ECRONICON OPEN ACCESS

EC ORTHOPAEDICS
Research Article

A Review of Major Animal Models Relevant to Contemporary Orthopaedic Repair of the Appendicular Skeleton in Humans

(Part 1: healing in the presence of bone defects, non-union and inter-current disease)

Reeve-Johnson L[1,2]* and Schuetz M[1, 2, 3]

[1]*Translational Research Institute, Brisbane, Australia*

[2]*Institute for Health and Biomedical Innovation, Queensland University of Technology, Brisbane*

[3]*Department of Trauma, Princess Alexandra Hospital, Brisbane, Australia*

***Corresponding Author:** Professor Lloyd Reeve-Johnson, Institute for Health and Biomedical Innovation Queensland University of Technology, Kelvin Grove, Brisbane, Australia.

Received: September 22, 2016; **Published:** October 22, 2016

Abstract

Animal models have long been used as a bridge from *in vitro* to the human patient to provide insights both as a precursor and valued addition to human-based research. Many animal models have been developed to investigate bone regeneration and repair. Animal models continue to have a place in orthopaedic innovation and development. Ethical constraints as well as reasons of experimental design limit direct progression to human clinical studies. Used with discretion to limit the impact on animal welfare, each species provides a unique perspective on the performance of prostheses, healing and repair processes in different scenarios. Although results often cannot be directly 'translated' to human patients, these provide invaluable windows of enlightenment to broaden our perspective on future innovative approaches. Some species such as the fish are cheap and bone changes can be directly visualised in living subjects. Others such as the mouse have been selectively bred for genotypic traits. Larger animal models may be more suitable in terms of size, to test scale versions of prosthetics and to evaluate mechanical properties. The objective of this paper is to review animal models relevant to contemporary orthopaedic surgery of the appendicular skeleton. To help guide readers to relevant data, we have structured this review by major animal species and separated the models into those considering either mechanical healing (including bone defects) and healing in the presence of other disease factors including infection to provide a resource to help future researchers locate definitive study references. Metabolic disease including osteoporosis and comparative imaging are considered separately in the follow-on review paper. The intention is to provide a resource for quick reference of established models in major non-primate species and to maximise the value of previous work on animals models used to study orthopaedic repair in the appendicular long bones.

Keywords: *Translational research; Bone healing; Animal models; One Health*

Introduction

The etymological basis: orthos (straight) and paidos (child) from Greek coined by Nicholas Andry (1741) was intended to describe 'preventing and correcting limb deformities of children'. The conceptual model he used to illustrate this was a twisted young tree tied to a straight pole. Knowledge of traction, reduction and the value of early mobilisation were understood centuries before this and there are detailed descriptions by Hippocrates around 400BC [1,2] and over subsequent centuries by Galen, Versalius, Morgagni, Roger of Padua,

Leonardo da Vinci, Havers, Fabricius and many others [3]. Comparative learning in orthopaedics has been a constant theme through the centuries. In the book "*De Motu Animalum*", published in Rome in 1608, Borelli who studied under Gallileo, compared muscle contraction of man and animals. He analysed the mechanics of walking, running, jumping, weight-lifting, bird flight, fish motion, and movement of insects. Renowned surgeon, John Hunter (1728-93) accumulated a substantive evidence-base from comparative anatomy and pathology. What is notable in the Huntarian Museum in London is his propensity to innovate and draw inspiration from multiple animal species and translate this to human patients.

Internal fixation began in 1895 when Lane introduced the metal plate. Treatment of fractures has evolved with the use of plates, screws, and other devices and introduction of high tensile strength inert alloys. The three-phlanged nail devised by Smith Petersen in 1931 represented an important advance for treatment of fractures in the neck of the femur. Intra- medullary nailing of fractures of the shaft of the femur with the Kiintscher nail was used extensively during the World War II. Internal fixation of shaft fractures however interferes with natural callus formation and delays healing.

Otto Stader, a veterinarian introduced external fixation in 1931 to stabilize fractures in dogs. During the 1940s and 50's veterinarian Jacques Jenny performed one of the first intramedullary pinning procedures in animals and his experiences with high tensile loads significantly advanced fracture repair strategies in horses and humans. In 1966, Sten-Erik Olsson and John L. Marshall, both of whom had medical and veterinary medical degrees, founded the first laboratory dedicated to comparative orthopaedic research at the Hospital for Special Surgery in New York. More recently, interlocking nailing has become an effective alternative to bone plating and plate-rod fixation as a standard technique in people. Indications for interlocking nailing have expanded to include treatment of periarticular fractures, corrections of angular deformities and revisions of failed plate osteosynthesis.

Improved surgical techniques and applied bioengineering have changed orthopaedic practice over the past 50 years, hip and knee arthroplasty being good examples. Arthoscopy, a revolution in its time, increased throughput massively while decreasing the cost of each intervention to the extent that it is widely and successfully used in man, horses and dogs.

Future changes in orthopaedics are likely to be driven by the ability to understand and alter cell function and apply innovation widely, safely and inexpensively to a much greater proportion of the global population. While much effort is being devoted to the study of skeletal connective tissue and their matrices, there is as much progress to be made in applying and disseminating innovation in a way that is simple and cheap enough to impact the larger proportion of the human population who even in the 21st century are denied interventions based on cost and access. Four billion people (the majority of the world's population) earn less than US $3000 per year, yet have the greatest potential to benefit from health care and medical innovations [4,5].

The use of animal models as a bridge from *in vitro* to the patient have been use in many situations [6] and these compliment inherent challenges of human-based research. Many animal models have been developed to investigate bone regeneration and repair. The objective of this paper is to review animal models relevant to contemporary orthopaedic surgery of the appendicular skeleton. To help guide readers to relevant data, we have structured this review by major animal species and separated the models into those considering either mechanical healing (including bone defects) and healing in the presence of other disease factors including infection. Metabolic disease including osteoporosis and comparative imaging are considered separately in the follow-on review paper.

Challenges of human-based research

Ioannidis [7] makes the point that there is increasing realization that human research findings often have low validity when applied to target populations. The probability that a research claim is true depends upon study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationship in study outcomes. He illustrates that a research finding is less likely to be true when the studies conducted are small; when effect sizes are small; when there is a greater number or less pre-selection of tested

relationships; where there is greater flexibility in designs, definitions, outcomes, analytical modes or selection of secondary variables where positive findings emerge; when there is greater financial and other interest or prejudice; and when more teams are involved in a scientific field pursuing statistical significance. Simulations show that for most study designs and settings, it is more likely for a research claim to be false than true. Moreover, for many current scientific fields, claimed research findings may often be simply accurate measures of the prevailing bias. Reeve-Johnson [8] points out that animal models offer the advantage of greater control over experimental design and particularly the randomisation between interventions, treatment compliance is not an issue and end-points can be predetermined. The facility to perform elective necropsy substantially increases our understanding of underlying pathology and the healing process.

A major problem is that it is impossible to know with absolute certainty what the truth is in any research question. There are several approaches to improve the post-study probability. Better powered evidence, e.g. large studies or low-bias meta-analyses, may help. However, a review of meta-analyses by Pigott., *et al*. [9] indicated a propensity to publish positive (rather than negative findings) and a tendency to either exclude pre-specified study variables from publication or highlight secondary outcomes when favourable, which introduces considerable bias into meta-analytical reviews of published data. Even large studies have biases which should be acknowledged and avoided. Large-scale evidence should be targeted for research questions where the pre-study probability is already high, so that a significant research finding will lead to a post-test probability that would be considered definitive. Large-scale evidence is particularly indicated to test major concepts in contrast to specific questions. However, there is a need to be cautious that extremely large studies may be more likely to find a 'statistically significant' difference for a trivial effect that is not meaningfully different from the null. Often research questions are addressed by many teams, and it is misleading to emphasize statistically significant findings of any single team. What matters is the totality of the evidence [7]. Rather than statistical significance, we should improve our understanding of *R* values (the pre-study odds) and before an experiment, investigators should consider what they believe the chances are that they are testing a true rather than a non-true relationship. Animal models can address many of these issues through being tightly controlled experiments specifically powered to focus on specific research questions in the absence of confounding factors such as concomitant pathology, variable compliance, lifestyle or medications.

Types of study design

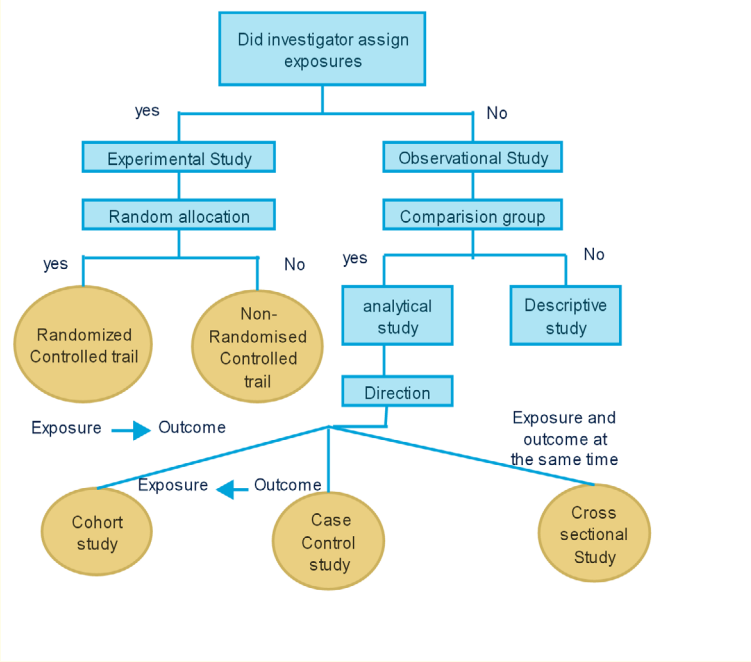

Figure 1, illustrates typical study designs, however in human ethical constraints limit the capacity to conduct truly randomised controlled experiments, especially when either the intervention (or lack of intervention) brings with it a known risk.

Bone regeneration

Ultimately, bone regeneration and strength underpins successful and timely fracture repair. The healing of sizeable bone deficits after trauma or tumour removal remains a significant challenge for surgeons. Bone repairs through a very efficient regenerative process in majority of healthy patients, yet many factors can cause delayed or impaired healing. To date, there are no reliable biological parameters to predict or diagnose bone repair defects. Orthopaedic surgeons mostly base their diagnoses on radiographic analyses. With the recent progress in our understanding of the bone repair process, new methods may be envisioned. Animal models allow us to define key steps of bone regeneration, the biological and mechanical factors that influence bone healing and evaluate new imaging techniques.

Fracture healing of long bones in various animal models can provide new perspectives relevant to humans, however, differences between the micro-and macrostructure need to be understood. There are also differences between osteotomy and artificial fracture models as well as the impacts of implanted material upon bone regeneration and the physiological stressors placed upon bone in different species. Clearly, there are limitations to the validity of each animal model.

A review of major contemporary animal studies relevant to long-bone healing are presented as an up-to-date reference source to help guide the selection of model.

Piscine models

Piscine models have the advantage of rapid healing, the ability to cheaply maintain many replicated experimental units with relative ease of manipulation and often direct visualisation, particularly of fin bones. However, the genomic and proteomic differences in taxonomic terms are a difference in class, implying that the validity of the data is challenged even if this type of model provides insight into the mechanism of healing.

One useful example is that while osteoblasts and osteoclasts have been shown to enter fracture sites it remains unknown how and where osteoclasts and osteoblasts are induced in a living animals. Takeyama., *et al*. [10] developed a fracture healing model by using fish (medaka). One side of lepidotrichia were fractured in a caudal fin ray without injuring the other soft tissues including blood vessels. Using the transgenic medaka in which osteoclasts and osteoblasts could be visualized by GFP and DsRed, respectively, two different types of functional osteoclasts were observed to have been induced before and after osteoblast callus formation. The early-induced osteoclasts resorbed the bone fragments and the late-induced osteoclasts remodelled the callus. Both types of osteoclasts were induced near the surface on the blood vessels, while osteoblasts migrated from adjacent fin ray. Transmission electron microscopy revealed that no significant ruffled border and clear zone were observed in early induced osteoclasts, whereas the late-induced osteoclasts had clear zones but did not have the typical ruffled border. In the remodelling the callus, the expression of Cox2 mRNA was upregulated at the fracture site around vessels, and the inhibition of Cox2 impaired the induction of the late-induced osteoclasts, resulting in abnormal fracture healing.

Murine models

Mouse models are frequently used for fracture healing studies. Many inbred strains are available. Standardized and mechanically controlled fracture healing models are strongly indicated, because mechanical conditions considerably influence the fracture healing outcome. Standardized fracture fixation techniques for the mouse are technical challenging due to the small skeleton. Several different fixation devices are commercially available for the mouse femur allowing increasingly controlled fracture healing studies. Intramedullary nails, plates and external fixators can be used. Each devices has various advantages and disadvantages. Deletion, over-expression or ectopic expression of a gene of interest help elucidate the physiological or pathological role. Mouse models represent a very worthwhile

tool for fracture healing research particularly where the genetic basis of fracture healing are of interest. Clearly, the limitation of size restricts the types of implants that can be usefully tested and mechanical differences challenge direct translation of data to humans and other species.

