further necessary as the stabilisation system acted rather like an internal fixator. The application of monocortical screws can further minimize screw-related intramedullary circulatory disturbances [25]. The development of such new biological techniques and implants has revived the interest towards open reduction and plate fixation. Nevertheless, the exact role of plate fixation in the treatment of tibial shaft fractures remains unclear, as the literature is lacking randomised control trials comparing plate fixation with the other established treatment concepts. In the area of tissue engineering and related animal experiments, defect fixation with internal fixators offers the great advantage of a minimal influence of the fixation device on the created defect site both concerning space for scaffold implantation and biological factors. When compared to external fixators or tibial nails, rates of infections (pin-track infection), infection related complications and non-union rates (6-25%) are lower. However, higher numbers of malalignment can be observed [26]. Especially in large animal models, defect fixation with eccentrically placed devices seems to be challenging and was used only in few studies [27-29] (Fig. 8). Since in most reports, complications, implant failures, postoperative treatment and animal care are not published and authors have described unconventional methods of defect fixation such as the use of overlapping plates [30] and the use of other supportive devices it can be assumed that implant modifications and the use of supportive devices were a necessary protective measure to overcome implant failures early after surgery resulting from critical loads/valgus stress occurring during the act of the animal uprising with the relatively long tibia serving as the lever arm of the force.

External fixator

In clinical settings, external fixation is often used as a temporary fixation device in patients with severe open or contaminated fractures or in the case of multiple traumas. Switch from external to internal fixation is best done as soon as the soft tissue problems are resolved [31, 32]. In animal models, external fixators have been widely used as they offer versatility and ease of application [33-35]. External fixators affect the surrounding soft tissue only marginally due to minimal intraoperative trauma. The most common complication when using external fixation systems is loosening of Schanz screws and following pin track infections. When compared to intramedullary nails, external fixators don't affect the defect site as extensively not limiting space for the implantation of biomedical, tissue engineered constructs. However, with external fixators healing periods are reported to be significantly longer when compared to other fixation devices [36]. Moreover, external fixators may be a burden for the animals as they represent an obstacle larger than the physiological circumference of the animal limb.

Mueller et al reported on a study designed to identify what levels of primary stability could be achieved with different forms of osteosynthesis in the treatment of diaphyseal fractures of the tibia. Treatment concepts included unreamed tibial nails (UTN), cannulated tibial nails (CTN), lateral tibial head buttress plates (LPO, Synthes), and 5-hole proximal lateral tibia less invasive stabilising systems (LISS), external fixators and hybrid fixators (Synthes) [37]. It was shown that the nailing systems as central load carriers tolerate higher maximum applied forces. The lowest axial deviations in varus and valgus direction were again described for the intramedullary nailing devices while the highest axial deviations were recorded for the plate fixations.

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