Despite growing knowledge on the mechanisms of fracture healing, delayed healing and nonunion formation remain a major clinical challenge. Small animal fracture models are very useful for fracture healing studies because they allow standardized, defined study conditions, with carefully controlled variable when designing fracture healing experiments in mammalian species. The genotype (strain), age and sex of the animals all influence the process of fracture healing. Furthermore, the choice of fracture fixation technique, intra- and extramedullary implants and open and closed surgical approaches also affect outcome. A variety of different, highly sophisticated implants for fracture fixation in small animals have been developed. The review by Hildebrand., *et al*. [11] is a useful critique of the advantages and pitfalls of the different fixation techniques in rats and mice. While that by Garcia., *et al*. [12] summaries the value of different approaches to study normal and delayed fracture healing as well as nonunion formation, and discusses different methods of data evaluation in mice and rats.

Rigid fixation with locking plates or external fixators have been shown to result in predominantly intramembranous healing in both mice and rats. Locking plates, external fixators, intramedullary screws, the locking nail and the pin-clip device allow different degrees of stability resulting in various amounts of endochondral and intramembranous healing. Analyses including biomechanical and histological evaluations as well as molecular mechanisms of fracture healing using widely available spectra of antibodies and gene targeted animals to study molecular mechanisms of fracture healing make these very flexible tools.

Healing, bone defects and non-union

The achievement of proper fixation with a murine model is challenging due to the small dimensions of the murine femur. Zwingenberger., *et al*. [13] attached an external fixation device to the right femur of 30 mice. Femoral bone defects of 1 mm (n = 10), 2 mm (n = 10), and 3 mm (n = 10) were created. Wounds were closed without any additional treatment. To investigate bone healing during the 12-wk observation period, x-ray analysis, histomorphology, immunohistochemistry, and mu CT scans were performed. Micro-CT analyses after 12 wk showed that 3/81-mm defects, 5/82-mm defects, and 8/83-mm defects remained as non-unions. The defect volumes were 0.36 +/- 0.42 mm (1-mm group), 1.40 +/- 0.88 mm (2-mm group), and 2.88 +/- 0.28 mm ($P < 0.001$, between all groups). They concluded that using external fixation, a defect size of 3 mm is necessary to reliably create a persisting femoral bone defect in nude mice.

Alford., *et al*. [14] showed that unilateral tibial fracture induced a time-dependent upregulation of the 200-kDa osteoblast species at the site of trauma. By contrast, relative levels of the 125-kDa osteoblast species at the fracture site were lower than in bones from naive control animals. In the contralateral untouched control tibia, the 200-kDa species was rapidly and substantially reduced compared to bone harvested from naive control mice. Levels of the 125-kDa species in the untouched tibia declined gradually with time post-fracture. TSP2 gene expression in uninjured control bone decreased modestly by 21 days post-fracture. On the day of fracture, the osteoblast differentiation potential of Mesenchymal Stem Cells harvested from uninjured bones decreased compared to those harvested from naive control animals. The presence of two isoforms suggests that TSP2 may undergo post-transcriptional or posttranslational processing in skeletal tissue. In the context of trauma, the two TSP2 isoforms appear to be differentially modulated at injured and non-injured skeletal sites in an animal undergoing fracture healing.

Kondo., *et al*. [15] used a murine model to demonstrate that C-type natriuretic peptide (CNP) is a potent stimulator of endochondral bone growth using elevated plasma CNP concentrations (SAP-CNP-Tg mice). Microcomputed tomography (CT) analysis revealed less bone in femurs, but not in lumber vertebrae, of young adult SAP-CNP-Tg mice than that of wild-type mice. Bone histomorphometry of the tibiae from 8-week-old SAP-CNP-Tg mice showed enhanced osteoblastic and osteoclastic activities, in accordance with elevated serum levels of osteocalcin and tartrate-resistant acid phosphatase-5b, respectively. They performed an open and stabilized femoral fracture

using 8-week-old SAP-CNP-Tg mice and compared the healing process with age-matched wild-type mice. Immunohistochemical study revealed that CNP and its receptors, natriuretic peptide receptor-B and natriuretic peptide clearance receptor, are expressed in hard calluses of wild-type mice, suggesting a possible role of CNP/natriuretic peptide receptor-B signaling in fracture repair, especially in bone remodeling stage. On micro-CT analysis, a rapid decrease in callus volume was observed in SAP-CNPTg mice, followed by a generation of significantly higher new bone volume with a tendency of increased bone strength. In addition, a micro-CT analysis also showed that bone remodeling was accelerated in SAP-CNP-Tg mice, which was also evident from increased serum osteocalcin and tartrate-resistant acid phosphatase-5b levels in SAP-CNP-Tg mice at the remodeling stage of fracture repair. These results indicate that CNP activates bone turnover and remodeling *in vivo* and possibly accelerates fracture healing.

Healing in the presence of inter-current disease

Impaired healing and non-union of skeletal fractures is a major public health problem, with morbidity exacerbated in patients with diabetes mellitus (DM). DM affects approximately 25.8 million US adults, with > 90% having obesity-related type 2 DM (T2DM). Bleedorn., *et al*. [16] used 5 week old male C57BL/6J mice and placed them on either a control lean diet or an experimental high-fat diet (HFD) for 12 weeks. A mid-diaphyseal open tibia was induced at 17 weeks of age and a spinal needle was used for intra-medullary fixation. Mice were necropsied at days 7, 10, 14, 21, 28, and 35 for micro-computed tomography (mu CT), histology-based histomorphometry and molecular analyses, and biomechanical testing. HFDfed mice displayed increased body weight and impaired glucose tolerance, both characteristic of T2DM. Compared to control mice, HFD-fed mice with tibia fractures showed significantly ($p < 0.001$) decreased woven bone at day 28 by histomorphometry and significantly ($p < 0.01$) decreased callus bone volume at day 21 by mu CT. Fracture calluses were found to contain markedly increased adiposity in HFD-fed mice at days 21, 28, and 35. HFD-fed mice also showed increased PPAR gamma immuno-histochemical staining at day 14. Calluses from HFD-fed mice at day 35 showed significantly ($p < 0.01$) reduced torsional rigidity compared to controls. This murine model of T2DM demonstrated delayed fracture healing and weakened biomechanical properties, characterized by increased callus adiposity. This suggests altered mesenchymal stem cell fate determination with a shift to the adipocyte lineage at the expense of the osteoblast lineage. The up-regulation of PPAR gamma in fracture calluses of HFD-fed mice may be involved in the proposed fate switching.

Sottnik., *et al*. [17] report that clinical studies over the past several years have indicate that metastasis-free survival times in humans and dogs with osteosarcoma are significantly increased in patients that develop chronic bacterial osteomyelitis at their surgical site.

However, the immunological mechanism by which osteomyelitis may suppress tumor growth has not been investigated. They used a mouse model of osteomyelitis to assess the effects of bone infection on innate immunity and tumor growth. A chronic Staphylococcal osteomyelitis model was established in C3H mice and the effects of infection on tumor growth of syngeneic DLM8 osteosarcoma were assessed. The effects of infection on tumor angiogenesis and innate immunity, including NK cell and monocyte responses, were assessed. They found that osteomyelitis significantly inhibited the growth of tumors in mice, and that the effect was independent of the infecting bacterial type, tumor type, or mouse strain. Depletion of NK cells or monocytes reversed the antitumor activity elicited by infection. Moreover, infected mice had a significant increase in circulating monocytes and numbers of tumor associated macrophages. Infection suppressed tumor angiogenesis but did not affect the numbers of circulating endothelial cells. Chronic localized bacterial infection was shown in this model to elicit significant systemic antitumor activity dependent on NK cells and macrophages.

Rat Models

Selection of rat versus mice models is generally determined by the availability of certain transgenic strains. Physically the increased size of rats can offer some advantages from a surgical implant perspective. De Giacomo., *et al*. [18] describe the most common procedure that has been developed for use in rats and mice to model fracture healing. The detailed surgical protocol to generate closed simple transverse fractures is presented, and general considerations when setting up an experiment using this model are described.

Healing, bone defects and non-union

Kaspar., *et al*. [19] established a model of bone atrophic non-union for investigating the process of bone regeneration by performing cauterization of the periosteum, removal of local bone marrow, and stabilization with external fixation. The model allows the modelling of atrophic non-union without the need for a critical size defect. Furthermore, it provides reproducible, defined mechanical conditions with minimized physical interference of the implant with the biological processes in the healing zone. Eighty adult Sprague-Dawley rats received an osteotomy of the left femur, stabilized with external fixators. In half of the animals, the periosteum proximal and distal to the osteotomy was destroyed by cauterization and the adjacent bone marrow was removed (nonunion group). At 2 and 8 weeks after surgery, radiological, biomechanical, histological histomorphometrical analyses showed a typical physiological healing in the control group while the non-union group was characterized by resorption of bone ends with callus formation distant to the osteotomy. At both time points, the callus was composed of significantly less bone and significantly more, connective tissue ($p < 0.001$). In addition, the torsional strength of the osteotomized femur was significantly less in the non-union group than in the control group, which was comparable to that of the intact femur ($p < 0.001$).

Fassbender., *et al*. [20] created 4 mm femur defects and stabilised these with a custom-made plate and filled them with either demineralised bone matrix (DBM) or DBX (DBX PuttyA (R)). Bone morphogenetic protein 2 (BMP-2)-loaded collagen and an empty defect served as controls. The outcome was followed after 21 and 42 days by radiology (Faxitron; micro-CT) and histology. Defect healing did not occur in any animal from the empty control, DBM or DBX group. Residuals of the implanted material were still found after six weeks, but only limited callus formation was visible. In contrast, the BMP-2 control demonstrated enhanced formation of callus tissue and undisturbed healing. After 21 days, 11 out of 16 and after 42 days, 7 out of 8 BMP-2-treated animals showed complete defect bridging by cancellous bone tissue. Demineralised bone grafts were not capable of defect reconstruction; only BMP-2 was able to provide sufficient stimulus to induce uneventful bridging under the specific experimental conditions.

Shapiro., *et al*. [21] investigated critical-size defects, in which bone loss is severe and a bone graft is required for healing to bridge the deficit. Faced with the shortcomings of grafts currently in use (i.e. autografts, allografts, and mineral-based bone substitutes) efforts are being made to establish new methods of bone regeneration. They investigated the use of exogenous gene-modified mesenchymal stem cells (MSCs), which can rapidly repair large bone defects in animal models. Unfortunately, ex vivo culture of MSCs may add certain complexity to the advancement of this cell therapy to the clinic. They cite having previously demonstrated efficient bone regeneration following direct gene delivery to endogenous MSCs that had been attracted to a fracture site. In that study, electroporation was used, which is an invasive method of gene transfection that may result in tissue damage. Unlike electroporation, sonoporation-the use of ultrasound for gene delivery-is non-invasive, considered safer, and relevant to the clinical setting. They evaluated the feasibility of ultrasound-based gene delivery to resident MSCs that had been recruited to a fracture site in different animal models. Results showed transient (up to 21 days) expression of a reporter gene in radial, vertebral, and tibial bone defects in mice, rats, and a minipig.

Montgomery., *et al*. [22] investigated osteogenic factors. These are often used in orthopedics to promote bone growth, improve fracture healing, and induce spine fusion. Osteogenic oxysterols are naturally occurring molecules that shown to induce osteogenic differentiation *in vitro* and promote spine fusion *in vitro*. They used a rat model to compare osteogenic oxysterol for clinical development and evaluate its ability to promote osteogenesis *in vitro* and spine fusion in rats *in vivo*. The study was compared two different demineralised bone matrices used clinically and considered to induce bone healing in a critical-size-defect rat model.

Statins, a class of naturally-occurring compounds that inhibit HMG-CoA reductase, are known to increase endogenous bone morphogenetic protein-2 (BMP-2) expression. Local administration of statins has been shown to stimulate fracture repair in vivo animal experiments. However, the ability of statins to heal more challenging critical-sized defects at the mid-diaphyseal region in long bones has not been investigated. Yuasa., *et al*. [23] examined the potential of injectable lovastatin microparticles combined with biodegradable

polyurethane (PUR) scaffolds in metaphyseal small plug defects and diaphyseal segmental bone defects in rat femora. Sustained release of lovastatin from the lovastatin microparticles was achieved over 14 days. The released lovastatin was bioactive, as evidenced by its ability to stimulate BMP-2 gene expression in osteoblastic cells. Micro-computed tomography (CT) and histological examinations showed that lovastatin microparticles, injected into PUR scaffolds implanted in femoral plug defects, enhanced new bone formation. Bi-weekly multiple injections of lovastatin microparticles into PUR scaffolds implanted in critical-sized femoral segmental defects resulted in increased new bone formation compared to the vehicle control. In addition, bridging of the defect with newly formed bone was observed in four of nine defects in the lovastatin microparticle treatment group, whereas none of the defects in the vehicle group showed bridging. These observations suggest that local delivery of lovastatin combined with PUR scaffold can be an effective approach for treatment of orthopaedic bone defects and that multiple injections of lovastatin may be useful for large defects.

Healing in the presence of inter-current disease

For prophylaxis and treatment of bone infections, antibiotics are used both systemically and locally. For several decades antibiotics mixed with bone cement (methylmethacrylate) have been used in prosthetic surgery and a gentamicin coated tibial nail is approved in Europe for fracture stabilization.

Fassbender., *et al*. [24] investigated whether gentamicin, locally applied from a polymeric coating of intramedullary nails, might interfere with the bone healing process. Female Sprague Dawley rats (n = 72) were used and the tibiae were intramedullary stabilized with Kirschner-wires (k-wires) after osteotomy. This model shows delayed healing with a prolonged inflammatory reaction. The open approach is clinically more relevant compared to a closed one because it mimics an open fracture, which has a higher risk of infection. The k-wire was either coated with the polymer poly(D,L-lactide) (control group) or with 10% gentamicin incorporated into the polymer (gentamicin group). *In vivo* mu CT analyses were performed at days 10, 28, 42, and 84 after osteotomy. Mechanical torsional testing and histological evaluation were done at the days of sacrifice: 28, 42, and 84. The mu CT analyses revealed an increase in tissue mineral density (TMD) over the healing period in both groups. In the control group, the torsional stiffness and maximum load did not reach the values of the intact contralateral side at any time point. At day 84 the gentamicin treated tibiae showed significantly better maximum load compared to the control group. Histology showed no bony bridging in the control, whereas in 2 of 5 calluses of the gentamicin group mineralized bridging occurred. Significantly more mineralized tissue was measured in the gentamicin group. In this study, local gentamicin application did not negatively interfere with the long-term healing process.

Management of soft tissue sarcoma involves multimodal treatment, including surgery and radiotherapy. Pathologic fracture of the femur after such treatment in the thigh is a serious, late complication and non-union rates of 80-90% are reported. Nicholls., *et al*. [25] hypothesize that the combination of radiotherapy and periosteal stripping (during tumor resection) leads to greater impairment of the fracture repair process than either intervention alone. Female Wistar retired breeder rats were randomized into four treatment groups (control, radiotherapy, surgery, and combination of radiotherapy and surgery) and three endpoints (21, 28, and 35 days post-fracture). Designated animals first underwent radiotherapy, followed by surgical stripping of the periosteum 3 weeks later and femoral fracture with fixation after another 3 weeks. Animals were necropsied and fractures examined using micro-CT and histomorphometry. Simple transverse or short oblique femoral fractures were produced. By 35 days, control animals formed unions, periosteum-stripped animals formed hypertrophic non-unions and irradiated animals formed atrophic non-unions.

Histomorphometry revealed an absence of chondroid and osteoid production in animals undergoing radiotherapy. The relative contribution of periosteal stripping to occurrence of non-union was statistically insignificant. Radiation prior to fracture reliably resulted in atrophic non-union in their model. The contribution of periosteal stripping was negligible.

Sclerostin is a negative regulator of bone formation. The effect of sclerostin monoclonal antibody (Scl-Ab) was evaluated in osteotomy healing. Suen., *et al*. [26] investigated the time course effects of systemic administration of Scl-Ab on fracture repair in rat femoral osteotomy model. 120 six-month-old male SD rats were subjected to transverse osteotomy at the right femur mid-shaft. Rats were treated with

vehicle or Scl-Ab treatment for 3, 6, or 9 weeks. Fracture healing was evaluated by radiography, micro-CT, micro-CT based angiography, 4-point bending mechanical test and histological assessment. Scl-Ab treatment resulted in significantly higher total mineralized callus volume fraction, BMD and enhanced neovascularization. Histologically, Scl-Ab treatment resulted in a significant reduction in fracture callus cartilage at week 6 and increase in bone volume at week 9, associated with a greater proportion of newly formed bone area at week 6 and 9 by fluorescence microscopy. Mechanical testing showed significantly higher ultimate load in Scl-Ab treatment group at week 6 and 9. This study demonstrated that Scl-Ab treatment enhanced bone healing in a rat femoral osteotomy model, and increased bone formation, bone mass and bone strength.

Lapine Models

Rabbits offer the advantage over small rodents of larger bones to accept prosthetic fixators as well as markedly increased physicodynamic stresses in the hind limbs. Increase in bone marrow volume for harvest, blood volumes and clearer imaging are all advantages.

Healing, bone defects and non-union

Open fractures with severe soft-tissue trauma are predisposed to poor bone healing. The vital coupling between osteo- and angiogenesis is disturbed. Frey., *et al*. [27] investigated use of cysteine-rich protein 61 (CYR61) which is an angiogenic inducer promoting vascularisation. Little is known about the effect of CYR61 on the callus regenerate after acute musculoskeletal trauma. They investigated whether local administration of CYR61 has an influence on callus formation and remodelling, increases bone volume, and, helps restore callus stability. Musculoskeletal trauma was created in 20 rabbits. To simulate fracture-site debridement, the limb was shortened. In the test group, a CYR61-coated collagen matrix was locally applied around the osteotomy. After ten days, gradual distraction was commenced (0.5 mm/12 h) to restore the original length. New bone formation was evaluated histomorphometrically, radiographically and biomechanically. Osseus consolidation occurred in all animals. Average maximum callus diameter was higher in the test group [1.39 mm; standard deviation (SD) = 0.078 vs 1.26 mm (SD = 0.14); p = 0.096]. In addition, bone volume was higher (p = 0.11) in the test group, with a mean value of 49.73 % (SD = 13.68) compared with 37.6 % (SD = 5.91). Torsional strength was significantly higher (p = 0.005) in the test group [105.43 % (SD = 31.68 %) vs. 52.57 % (SD = 24.39)]. Instead, stiffness of the newly reconstructed callus decreased (64.21 % (SD = 11.52) vs. 71.30 % (SD = 32.25) (p = 0.81)). CYR61 was observed to positively influences callus regeneration after acute trauma, histologically, radiographically and biomechanically, probably by a CYR61-associated pathway.

The most important issue in the assessment of fracture healing is to acquire information about the restoration of the mechanical integrity of bone. Tobita., *et al*. [28] set out to determine the relationship between bending stiffness and strength using mechanical testing at different times during the healing process. Unilateral, transverse, mid-tibial osteotomies with a 2-mm gap were performed in 28 rabbits. The osteotomy site was stabilized using a double-bar external fixator. The animals were divided into four groups (n = 7/group/time point; 4, 6, 8 and 12 weeks). A series of images from micro-computed tomography of the gap was evaluated to detect the stage of fracture healing and a 4-point bending test was performed to measure stiffness and strength. Relative stiffness and strength values were also acquired from calculation of ratios relative to those of the non-osteotomized contralateral bones. Formation of cortex and medullary canal at the gap was seen in the 12-week group and would represent the remodelling stage. In addition, the relationship between stiffness and strength remained almost linear until at least 12 weeks. However, stiffness recovered much more rapidly than strength. Strength was not fully restored until the later stages of fracture healing. The current study suggests that stiffness could be monitored as a surrogate marker of strength until at least the remodelling stage.

Correct choice of osteosynthesis method is a very important factor in providing the optimal conditions for appropriate healing of the fracture. There are still disagreements about the method of stabilization of some long bone fractures. Critically observed, no method of fracture fixation is ideal. Each osteosynthesis method has both advantages and weaknesses. Gajdobransk., *et al*. [29] compared the results of the experimental application of three different internal fixation methods: plate fixation, intramedullary nail fixation and self dynamis-

able internal fixator (SIF). A series of 30 animals were used (*Lepus cuniculus*) as experimental animals, divided into three groups of ten rabbits each. Femoral diaphysis of each animal was osteotomized and fixed with one of three implants. Ten weeks later all animals were necropsied and each specimen underwent histological and biomechanical testing. Histology showed that the healing process with SIF was more complete and bone callus was more mature in comparison to other two methods. During biomechanical investigation (computerized bending stress test), statistically significant differences indicated that using SIF led to stronger healing ten weeks after the operation and that SIF is a suitable method for fracture treatment.

Microwave is a method for improving fracture repair. However, one of the contraindications for microwave treatment listed in the literature is surgically implanted metal plates in the treatment field. The reason is that the reflection of electromagnetic waves and the eddy current stimulated by microwave increases the temperature of magnetic implants and can cause heat damage in tissues. Comparing with traditional medical stainless steel, titanium alloy has low magnetic permeability and electric conductivity. The effects of microwave treatment on fracture with titanium alloy internal fixation were investigated by Ye., *et al*. [30]. Titanium alloy internal fixation systems were implanted in New Zealand rabbits with a 3.0 mm bone defect in the middle of femur. A 30-day microwave treatment (2,450MHz, 25W, 10 min per day) was applied to the fracture 3 days after operation. Temperature changes of muscle tissues around implants were measured during the irradiation. Normalized radiographic density of the fracture gap was measured on the 10^{th} day and 30^{th} day of the microwave treatment. All of the animals were necropsied after 10 and 30 days microwave treatment with histologic and histomorphometric examinations performed on the harvested tissues. The temperature did not increase significantly in animals with titanium alloy implants. Histology of muscles, nerve and bone around the implants revealed no deleterious effects. Radiographic assessment, histologic and histomorphometric examinations revealed significant improvement in the healing bone. Their suggestion is that, in the healing of fracture with titanium alloy internal fixation, a low dose of microwave treatment may be a promising adjunct.

Southwood., *et al*. [31] evaluated the use of adenoviral transfer of the BMP-2 gene (AdBMP-2) for enhancing healing in an infected defect fracture model. A femoral defect stabilized with plates and screws was surgically created in sixty-four skeletally mature New Zealand white rabbits. Experimental groups were: (1) non-infected Ad-luciferase (Ad-LUC, NONLUC), (2) non-infected Ad-BMP-2 (NONBMP), (3) infected Ad-LUC (INFLUC), and (4) infected Ad-BMP-2 (INFBMP). A sclerosing agent was applied to the ends of the bone at surgery to facilitate the development of osteomyelitis. Fracture healing was evaluated radiographically and histologically. Rabbits in the non-infected and infected groups that were treated with Ad-BMP-2 had earlier initial- and bridging-callus formation, and a higher overall external callus grade compared to rabbits in the Ad-LUC groups. Rabbits in the AdLUC groups had more defect ossification compared to rabbits in the Ad-BMP-2 groups. There was a trend for rabbits in the Ad-BMP-2 group that were euthanized at 2 and 4 weeks after surgery to have more bone and cartilage compared to rabbits in the Ad-LUC group. The results of this study suggest that Ad-BMP-2 enhances the early stages of healing in an infected defect fracture.

Healing in the presence of inter-current disease

Arens., *et al*. [32] investigated healing in the presence of *Staphylococcus aureus* osteomyelitis. Higher bacterial doses led to an increasing infection rate and in infected groups of animals there was a complete lack of closure of osteotomy at 4 weeks. C-reactive protein (CRP), lymphocyte: granulocyte ratio and weight loss were increased in infected animals receiving IM nails in comparison with non-inoculated equivalents, although this was less evident in the plate group. In a 10-week infection group, healing did not occur in the plated rabbits.

Akkaya., *et al.* (2012) investigated whether cefazolin-sodium had any adverse effect on fracture healing in an experimental model using 50 New-Zealand white rabbits. Under general anesthesia, closed double fracture of middle one-third of the tibia-fibula of the left lower extremity of the subjects was produced by manual compression followed by closed reduction of fracture and long leg circular cast was applied. Subjects were divided into five groups of 10 rabbits. The first and second group were administered ciprofloxacin 50 mg/kg SC bid and cefazolin-sodium 50 mg/kg IM on the seventh day of fracture. The third group was applied a single high-dose of vitamin D (50.000 IU/kg) IM following fracture. The fourth group was applied daily vitamin E (alpha tocopherol) 20 mg/kg IM for five days from one hour

before the production of fracture. Control group did not receive any treatment before and after fracture. Initial and control X-ray examinations were performed immediately and four weeks after production of fracture, respectively. At the end of the fourth week, animals were necropsied. Histological evaluation showed that the grade of the fracture healing was significantly lower in the ciprofloxacin group, while it was significantly higher in the cefazolin-sodium, vitamin D and vitamin E groups, compared to control group ($p < 0.005$).

Mutsuzuki., *et al*. [33] investigated pin-tract infections, which are the most common complications of external fixation. They developed a fibroblast growth factor-2 (FGF-2) apatite composite layer for coating titanium screws. The purpose was to elucidate the mechanism of the improvement in infection resistance associated with FGF-2-apatite composite layers. They analyzed FGF-2 release from the FGF-2-apatite composite layer and the mitogenic activity of the FGF-2-apatite composite layer. They evaluated time-dependent development of macroscopic pin-tract infection around uncoated titanium control screws (n = 10). Screws coated with the apatite layer (n = 16) and FGF-2-apatite composite layer (n = 16) were percutaneously implanted for 4 weeks in the medial proximal tibia in rabbits. They found that FGF-2-apatite composite layer coated on the screws led to the retention of the mitogenic activity of FGF-2. FGF-2 was released from the FGF-2-apatite composite layer in vitro for at least 4 days, which corresponds to a period when 30% of pin-tract infections develop macroscopically in the percutaneous implantation of uncoated titanium control screws. The macroscopic infection rate increased with time, reaching a plateau of 80-90% within 12 days. This value remained unchanged until 4 weeks after implantation. The screws coated with an FGF-2-apatite composite layer showed a significantly higher wound healing rate than those coated with an apatite layer (31.25 vs. 6.25%, $p < 0.05$). The interfascial soft tissue that bonded to the FGF-2-apatite composite layer is a Sharpey's fiber-like tissue, where collagen fibers are inclined at angles from 30 to 40° to the screw surface. The Sharpey's fiberlike tissue is rich in blood vessels and directly bonds to the FGF-2-apatite composite layer via a thin cell monolayer (0.8-1.7 μm thick). It is suggested that the enhanced wound healing associated with the formation of Sharpey's fiber-like tissue triggered by FGF-2 released from the FGF-2-apatite composite layer leads to the reduction in the pin-tract inflammation rate.

Wellisz., *et al*. [34] evaluated the effects of using a water-soluble polymer bone hemostatic material in a contaminated environment in a rabbit tibial defect model. Infection rates and healing of polymer-treated bone were compared with the infection and healing of bone wax treated bone and untreated controls after a bacterial challenge. Defects created in 24 rabbit tibias were treated with the polymer or bone wax, or left without a hemostatic agent. The defects were inoculated with Staphylococcus aureus ATCC-29213 (2.5 x 10(4) colony forming units). After 4 weeks, all defects treated with bone wax were infected and osteomyelitis had developed, and none had evidence of bone healing. In the polymer and control groups, two defects in each group (25%) had osteomyelitis develop. The remaining six defects in each group (75%) showed no osteomyelitis and exhibited normal bone healing. The polymer-treated defects had a considerably lower rate of osteomyelitis and positive bone cultures compared with the bone wax-treated group. There were no differences between the polymer-treated and control groups in the rates of osteomyelitis, positive cultures, or bone healing. The use of a soluble polymer as an alternative to bone wax may decrease the rates of postoperative bone infections.

Kaplan., *et al*. [35] monitored fracture site axial rigidity at weekly intervals during healing of tibial osteotomies in adult rabbits. Two groups of 20 rabbits each were treated with external fixators of two different rigidities. Four animals from each group were necropsied at 3, 5, 6, 7, and 8 weeks to determine the bending moments at failure of the healing fractures. Normal fracture healing was accompanied by characteristic phases in the development of fracture site axial rigidity. From 0 to 3 weeks there was a period of low and approximately constant rigidity, followed by a linear increase during 3 to 5 weeks to an approximately three to four times greater rigidity. The maximum average normalized axial rigidities were reached at 6 weeks and were 57% (high rigidity group) and 77% (low rigidity group) of the untreated contralateral controls. The maximum average normalized failure moments occurred at 8 weeks and were 48% (high rigidity) and 44% (low rigidity) of controls. The differences due to fixator rigidity were not statistically significant except for a large increase in failure moments at 3 weeks for the low rigidity group. Axial rigidities were correlated ($r2 = 0.74$ and 0.53, respectively) with failure moments, but only during the first 6 weeks. The monitoring technique provides a nondestructive means for following the biomechanical progress

of fracture healing in an animal model. The occurrence of the characteristic increase in fracture site axial rigidity at 3 to 5 weeks can also be used to distinguish between normal and abnormal healing.

Cavine models

Guinea pigs provide an alternative to rat models although availability, hardiness and fecundity limit use. Guinea pigs are also less mobile or agile than rats.

Healing, bone defects and non-union

Kdolsky., *et al*. [36] used a guinea pig model to validate the hypothesis that healing of fractures can be accelerated by oral administered L-arginine. A diaphyseal defect was established in the right femur of each of the 32 animals and stabilized. The measurement for the healed femur was individually compared with that of the uninjured femur in each animal for bending stiffness, force until failure and energy necessary for re-fracture. The bending stiffness reached 73% by the control group and 88% by the 4-week treatment group. The force necessary for refracture was 52% in the control compared with 65% in the 4-week treatment group. The energy necessary for re-fracture was 36% in the control compared with 73% in the group treated for 4 weeks. The 2 week treatment group showed no statistical significant differences to the control, but the femora from the 4 week treatment group required statistically significant higher energy for refracture than the femora from the control.

Omeroğlu., *et al*. [37] examined the benefits of a single high dose of vitamin D3 on fracture healing in a healthy animal model. Twenty healthy young adult guinea pigs were randomly divided into groups as 'control' and 'vitamin D', and their right tibias were fractured with digital manipulation. Guinea pigs in vitamin D group were injected intramuscularly with 50,000 (IU/kg) of vitamin D3. The animals were killed at 7, 14, 21 and 28 days following fracture. Ultrastructural analysis of the harvested tibias revealed that a single high dose of vitamin D3 stimulated fracture healing. The observed effects at the fracture zone in a healthy animal model included advancement of blood supply, acceleration of synthesis and organization of collagen fibres, acceleration of the proliferation and differentiation of osteoprogenitor cells, and activation of the mineralization of the matrix.

Healing in the presence of inter-current disease

Passl., *et al*. [38] report on a model of experimental post-traumatic osteomyelitis in which the femur of guinea pigs was fractured and infected with *E. coli* (10(5)) or *Staphylococcus aureu*s (10(4)). Traumatized uninfected animals served as controls. The animals were further divided within each group by treating the fractured site with an intramedullary wire in one half. Osteomyelitis developed and became chronic in all guinea pigs infected with *Staph. aureus*, and in nine of 12 infected with *E. coli*. All animals infected with *E. coli* treated with an intramedullary wire developed chronic osteomyelitis; only four of seven from *E. coli* infected animals with fractures developed this disease. Moreover, *Staph. aureus* could be recovered from the osseous tissue in the chronic stage of the disease regularly, while *E. coli* was only present in the early weeks after operation, but not in the chronic stage.

Ovine Models

Sheep offer examples of larger bone sizes, not greatly dissimilar to those required for human prosthetic implants. Unlike rabbits where mechanical stresses differ greatly between fore and hind limbs, sheep offer more even and constant physical stressors and are a readily obtainable animal source that can be easily and cheaply maintained in animal facilities.

Healing, bone defects and non-union

Delayed and non-union fractures are challenging problems. Lienau., *et al*. [39] used a sheep model to examine the endogenous mRNA expression of genes regulating cartilage formation, bone formation, endochondral ossification, and bone remodelling during mechanically induced delayed bone healing. A tibial osteotomy was performed in two groups of sheep and stabilized with either a rigid external

fixator leading to standard healing or with a rotationally unstable fixator leading to delayed healing. At days 4, 7, 9, 11, 14, 21, and 42 after surgery, total RNA was extracted from the callus. Gene expressions of several molecules functionally important for bone healing were studied by quantitative reverse transcriptase-polymerase chain reaction. The expression profiles were related to callus tissue composition, analyzed by histomorphometry. Histomorphometry demonstrated a delayed, prolonged chondral phase and a reduction in bone formation in the experimental group. There was no differential expression of Runx2 between both groups until day 42, but mRNA expression levels of BMP2, BMP4, BMP7, noggin, Col1a1, IGF1, TGFb1, OPN, MMP9, MMP13, TIMP3, TNF alpha, MCSF, RANKL, and OPG were lower in the delayed healing group at several time points. This study provides insight into the temporal periods during which various factors may be deficient during a compromised bone-healing situation.

Piorek., *et al*. [40] examined the treatment of transverse tibial shaft fractures in six sheep with the use of interlocking nails and type I external fixators. During surgery, tibial osteotomy was performed to induce an experimental fracture which was stabilized using a type I external fixator. Osteosynthesis was monitored for nine weeks with clinical tests, observing the degree of lameness and subjecting the patients to weekly radiological examinations. After nine weeks, the animals were euthanized, and samples of bone callus were sampled for histopathological analyses. Weight bearing on the fractured limb began on day 2 to 4 after treatment. Limb function was fully restored around five weeks after surgery. Radiographs taken during the observation period revealed gradual hyperplasia and progressing mineralization of bone callus at different stages of healing. The histopathological picture of the bone callus was characteristic of the phase of bone turnover and remodelling.

In an effort to obtain a high-quality bone-implant interface, several methods involving alteration of surface morphological, physicochemical, and biochemical properties are being investigated. Dergin., *et al*. [41] examined methods to increase the osseointegration rate and quality and decrease the waiting period of dental implants before loading by using a micro electric implant stimulator device. This was done to imitate micro electrical signals, which occur in bone fractures described in terms of piezoelectric theory. A single dental implant (Zimmer Dental), 3.7 mm in diameter, was inserted into the tibia of sheep bilaterally.

Twenty-four dental implants were inserted into 12 sheep. Implant on the tibia of each sheep was stimulated with 7.5 mu A direct current (DC), while the other side did not receive any stimulation and served as a control. Animals were necropsied 1, 2, and 3 months after implantation. No statistically significant difference in bone-to-implant contact (BIC) ratio, osteoblastic activity, and new bone formation was found between the stimulation group and the control group at the late phase of healing (4, 8, and 12 weeks). No evidence was found that electric stimulation with implanted 7.5 mu A DC is effective at late phase implant osseointegration on a sheep experimental model.

Secondary fracture healing in long bones leads to the successive formation of intricate patterns of tissues in the newly formed callus. Vetter., *et a*l. [42] quantitatively described the topology of tissue patterns at different stages of the healing process to generate averaged images of tissue distribution. This averaging procedure was based on stained histological sections (2, 3, 6, and 9 weeks post-operatively) of 64 sheep with a 3 mm tibial mid-shaft osteotomy, stabilized either with a rigid or a semi-rigid external fixator. Before averaging, histological images were sorted for topology according to six identified tissue patterns. The averaged images were obtained for both fixation types and the lateral and medial side separately. For each case, the result of the averaging procedure was a collection of six images characterizing quantitatively the progression of the healing process. In addition, quantified descriptions of the newly formed cartilage and the bone area fractions (BA/TA) of the bony callus are presented. For all cases, a linear increase in the BA/TA of the bony callus was observed. The slope was greatest in the case of the most rigid stabilization and lowest in the case of the least stiff. This topological description of the progression of bone healing will allow quantitative validation (or falsification) of current mechano-biological theories.

Woodruffa., *et al*. [43] created 3 cm and 6 cm ovine tibial bone defects in sheep aged 5 or more years and slow biodegradable composite scaffolds comprised of medical grade polycaprolactone and calcium phosphates (hydroxyapatite and tricalcium phosphate) were implanted with or without bone morphogenic proteins (BMP-which stimulate growth, maturation and regulation of bone). Both 3 cm

and 6 cm bone defects were regenerated by recruitment and stimulation of endogenous cells using a matrix scaffold containing relevant growth factors. The regenerative potential of a scaffold system with BMP outperformed the best available autografts after 12 months of implantation. This was verified with x-ray, CT and biomechanical and histological assessment. A composite scaffold loaded with 40 ml bone marrow-derived mesenchymal precursor cells stimulated more bone formation than the scaffold alone; however, there was significantly less bridging and bone volume than the scaffold plus BMP group. Ongoing studies focus on increasing cell implantation number and adapting scaffold design to minimize the invasive injection of cells at 4-6 weeks after the implantation of the scaffold to help avoid the initial inflammatory phase related to surgery and to synchronize implantation with the early vascularization of the implanted matrix.

Healing in the presence of intercurrent disease

Implant-associated infections contribute to patient morbidity and health care costs. Stewart., *et al*. [44] hypothesized that surface modification of titanium fracture hardware with vancomycin would support bone-healing and prevent bacterial colonization of the implant in a large-animal model. A unilateral transverse mid-diaphyseal tibial osteotomy was performed and repaired with a titanium locking compression plate in nine sheep. Four control animals were treated with an unmodified plate and five experimental animals were treated with a vancomycin-modified plate. The osteotomy was inoculated with 2.5 x 10^6 colony forming units of *Staphylococcus aureus*. The animals were necropsied at three months postoperatively, and implants were retrieved aseptically. Microbiologic and histologic analyses, scanning electron and confocal microscopy, and microcomputed tomography were performed. All animals completed the study. Compared with the treatment cohort, control animals exhibited protracted lameness in the operatively treated leg. Gross findings during necropsy were consistent with an infected osteotomy accompanied by a florid and lytic callus. Microcomputed tomography and histologic analysis of the tibiae further supported the presence of septic osteomyelitis in the control cohort. Thick biofilms were also evident, and bacterial cultures were positive for *Staphylococcus aureus* in three of four control animals. In contrast, animals treated with vancomycin-treated plates exhibited a healed osteotomy site with homogenous remodeling, there was no evidence of biofilm formation on the retrieved plate, and bacterial cultures from only one of five animals were positive for *Staphylococcus aureus*. Vancomycin-derived plate surfaces inhibited implant colonization with *Staphylococcus aureus* and supported bone-healing in an infection model using sheep.

Caprine Models

Goats provide an alternative to sheep with longer and often wider appendicular skeletal bones in some breeds and also reflect a broader range of physical stresses due to the greater range of movement of goats compared to sheep. In some geographies goats, may be cheaper and easier to obtain.

Healing, bone defects and non-union

Since the introduction of uncemented hip implants, there has been a search for the best surface coating to enhance bone apposition in order to improve retention. The surface coating of the different stems varies between products. Harboe., *et al*. [45] assessed retention forces and bone adaption in two differently coated stems in a weight-bearing goat model.

Hydroxyapatite (HA) and electrochemically deposited calcium phosphate (CP; Bonit (R)) on geometrically comparable titanium-based femoral stems were implanted into 12 (CP group) and 35 (HA group) goats. This animal model included physiological loading of the implants for 6 months. The pull-out force of the stems was measured, and bone apposition was microscopically evaluated. After exclusion criteria were applied, the number of available goats was 4 in the CP group and 11 in the HA group. The CP-coated stems had significantly lower retention forces compared with the HA-coated ones after 6 months (CP median 47 N, HA median 1,696 N, $p = 0.003$). Bone sections revealed a lower degree of bone apposition in the CP-coated stems, with more connective tissue in the bone/implant interface compared with the HA group. In this study, HA had better bone apposition and needed greater pull-out force in loaded implants. The application of CP on the loaded titanium surface to enhance the apposition of bone is questioned.

Kon., *et al*. [46] developed a study to examine whether different mechanical modifications and/or impregnation of hyaluronic acid (HA) might enhance aragonite-based scaffold properties for the regeneration of cartilage and bone in an animal model. Bi-phasic osteochondral scaffolds were prepared using coralline aragonite with different modifications, including 1- to 2-mm-deep drilled channels in the cartilage phase (Group 1, n = 7) or in the bone phase (Group 2, n = 8), and compared with unmodified coral cylinders (Group 3, n = 8) as well as empty control defects (Group 4, n = 4). In each group, four of the implants were impregnated with HA to the cartilage phase. Osteochondral defects (6 mm diameter, 8 mm depth) were made in medial and lateral femoral condyles of 14 goats, and the scaffolds were implanted according to a randomization chart. After 6 months, cartilage and bone regeneration were evaluated macroscopically and histologically by an external laboratory. Group 1 implants were replaced by newly formed hyaline cartilage and subchondral bone. In this group, the cartilaginous repair tissue showed a smooth contour and was well integrated into the adjacent native cartilage, with morphological evidence of hyaline cartilage as confirmed by the marked presence of proteoglycans, a marked grade of collagen type II and the absence of collagen type I. The average scores in other groups were significantly lower (Group 2 (n = 8) 28.8 +/- A 11, Group 3 (n = 8) 23 +/- A 9 and Group 4 (empty control, n = 4) 19.7 +/- A 15). The implants with the mechanical modification and HA impregnation in the cartilage phase outperformed all other types of implant. Although native coral is an excellent material for bone repair, as a stand-alone material implant, it does not regenerate hyaline cartilage. Mechanical modification with drilled channels and impregnation of HA within the coral pores enhanced the scaffold's cartilage regenerative potential. The modified implant shows young hyaline cartilage regeneration. This implant was postulated to be of use for the treatment of chondral and osteochondral defects in humans.

Stephens., *et al*. [47] evaluated a novel humeral fixation device, the insertion technique, healing of humeral osteotomies, and clinical outcomes in a caprine model over a six month period. Fourteen mature female Boer/Nubian cross goats with a mean body weight of 50.7 kg were implanted with a proprietary segmented interlocking nail (SILN) in both humeri. Each goat had one humerus randomly selected for mid-diaphyseal osteotomy. Immediately after surgery all but one goat was able to stand, although none of the goats were weight bearing on the osteotomy limb. During the six-month study, clinical lameness was always associated with the osteotomy limb. One month after surgery, lameness for twelve of the goats was grade 2/5 or better. At three months, 11 of the 14 did not exhibit any signs of lameness. On radiographic images, notable malalignment of the osteotomy was observed, although all osteotomies went to bone union. The results suggest that despite misalignment, the SILN maintained adequate osteotomy fixation to achieve bone union in the model studied, with reduced morbidity and early return to function with bilateral implantation. The SILN used in this study allowed intramedullary fixation of humeral diaphyseal osteotomies with a limited and safe surgical approach.

Tissue-engineered bone (TEB) has shown to be an effective alternative to conventional 'goldstandard' autogenous bone for the repair of critically sized bone defects (CSBD). Moderate axial interfragmentary movement (IFM) has been shown to promote bone healing in conventional models. Hou., *et al*. [48] explored the use of IFM to enhance the capacity of TEB in the repair of CSBD using a goat model. Dynamic intramedullary rods designed to supply axial IFMs within 10% of the interfragmentary strain were used to stabilize CSBD goat femur models, whose bone defects were filled with TEB. Bone regeneration was evaluated using radionuclide bone imaging, roentgenographic analysis, periosteal callus area, computed tomography value score, biomechanical analysis, and histological observation. Compared with the static intramedullary rods, the dynamic intramedullary rod group showed an increase in early-stage callus formation and blood supply to the callus tissue, better differentiation of fibrous and cartilaginous tissue into bone tissue, improved strength and stiffness of callus tissue in late-stage healing, and overall better functional recovery of the goat femur. This showed that moderate axial IFM could promote the osteogenesis and reconstruction of TEB *in vivo*.

To determine whether washing morselized cancellous bone allograft in impaction grafting for revision hip arthroplasty would improve mechanical and biologic performance Voor., *et al*. [49] performed left hip hemiarthroplasty with a collarless stem cemented into impacted morselized cancellous bone was performed in 22 goats. Washed allograft was used in the experimental group, and standard allograft was used in the control group. One of 11 experimental and 4 of 11 control implants were observed to be loose at 8 weeks. Washing allowed significantly more morselized cancellous bone to be placed in the experimental group compared to the control group (7.7

+/- 1.9 and 6.2 +/- 2.0 g, respectively, P < 0.05). Significantly less *in vivo* subsidence over the 8-week study period also was demonstrated in the experimental group compared to the control group (0.4 +/- 0.4 and 2.2+/-2.3 mm, respectively, P < .05). Angular motion during cyclic +/- 1.5 Nm loading demonstrated significant differences between the 2 groups at time zero (2.67 degrees +/- 1.02 degrees for the control group and 1.98 degrees +/- 0.47 degrees for the experimental group, P < .05) and at 8 weeks (2.40 degrees +/- 0.38 degrees for the control group and 1.74 degrees +/- 0.55 degrees for the experimental group, P < .05). Histology showed little difference between the 2 groups, but there was a trend toward less inflammation in the experimental group.

Nandi., *et al*. [50] evaluated porous hydroxyapatite (HAp)as a bone substitute in healing bone defects in vivo, as assessed by radiologic and histopathologic methods, oxytetracycline labeling, and angiogenic features in Bengal goats. Bone defects were created in the diaphysis of the radius and either not filled (group I) or filled with a HAp strut (group II). The radiologic study in group II showed the presence of unabsorbed implants which acted as a scaffold for new bone growth across the defect, and the quality of healing of the bone defect was almost indistinguishable from the control group, in which the defect was more or less similar, although the newly formed bony tissue was more organized when HAp was used. Histologic methods showed complete normal ossification with development of Haversian canals and well-defined osteoblasts at the periphery in group II, whereas the control group had moderate fibro-collagenization and an adequate amount of marrow material, fat cells, and blood vessels. An oxytetracycline labeling study showed moderate activity of new bone formation with crossing-over of new bone trabeculae along with the presence of resorption cavities in group II, whereas in the control group, the process of new bone formation was active from both ends and the defect site appeared as a homogenous non-fluorescent area. Angiograms of the animals in the control group showed uniform angiogenesis in the defect site with establishment of trans-transplant angiogenesis, whereas in group II there was complete trans-transplant shunting of blood vessel communication. Porous HAp ceramic prepared by an aqueous combustion technique promoted bone formation over the defect, confirming their biologic osteo-conductive property.

Chen., *et al*. [51] observed the long-term effect of tissue engineering-based repair of large weight-bearing bone defect in goats, and the final outcome of the scaffold material coral hydroxyapatite (CHAP) in vivo. Fifteen Chinese goats were subjected to operations to induce a 2 cm left tibial diaphyseal defect, which was filled subsequently with CHAP and bone marrow stromal stem cells (BMSCs). The repaired defects were evaluated by ECT, X-ray and histology in the early stage and at 6, 12, 18, and 24 months postoperatively. ECT showed good bone regeneration and revascularization within 2 months postoperatively. X-ray and histology displayed eccentric and gradual bone regeneration in the early stage, and the tissue engineered bone graft was firmly healed with the goat tibia. X-ray and histological examination at 6, 12, 18, 24 months postoperatively revealed moulding of the new bones and medullary cavity recanalization, and the structure of CHAP disappeared and gradually integrated into the new bones. They concluded that tissue-engineered bone is capable of total repair of large bone defect in goats by forming normal functional new bones. CHAP can be eventually degraded completely and become the component of the newly generated bones.

Zhou., *et al*. [52] studied the outcome of reconstructing goat femoral fracture by use of cortical bone plates allografts that have been kept by deep-freezing at -70 degrees C for 4 weeks after being treated with 48 degrees C ethylene oxide. The recipients, sixteen 10-12month-old goats with fractures of right femur were subjected the operation for transplanting the cortical bone plates allografts in the medial, lateral and back sides of fractured femurs. The goats were necropsied and the specimens were procured at 3, 6, 12, and 24 weeks after surgery for X-ray photography, Chinese ink perfusion, tetracycline fluorescence labeling and histological observation in order evaluate the healing of fracture and the incorporation of cortical bone plates allografts. The allograft strut was found revascularized at 6 weeks after surgery in the fracture group, whereas at 3 weeks in the control group. The tetracycline fluorescence labeling was poor in the fracture group as compared with that in the control group from 3 weeks to 6 weeks, but it was better in the fracture group than in the control group beyond 6 weeks after surgery. Fracture was healed and bone conjunction between allograft strut and host bone was seen at weeks after operation. The allograft strut was incorporated in host bone, the ability of remodeling of allograft strut and the size of femoral cortex were better in the fracture group than in the control group at 24 weeks after surgery.

The fracture was displaced in 19% animals and the allograft bone plates were not fractured.

They concluded that the use of allograft strut pre-treated by ethylene oxide sterilization and deep-freezing could underpin fixation and promote healing of femoral fracture, and it can increase bone reservation and augment the strength of femur once the allograft strut is incorporated in the host bone.

Welch., *et al*. [53] studied the effects of rhBMP-2 in an absorbable collagen sponge (ACS) on bone healing in a large animal tibial fracture model. Bilateral closed tibial fractures were created in 16 skeletally mature goats and reduced and stabilized using external fixation. In each animal, one tibia received the study device (0.86 mg of rhBMP-2/ACS or buffer/ACS), and the contralateral fracture served as control. The device was implanted as a folded onlay or wrapped circumferentially around the fracture. Six weeks following fracture, the animals were necropsied and the tibiae harvested for torsional testing and histomorphologic evaluation. Radiographs indicated increased callus at 3 weeks in the rhBMP-2/ACS treated tibiae. At 6 weeks, the rhBMP-2/ACS wrapped fractures had superior radiographic healing scores compared with buffer groups and controls. The rhBMP-2/ACS produced a significant increase in torsional toughness ($p = 0.02$), and trends of increased torsional strength and stiffness ($p = 0.09$) compared with fracture controls. The device placed in a wrapped fashion around the fracture produced significantly tougher callus ($p = 0.02$) compared with the onlay application. Total callus new bone volume was significantly increased ($p = 0.02$) in the rhBMP-2/ACS fractures compared with buffer groups and controls regardless of the method of device application. The rhBMP-2/ACS did not alter the timing of onset of periosteal/endosteal callus formation compared with controls. Neither the mineral apposition rates nor bone formation rates were affected by rhBMP-2/ACS treatment. The increased callus volume associated with rhBMP-2 treatment produced only moderate increases in strength and stiffness.

Healing in the presence of inter-current disease

Bone infection remains a significant concern. Tran., *et al*. [54] evaluated antibacterial coatings *in vitro* and developed a caprine model to assess coated bone implants. The coating consisted of titanium oxide and siloxane polymer with silver and tested *in vitro* using rapid screening techniques to determine compositions which inhibited *Staphylococcus aureus* growth, while not affecting osteoblast viability. The coating was then applied to intramedullary nails and evaluated *in vivo* in a caprine model. A fracture was created in the tibia of the goat, and *Staphylococcus aureus* was inoculated directly into the bone canal. The fractures were fixed by either coated (treated) or non-coated intramedullary nails (control) for 5 weeks. Clinical observations as well as microbiology, mechanical, radiology, and histology testing were used to compare the animals. The treated goat was able to walk using all four limbs after 5 weeks, while the control was unwilling to bear weight on the fixed leg. While this model is too small to be considered any more than a pilot study, these results support antimicrobial potential of hybrid coating intra-medullary implants.

Pin tract infection is a common complication of external fixation. An anti-infective external fixator pin might help to reduce the incidence of pin tract infection and improve pin fixation. DeJong., *et al*. [55] used stainless steel and titanium external fixator pins, with and without a lipid stabilized hydroxyapatite/chlorhexidine coating in a goat model. Two pins contaminated with an identifiable *Staphylococcus aureus* strain were inserted into each tibia of 12 goats. The pin sites were examined daily. On day 14, the animals were killed, and the pin tips cultured. Insertion and extraction torques were measured. Infection developed in 100% of uncoated pins, whereas coated pins demonstrated 4.2% infected, 12.5% colonized, and the remainder, 83.3%, had no growth ($p < 0.01$). Pin coating decreased the percent loss of fixation torque over uncoated pins ($p = 0.04$). They found that the lipid stabilized hydroxyapatite/chlorhexidine coating was successful in decreasing infection and improving fixation of external fixator pins.

Hou., *et al*. [56] investigated the delivery of vancomycin via alginate beads embedded in a fibrin gel (Vanco-AB-FG) to treat bone infections, with the addition of bone marrow-derived mesenchymal stem cells (BMMSCs) seeded in the fibrin gel to promote bone formation. The proliferation of BMMSCs was measured under different conditions of three-dimensional (3D) gel or monolayer, with or without Vanco-AB; cells were labelled by enhanced green fluorescence protein, and their morphology and distribution were observed. The alka-

line phosphatase (ALP) activity, real-time RT-PCR, and von Kossa staining were used for determining the osteogenic differentiation of BMMSCs. The concentrations of vancomycin resulting from the antibiotic delivery were determined; the antibiotic activity was evaluated by an assay with standard *Staphylococcus aureus* (ATCC 25923) as a biological target. The results showed that for Vanco-AB-FG, vancomycin concentrations remained above the breakpoint sensitivity for 22 days. The 3D culture within the gel and the addition of Vanco-AB affected the cell behavior. The morphology of BMMSCs within the 3D gel was different from that in monolayer. The proliferation of the cells within the 3D gel was lower than that in monolayer in early stage, but in later stage the number of BMMSCs in Vanco-AB-FG was similar to that in monolayer. The ALP activity was higher in the 3D gel, and the addition of Vanco-AB slightly increased ALP activity. The osteogenic gene expression levels of ALP, osteopontin, and alpha1 chain of collagen I were higher in the 3D gel than those in monolayer, and additional Vanco-AB could also increase their expression. The von Kossa staining showed that the deposition of mineralization was observed in both the 3D gel and monolayer cultures, but the mineralization nodule size in monolayer was bigger and the number of them in 3D gel was greater. They proposed this system could be useful for treating for bone infections and defects.

Porcine Models

Pigs are metabolically most similar to humans of the non-primate models in respect of pharmacokinetics, the structure and function of the organs, the morphology of bone and the overall metabolic nature.

Healing, bone defects and non-union

The mechanical properties (resistance to bending forces) of flexible buttress osteosynthesis using two different bone-implant constructs stabilizing experimental segmental femoral bone defects (segmental ostectomy) was investigated by Urbanova., *et al*. [57] in a miniature pig ex vivo model using 4.5 mm titanium LCP and a 3 mm intramedullary pin (plate and rod construct) (PR-LCP), versus the 4.5 mm titanium LCP alone (A-LCP). The plate and rod fixation (PR-LCP) of the segmental femoral defect is significantly more resistant ($p < 0.05$) to bending forces (200 N, 300 N, and 500 N) than LCP alone (A-LCP). Stabilization of experimental segmental lesions of the femoral diaphysis in miniature pigs by flexible bridging osteosynthesis 4.5 mm LCP in combination with the plate and rod construct appears to be a suitable fixation of non-reducible fractures where considerable strain of the implants by bending forces can be assumed.

During orthopaedic surgery, small bicortical circular bone defects are often produced as a result of internal fixation of fractures. Ho., *et al*. [58] investigated the amount of torsional strength reduction in animal bone with a bicortical bone defect and how much residual strength remains if the bicortical bone defect was occluded. Forty pig femurs were divided into four groups. Group 1 femurs were left intact. Group 2 femurs were given a 4 mm bicortical bone defect. Group 3 were prepared as in Group 2, but occluded with a 4.5 mm cortical screw. Group 4 were prepared as in Group 2, but occluded with plaster of paris. Measurements including the length of the bone, working length of the bone, mid-diaphyseal diameter and cortical thickness were recorded. All specimens were tested until failure under torsional loading. Peak torque at failure and angular deformation were recorded. One-way analysis of variance was used to test the sample groups, with a value of $P < 0.05$ considered to be statistically significant. When compared with Group 1, all of the other groups showed a reduction in peak torque at failure point. Only the difference in peak torque between Groups 1 and 2 was statistically significant ($P = 0.007$). Group 2 showed the most reduction with 23.11% reduction in peak torque and 38.19% reduction in total energy absorption. No significant difference was found comparing the bone length, bone diameter and the cortical thickness. The presence of the defect remains the major contributing factor in long bone strength reduction. It has been shown that a 10% bicortical defect was sufficient to produce a reduction in peak torque and energy absorption under torsional loading. By occluding this defect using a screw or plaster of paris, an improvement in bone strength was achieved. These results may translate clinically to an increased vulnerability to functional loads immediately following screw removal and prior to the residual screw holes healing.

Biomechanics of fracture fixation and testing of mechanical properties of bone/implant construct were investigated using computer aided mathematical modelling. Necas., *et al*. [59] used this to obtain a 3D model to characterize forces acting on the implant and analyze

the forces causing the implant failure (broken plate). Their study employed mathematical-statistical modelling for determination of forces that caused failure (broken implant) of a five-hole titanium 4.5 mm Locking Compression Plate. This plate has been used for flexible bridging osteosynthesis of segmental femoral diaphyseal defect in a miniature pig to investigate bone healing after transplantation of mesenchymal stem cells in combination with biocompatible scaffolds. Mathematical modelling has been performed with COMSOL Multiphysics software. Numerical study described deformation processes taking place in implant failure and demonstrated possibilities of deformation of five-hole titanium 4.5 mm LCP in the case of exceeding the elastic limits of a material. Knowledge of the forces acting on implants used for fracture fixation acquired from mathematical modelling could potentially be used in human practice in order to prevent undesirable implant failure.

Necas., *et al*. [60] described types, absolute and relative numbers of implant failures in flexible bridging osteosynthesis using a six-hole 3.5 mm titanium Locking Compression Plate (n = 9) or a five-hole LCP 4.5 mm titanium (n = 40) selected for the fixation of segmental ostectomy of femoral diaphysis in the miniature pig used as an *in vitro* model in a study on the healing of a critically sized bone defect using transplantation of mesenchymal stem cells combined with biocompatible scaffolds within a broader research project. Occasional implant failure was evaluated based on radiographic examination of femurs of animals 2, 4, 8, 12 and 16 weeks after surgery. When bone defect was stabilized using 3.5 mm LCP, in 6 cases (66.7%) the screw was broken/lost in the proximal fragment of the femur 2 weeks after implantation (n = 4) and 4 weeks after implantation (n = 2). In 4 of these, the implant failure was accompanied by loosening of the screw in position 3 in the proximal fragment of the femur. During ostectomy stabilization with 4.5 mm LCP, in 3 cases (7.5%) LCP was broken at the place of the empty central plate hole (without inserted screw) at the level of the segmental bone defect. Compared to the six-hole 3.5 mm LCP, the five-hole titanium 4.5 mm LCP is more suitable implant for flexible bridging osteosynthesis of a critically sized segmental defect of femoral diaphysis in the miniature pig.

Mesenchymal stem cells (MSCs) are the key repair cells in bone healing and implant osseointegration, but the osteogenic capacity of minipig MSCs is incompletely known. Heino., *et al*. [61] aimed to isolate and characterize minipig bone marrow (BM) and peripheral blood (PB) MSCs in comparison to human BM-MSCs. BM sample was aspirated from posterior iliac crest of five male Gottingen minipigs (age 15 +/- 1 months). PB sample was drawn for isolation of circulating MSCs. MSCs were selected by plastic-adherence as originally described by Friedenstein. Cell morphology, colony formation, proliferation, surface marker expression, and differentiation were examined. Human BM-MSCs were isolated and cultured from adult fracture patients (n = 13, age 19 - 60 years) using identical techniques. MSCs were found in all minipig BM samples, but no circulating MSCs could be detected. Minipig BM-MSCs had similar morphology, proliferation, and colony formation capacities as human BM-MSCs. Unexpectedly, minipig BM-MSCs had a significantly lower ability than human BM-MSCs to form differentiated and functional osteoblasts. This observation emphasizes the need for species-specific optimization of MSC culture protocol before direct systematic comparison of MSCs between human and various preclinical large animal models can be made.

Management of pain in research swine used for studies involving painful procedures is a considerable challenge. Royal., et al. [62] assessed whether a regional anesthesia method is effective for pain control of hindlimb injuries in pigs used for research in bone fracture healing. For this randomized controlled study, they administered regional anesthesia before an experimental femoral injury was produced. Using ultrasound guidance, sterile infusion catheters were placed near the sciatic and femoral nerves and administered local anesthetic (bupivacaine) for the first 24h after surgery. They evaluated Behavioural and physiologic parameters to test the hypothesis that this regional anesthesia would provide superior analgesia compared with systemic analgesia alone. They also collected blood samples to evaluate serum levels of cortisol and fentanyl postoperatively. At the end of the study period, they harvested sciatic and femoral nerves and surrounding soft tissues for histopathologic evaluation. Treatment pigs had lower subjective pain scores than did control animals. Control pigs had a longer time to first feed consumption and required additional analgesia earlier in the postoperative period than did treatment pigs. Ultrasound-guided regional anesthesia is a viable and effective adjunct to systemic analgesics for providing pain control in swine with experimental femoral fractures.

Healing in the presence of inter-current disease

It is generally accepted that surgery is necessary for the proper treatment of chronic haematogenous osteomyelitis (HO) in children. However, the correct timing of surgery and the technique most effective for debridement of infectious bone tissue is debated. Johansen., *et al*. [63] report, a porcine model of HO exposed to surgical treatment and compare this with their surgical experiences with Angolan children suffering from chronic HO. Surgically-debrided bone tissue from the children and pigs were analyzed microbiologically and histopathologically together with the entire operated bones from the pigs. It was illustrated that surgical intervention on porcine bones with experimentally-induced HO is representative of the handling of the condition in children. The porcine HO model was used for refinement and application of surgical techniques used in order to cure children with HO. Johansen., *et al*. [64] also report on their inoculation technique for the model and which was based on inoculation into the femoral artery mimicking hematogenous osteomyelitis involving femur and tibia in children.

Canine Models

The dog provides a more athletic model with concomitantly different physical forces on limbs and metabolic differences to herbivorous animals. The dog also provides conveniently sized bone morphology for operative treatment and well characterized physiological and pharmacological subjects.

Healing, bone defects and non-union

Conventional nails are being used for an expanding range of fractures from simple to more complex. Angle stable designs are a relatively new innovation; however, it is unknown if they will improve healing for complex fractures. Kubacki., *et al*. [65] compared traditional and angle stable nails to treat complex open canine femur fractures addressing whether two constructs differ in: radiographic evidence of bone union across the cortices; stability as determined by toggle (torsional motion with little accompanying torque) and angular deformation; biomechanical properties, including stiffness in bending, axial compression, and torsional loading, and construct failure properties in torsion; and, degree of bone tissue mineralization. Ten dogs with a 1 cm femoral defect and periosteal stripping were treated with a reamed titanium angle stable or non-angle stable nail after the creation of a long soft tissue wound. Before the study, animals were randomly assigned to receive one of the nails and to be evaluated with biomechanical testing or histology. After euthanasia at 16 weeks, all operative femora were assessed radiographically. Histological and biomechanical evaluation was conducted of the operative bones with nails left *in situ* compared with the non-operative contralateral femora. Radiographic and gross inspection demonstrated hypertrophic non-union in all 10 animals treated with the nonangle stable nail, whereas six of 10 animals treated with the angle stable nail bridged at least one cortex ($p = 0.023$). The angle stable nail construct demonstrated no toggle in nine of 10 animals, whereas all control femora exhibited toggle. The angle stable nail demonstrated less angular deformation and toggle ($p = 0.005$) and increased compressive stiffness ($p = 0.001$) compared with the conventional nonangle stable nail. Histology demonstrated more non-mineralized tissue in the limbs treated with the conventional nail ($p = 0.005$). Angle stable nails that eliminate toggle lead to enhanced yet incomplete fracture healing in a complex canine fracture model. Thus it was concluded that care should be taken in tailoring the nail design features to the characteristics of the fracture and the patient.

Volpon., *et al*. [66] assessed the repair of transverse, 3 mm wide bone gaps created at the distal radius in 28 dogs randomly divided into two 14-animal groups; one was the control group and the other received a daily, 20-min application of low-intensity pulsed ultrasound for 100 days. Sequential radiographs, histomorphometrics, bone fluorescent histology and bone vascularity assessments found that all animals from both groups obtained a stage of hypertrophic-type non-union with fibrocartilage tissue formation throughout the region of osteotomy. However, treated animals exhibited areas of endochondral ossification within the fibrocartilage region. There was no difference in type of vascularity or the newly formed bone process obtained by tetracycline labelling. Application of low-intensity ultrasound was not capable of significantly changing the reparative process and it may not be sufficiently powerful to overcome a combination

of local deleterious effects on bone healing, created by gapping, excessive motion and periosteal resection.

Heo., *et al*. [67] investigated the effect of xenogenic cortical bone (XCB) on fracture repair in the canine ulna. The entire group of animals (n = 12) had a transverse resection of 5 mm length at the middle part of the right ulnar diaphysis. In Group A (eight beagles), the fracture was treated with XCB and metal bone screw. In Group B (four beagles), the fracture was treated with metal bone plate and screw. Radiological, micro-computed tomography (micro-CT), histological examination and mechanical testing were employed to evaluate bone healing and reaction of XCB in the host bone. In Group A, bone union was noticed in 6 out of 8 dogs (75%), starting from the 4th week onwards. Micro-CT and histological examinations showed that the XCB was absorbed and incorporated into the host bone. Incorporation of XCB was observed in 7 cases (88%); it started from the 10th week onwards and continued to week 32 after surgery. Biomechanical strength of the bone fracture site was higher in Group A than in Group B, and was similar to that of normal bone. XCB enhances the bone healing process and can be used as absorbable internal fixation for the management of long Dejardin bone fractures in dogs.

Dejardin., *et al*. [68] compared clinical outcome and callus biomechanical properties of a novel angle stable interlocking nail (AS-ILN) and a 6 mm bolted standard ILN (ILN6b) in a canine tibial fracture model using dogs (n = 11). A 5 mm mid-diaphyseal tibial ostectomy was stabilized with an AS-ILN (n = 6) or an ILN6b (n = 5). Orthopedic examinations and radiographs were performed every other week until clinical union (18 weeks). Paired tibiae were tested in torsion until failure. Callus torsional strength and toughness were statistically compared and failure mode described. Total and cortical callus volumes were computed and statistically compared from CT slices of the original ostectomy gap. Statistical significance was set at $P < 0.05$ From 4 to 8 weeks, lameness was less pronounced in AS-ILN than ILN6b dogs ($P < 0.05$). Clinical union was reached in all AS-ILN dogs by 10 weeks and in 3/5 ILN6b dogs at 18 weeks. Callus mechanical properties were significantly greater in AS-ILN than ILN6b specimens by 77% (failure torque) and 166% (toughness). Failure occurred by acute spiral (control and AS-ILN) or progressive transverse fractures (ILN6b). Cortical callus volume was 111% greater in AS-ILN than ILN6b specimens ($P < .05$). Earlier functional recovery, callus strength and remodeling suggest that the AS-ILN provides a postoperative biomechanical environment more conducive to bone healing than a comparable standard ILN.

Experimental studies have shown the ability of statins to stimulate bone formation when delivered locally or in large oral doses, however most have been studied in rodents. This anabolic effect is through the selective activation of BMP-2. Bleedorn., *et al*. [16] administered lovastatin (6 mg/kg) by percutaneous injection to a canine tibial osteotomy stabilized with external fixation. They found that lovastatin improved bone healing after a single injection into the fracture site assessed by serial radiography and histology at bone union. However, lovastatin treatment resulted in adverse local soft tissue inflammation. These results suggest that percutaneous lovastatin injection may be a useful adjuvant treatment over the course of bone healing to augment fracture repair, although further investigation into the mechanism of soft tissue adverse effects is warranted.

Healing in the presence of inter-current disease

Varshney., *et al*. [69] induced osteomyelitis in 45 male dogs by inoculating hemolytic strain of Staphylococcus aureus alone into the tibial marrow cavity. Clinical, radiological and bacteriological studies were conducted to evaluate the progress of disease up to 15 weeks. Clinical signs consisted of localized soft tissue swelling, pain, pyrexia and lameness which later developed an open wound with purulent exudation. Predominant radiographic features were extensive periosteal reaction, cortical lysis, new bone formation, frequent development of sequestrum and formation of localized abscess pockets in advanced cases. Staphylococci were recovered from the tibial marrow cavity for as long as 15 weeks after onset of the infection.

Equine Models

Whilst larger and more expensive, equine models provide an ultimate test of load bearing and torsional mechanical properties. Anatomically the equine lower limb is comprised of an elongated load bearing metacarpal III or metatarsal III. By contrast this naturally splints non load bearing metatarsals II and IV. A complication of the equine limb is reliance on the frog mechanism of the foot for venous

return. Non-weight bearing lameness can result in vascular pooling as a significant complication to surgery. The equine lower limb also has few muscles and is mainly comprised of tendons running from the upper leg as pulley systems via the metacarpus to the hoof. Collateral circulation is therefore also poor and cannot be relied upon to aid the healing process, however surgical access is relatively bloodless and simple.

Healing, bone defects and non-union

Southwood., *et al*. [70] used an equine metacarpal IV (MCIV) ostectomy model and adenoviral vectors encoding the human bone morphogenetic protein-2 and protein-7 gene (Ad-BMP-2/-7) to evaluate gene transfer using healthy adult horses (n = 15). A plate stabilized, critical size 1.5 cm ostectomy was created in left and right MCIV. The ostectomy site was injected with either Ad-green fluorescent protein (Ad-GFP) or Ad-hBMP-2/-7 at completion of surgery; the same treatment was assigned to both the left and right forelimb of each horse (n = 5 horses/group). Bone healing was evaluated radiographically every 2 weeks for 16 weeks. Horses in a pilot study (n = 5) were used as untreated controls for radiographic evaluation to 8 weeks. After euthanasia at 16 weeks bone healing was evaluated using dual energy X-ray absorptiometry (DEXA) and histomorphometry. Data were analyzed using an ANOVA or Kruskal-Wallis test. Level of significance was $P < 0.05$. At 4 and 6 weeks, the Ad-GFP group had a significantly lower percentage defect ossification compared with the untreated control group. There was no significant difference between untreated and AdhBMP-2/-7 groups at any time point and no significant difference in bone healing radiographically, histologically, or using DEXA between any groups at 16 weeks. They concluded that Ad-hBMP-2/-7 did not improve bone healing in horses at 16 weeks.

McDuffee., *et al*. [71] compared the efficacy of osteoprogenitors in fibrin glue to fibrin glue alone in bone healing of surgically induced ostectomies of the fourth metacarpal bones in an equine model using adult horses (n = 10). Segmental ostectomies of the 4th metacarpal bone (MC4) were performed bilaterally in 10 horses. There was 1 treatment and 1 control limb in each horse. Bone defects were randomly injected with either fibrin glue and osteoprogenitor cells or fibrin glue alone. Radiography was performed every week until the study endpoint at 12 weeks. After euthanasia, bone healing was evaluated using radiography and histology. Analysis of radiographic data was conducted using a linear-mixed model. Analysis of histologic data was conducted using a general linear model. Statistical significance was set at $P < 0.05$. Radiographic grayscale data as a measure of bone healing revealed no significant difference between treatment and control limbs. Radiographic scoring results also showed that the treatment effect was not significant. Histologic analysis was consistent with radiographic analysis showing no significant difference between the area of bone present in treatment and control limbs. Injection of periosteal-derived osteoprogenitors in a fibrin glue carrier into surgically created ostectomies of MC4 does not accelerate bone healing when compared with fibrin glue alone.

Selzer., *et al*. [72] investigated torsional monotonic structural material properties of equine metacarpi with or without, either a 5/16 inch or 3/8-inch diameter bicortical lateromedial middiaphyseal hole were assessed to determine the effect of a hole on metacarpal strength. Torsional stiffness was not significantly effected by the presence of a bicortical hole, whereas yield and failure angles, torques and energies of metacarpi with a hole were 51% to 97% of those of intact bones. Significant differences were not apparent for yield and failure mechanical properties between metacarpi with a 5/16 inch diameter hole and metacarpi with a 3/8 inch diameter hole; however, post yield mechanical properties were lower for metacarpi with a 3/8 inch hole. Whereas some metacarpi with a 5/16 inch hole were capable of plastic deformation before failure, metacarpi with a 3/8 inch diameter hole appeared to have sufficient stress concentration to propagate complete fracture on structural yield.

Knowledge of the forces that act upon the equine humerus while the horse is standing and the resulting strains experienced by the bone is useful for the prevention and treatment of fractures and for assessing the proximolateral aspect of the bone as a site for obtaining autogenous bone graft material. Pollock., *et al*. [73] developed a mathematical model to predict the loads on the proximal half of the humerus created by the surrounding musculature and ground reaction forces while the horse is standing. They calculated surface bone

stresses and strains at three cross sections on the humerus corresponding to the donor site for bone grafts, a site predisposed to stress fracture, and the middle of the diaphysis. A three dimensional mathematical model employing optimization techniques and asymmetrical beam analysis was used to calculate shoulder muscle forces and surface strains on the proximal and mid-diaphyseal aspects of the humerus. The active shoulder muscles, which included the supraspinatus, infraspinatus, subscapularis, and short head of the deltoid, produced small forces while the horse is standing; all of which were limited to 4.3% of their corresponding maximum voluntary contraction. As a result, the strains calculated at the proximal cross sections of the humerus were small, with maximum compressive strains of -104 microepsilon at the cranial aspect of the bone graft donor cross section. The middle of the diaphysis experienced larger strain magnitudes with compressive strains at the lateral and the caudal aspects and tensile strains at the medial and cranial aspects (377 microepsilon and 258microepsilon maximum values, respectively) while the horse is standing. Small strains at the donor bone graft site do not rule out using this location to harvest bone graft tissue, although strains while rising to a standing position during recovery from anesthesia are unknown. At the site common to stress fractures, small strains imply that the stresses seen by this region while the horse is standing, although applied for long periods of time, are not a cause of fracture in this location. Knowing the specific regions of the middle of the diaphysis of the humerus that experience tensile and compressive strains is valuable in determining optimum placement of internal fixation devices for the treatment of complete fractures.

Discussion

The sub-cellular biochemical end stage (proteomics) of bone repair can be elucidated in *ex vivo* and *in vitro* models. However, the expression of biochemical pathways is driven by genetic polymorphisms (genomics) within species that become magnified to the level that the validity of interspecies extrapolation is questionable. Superimposed upon this are phenotypic demands to support external physical stressors.

Animal models have been long being used and continue to have a place in orthopaedic innovation and development. Ethical constraints as well as reasons of experimental design and control limit direct progression to human clinical studies. Used with discretion to limit the impact on animal welfare, each species provides a unique perspective on the performance of prosthesis, healing and repair processes in different scenarios. Whilst generally results cannot be directly 'translated' to human patients, these provide invaluable windows of enlightenment to broaden our perspective on future innovative approaches. Some species such as the fish are cheap and bone changes can be directly visualised in living subjects. Others such as the mouse have been selectively bred for genotypic traits. Larger animal models may be more suitable in terms of size, to test scale versions of prosthetics and to evaluate mechanical properties.

Murine models have traditionally provided powerful tools to understand the genetic basis of normal and impaired bone healing and define the role of inflammation, skeletal cell lineages, signalling pathways, the extracellular matrix, osteoclasts and angiogenesis. Murine models for delayed repair and non-union provide inspiration for greater understanding of human conditions yet the massive gulf in physiological evolution restricts these to proof of concept studies.

Larger animal models are generally required to validate conceptual interventions and accommodate the demand for limbs large enough size to scale up prosthetic implants and where there are comparable physical forces to test interventions.

Animal models are also useful for investigation of diseases such as osteomyelitis and osteoporosis, both of which can be conveniently reproduced. Commonly used bones for creating local osteomyelitis include tibia, femur, and radius, and, less frequently, mandible and spine. When designing a specific model, one should consider which animal species, which bone, the route for inoculation (e.g. local or systemic), bacterial species and infection dose, sclerosing agent if applicable, whether a foreign body or implant should be employed, and if local trauma is needed.

Current clinical therapeutic approaches for bone reconstruction focus on transplantation of autografts and allografts, and the implantation of metal devices or ceramic-based implants to assist bone regeneration. Bone grafts possess osteo-conductive and osteo-inductive properties, however they are limited in access and availability and associated with donor site morbidity, haemorrhage, risk of infection, insufficient transplant integration, graft devitalisation, and subsequent resorption resulting in decreased mechanical stability. Recent research focuses on the development of alternative therapeutic concepts for the development of tissue engineered constructs for bone regeneration. Approval by regulatory bodies is a protracted and costly process requiring comprehensive *in vitro* and *in vitro* studies. In translational orthopaedic research, the utilisation of preclinical animal studies is prerequisite.

Comparison between studies and outcomes are confounded by their differences. Direct translation from animal-models to human intervention is infrequent. Ideally animal models, fixation devices, surgical procedures and methods of taking measurements would be standardized to produce reliable data pools as a base for further research directions, practically this is unlikely to occur in a range of facilities with investigators with different objectives. In this paper, we have categorized orthopaedic repair models by animal species and focused upon models for weight-bearing limbs of the appendicular skeleton, including investigations of fracture-healing, segmental bone defects, fracture non-union and intercurrent disease in the second part of this review series we consider osteoporotic bone and imaging, while in subsequent review papers we take the same approach to consider the axial skeleton and joints and cartilage repair.

The objective of this paper has been to provide a resource for quick reference of established models in major non-primate species and to maximize the value of previous work on animals models used to study orthopaedic repair in the appendicular long bones. This review has therefore been organized within species of animal by topic to aid as a reference text and to provide a resource to help future researchers locate definitive study references.

Bibliography

1. Anon. "Opera Omnia Quae Extant Frankfurt". (1595).
2. Anon. "The Genuine Works of Hippocrates by Francis Adams, London". (1842).
3. Ponseti IV. "History of Orthopaedic Surgery". *Iowa Orthopaedic Journal* 11 (1991): 59-64.
4. Anon. "The Next 4 billion: Market Size and Business Strategy at the Base of the Pyramid". *World Resources Institute (2015)*.
5. Reeve-Johnson L. "In Forensic Veterinary Medicine ed Bailey D. Macro-economic incentives for Translational research and One Health collaborations". *CABI publishing Cambridge* (2016).
6. Reeve-Johnson L. "Use of experimentally induced diseases in the development and evaluation of therapeutic agents". *Veterinary Record* 142.23 (1998): 638-642.
7. Ioannidis JPA. "Why most published research findings are false". *PLoS Med* 2.8 2005.: e124.
8. Reeve-Johnson L. "Evidence Based Medicine". *Veterinary Record* 177.10 (2015): 266
9. Pigott HE., *et al*. "Efficacy and Effectiveness of Antidepressants: Current Status of Research". *Psychother Psychosom* 79.5 (2010): 267-279.
10. Takeyama K., *et al*. "*In-vivo* imaging of the fracture healing in medaka revealed two types of osteoclasts before and after the callus formation by osteoblasts". *Developmental Biology* 394.2 (2014): 292-304.

11. Hildebrand F., *et al*. "Animal models to assess the local and systemic effects of nailing: Review of the literature and considerations for future studies". *Journal of Trauma and Acute Care Surgery* 76.6 (2014): 1495-1506.

12. Garcia P., *et al*. "Rodent Animal Models of Delayed Bone Healing and non-union formation: A comprehensive review". *European Cells & Materials* 26 (2013): 1-12.

13. Zwingenberger S., *et al*. "Establishment of a femoral critical-size bone defect model in immunodeficient mice". *Journal of Surgical Research* 181.1 (2013): e7-e14.

14. Alford AI., *et al*. "Two Molecular Weight Species of Thrombospondin- Are Present in Bone and Differentially Modulated in Fractured and Nonfractured Tibiae in a Murine Model of Bone Healing". *Calcified Tissue International* 90.5 (2012): 420-428.

15. Kondo E., *et al*. "Increased Bone Turnover and Possible Accelerated Fracture Healing in a Murine Model With an Increased Circulating C-Type Natriuretic Peptide". *Endocrinology* 156.7 (2015): 2518-2529.

16. Bleedorn JA., *et al.* "Percutaneous Lovastatin Accelerates Bone Healing but Is Associated with Periosseous Soft Tissue Inflammation in a Canine Tibial Osteotomy Model". *Journal of Orthopaedic Research* 32.2 (2014): 210-216.

17. Sottnik JL., *et al*. "Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses". *Cancer Immunology Immunotherapy* 59.3 (2010): 367-378.

18. De Giacomo A., *et al*. "Generation of Closed Transverse Fractures in Small Animals In: Hilton MJ, ed". *Skeletal Development and Repair: Methods and Protocols* (2014): 35-44.

19. Kaspar K., *et al*. "A New Animal Model for Bone Atrophic Nonunion: Fixation by External Fixator". *Journal of Orthopaedic Research* 26.12 (2008): 1649-1655.

20. Fassbender M., *et al*. "Efficacy of two different demineralised bone matrix grafts to promote bone healing in a critical-size-defect: a radiological, histological and histomorphometric study in rat femurs". *International Orthopaedics* 38.9 (2014): 1963-1969.

21. Shapiro G, *et al*. "Ultrasound-mediated transgene expression in endogenous stem cells recruited to bone injury sites". *Polymers for Advanced Technologies* 25.5 (2014): 525-531.

22. Montgomery SR., *et al*. "A Novel Osteogenic Oxysterol Compound for Therapeutic Development to Promote Bone Growth: Activation of Hedgehog Signaling and Osteogenesis Through Smoothened Binding". *Journal of Bone and Mineral Research* 29.8 (2014): 1872-1885.

23. Yuasa M., *et al*. "The temporal and spatial development of vascularity in a healing displaced fracture". *Bone* 67 (2014): 208-221.

24. Fassbender M., *et al*. "Local gentamicin application does not interfere with bone healing in a rat model". *Bone* 55.2 (2013): 298-304.

25. Nicholls F., *et al.* "Effects of radiation and surgery on healing of femoral fractures in a rat model". *Journal of Orthopaedic Research* 31.8 (2013): 1323-1331.

26. Suen PK., *et al*. "Sclerostin Monoclonal Antibody Enhanced Bone Fracture Healing in an Open Osteotomy Model in Rats". *Journal of Orthopaedic Research* 32.8 (2014): 997-1005.

27. Frey SP., *et al*. "Cysteine-rich matricellular protein improves callus regenerate in a rabbit trauma model". *International Orthopaedics* 36.11 (2012): 2387-2393.

28. Tobita K., *et al*. "Measurement of mechanical properties on gap healing in a rabbit osteotomy model until the remodeling stage". *Clinical Biomechanics* 27.1 (2012): 99-104.

29. Gajdobranski D., *et al*. "Influence of Different Methods of Internal Bone Fixation on Characteristics of Bone Callus in Experimental Animals". *Srpski Arhiv Za Celokupno Lekarstvo* 142.1-2 (2014): 40-47.

30. Ye DM., *et al*. "Effects of Low-Dose Microwave on Healing of Fractures with Titanium Alloy Internal Fixation: An Experimental Study in a Rabbit Model". *Plos One* 8.9 (2013): e75756.

31. Southwood LL, *et al.* "Evaluation of Ad-BMP-2 for enhancing fracture healing in an infected defect fracture rabbit model". *Journal of Orthopaedic Research* 22.1 (2004): 66-72.

32. Arens D, *et al*. "A rabbit humerus model of plating and nailing osteosynthesis with and without Staphylococcus aureus osteomyelitis". *European Cells & Materials* 30 (2015): 148-162.

33. Mutsuzaki H., *et al*. "Enhanced wound healing associated with Sharpey's fiber-like tissue formation around FGF-2-apatite composite layers on percutaneous titanium screws in rabbits". *Archives of Orthopaedic and Trauma Surgery* 132.1 (2012): 113-121.

34. Wellisz T., *et al*. "Infection rates and healing using bone wax and a soluble polymer material". *Clinical Orthopaedics and Related Research* 466.2 (2008): 481-486.

35. Kaplan SJ., *et al*. "Monitoring the healing of a tibial osteotomy in the rabbit treated with external fixation". *Journal of Orthopaedic Research* 3.3 (1985): 325-330.

36. Kdolsky R, *et al*. "Biomechanical analysis of fracture healing in guinea=-pigs". *Studies in Health Technology and Informatics* 133 (2008): 141-147.

37. Omeroğlu S., *et al*. "Effects of single high-dose vitamin D3 on fracture healing. An ultrastructural study in healthy guinea pigs". *Archives of Orthopaedic and Trauma Surgery* 116.1-2 (1997): 37-40.

38. Passl R., *et al*. "A model of experimental post-traumatic osteomyelitis in guinea pigs". *Journal of trauma* 24.4 (1984): 323-326.

39. Lienau J., *et al*. "Insight into the Molecular Pathophysiology of Delayed Bone Healing in a Sheep Model". *Tissue Engineering Part A* 16.1 (2010): 191-199.

40. Piorek A., *et al*. "Treatment of tibial shaft fractures in sheep using interlocking nails, Schanz screws and type I external fixators". *Polish Journal of Veterinary Sciences* 15.4 (2012): 651-659.

41. Dergin G., *et al*. "Direct Current Electric Stimulation in Implant Osseointegration: An Experimental Animal Study with Sheep". *Journal of Oral Implantology* 39.6 (2013): 671-679.

42. Vetter A., *et al*. "Temporal Tissue Patterns in Bone Healing of Sheep". *Journal of Orthopaedic Research* 28.11 (2010): 1440-1447.

43. Woodruffa MA., *et al*. "Bone tissue engineering: from bench to bedside 15:10 (2012): 430-435.

44. Stewart S., et al. "Vancomycin-Modified Implant Surface Inhibits Biofilm Formation and Supports Bone-Healing in an Infected Osteotomy Model in Sheep A Proof-of Concept Study". *Journal of Bone and Joint Surgery-American* 94.15 (2012): 1406-1415.

45. Harboe K., *et al*. "Assessment of retention force and bone apposition in two differently coated femoral stems after 6 months of loading in a goat model". *Journal of Orthopaedic Surgery and Research* 9 (2014): 69.

46. Kon E., *et al*. "Osteochondral regeneration using a novel aragonite-hyaluronate bi-phasic scaffold in a goat model". *Knee Surgery Sports Traumatology Arthroscopy* 22.6 (2014): 1452-1464.

47. Stephens TJ., *et al*. "Segmented interlocking nail: An in vivo evaluation of a novel humeral osteotomy fixation device in a caprine model". *Veterinary and Comparative Orthopaedics and Traumatology* 25.1 (2012): 28-35.

48. Hou T., et al. "Controlled dynamization to enhance reconstruction capacity of tissue-engineered bone in healing critically sized bone defects: an in vivo study in goats". Tissue Engineering Part A 16.1 (2010): 201-212.

49. Voor MJ., *et al*. "Impaction grafting for femoral component revision in a goat model using washed morselized cancellous allograft". *Orthopedics* 31.5 (2008): 443.

50. Nandi SK., et al. "Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goats". Journal of Veterinary Science 9.2 (2008): 183-191.

51. Chen B., *et al*. "Long-term observation of large weight-bearing bone defect in goats repaired with tissue engineering technique". *Nan Fang Yi Ke Da Xue Xue Bao* 26.6 (2006): 770-773.

52. Zhou ZK., *et al*. "Use of cortical bone plates allografts for reconstruction of femoral fracture: an experimental study in goats". *Sichuan Da Xue Xue Bao Yi Xue Ban* 36.6 (2005): 880-884.

53. Welch RD., *et al*. "Effect of recombinant human bone morphogenetic protein-2 on fracture healing in a goat tibial fracture model". *Journal of Bone and Mineral Research* 13.9 (1998): 1483-1490.

54. Tran N., *et al*. "In vivo caprine model for osteomyelitis and evaluation of biofilm-resistant intramedullary nails". *BioMed Research International* (2013).

55. DeJong ES., *et al*. "Antimicrobial efficacy of external fixator pins coated with a lipid stabilized hydroxyapatite/chlorhexidine complex to prevent pin tract infection in a goat model". *Journal of trauma* 50.6 (2001): 1008-1014.

56. Hou T., *et al*. "In vitro evaluation of a fibrin gel antibiotic delivery system containing mesenchymal stem cells and vancomycin alginate beads for treating bone infections and facilitating bone formation". *Tissue Engineering Part A* 14.7 (2008): 1173-1182.

57. Urbanova L., *et al*. "Comparison of the Resistance to Bending Forces of the 4.5 LCP Plate-rod Construct and of 4.5 LCP Alone Applied to Segmental Femoral Defects in Miniature Pigs". *Acta Veterinaria Brno* 79.4 (2010): 613-620.

58. Ho KWK., *et al*. "The effect of 4 mm bicortical drill hole defect on bone strength in a pig femur model". *Archives of Orthopaedic and Trauma Surgery* 130.6 (2010): 797-802.

59. Necas A., *et al*. "Radiographic Assessment of Implant Failures of Titanium 3.5 LCP vs. 4.5 LCP Used for Flexible Bridging Osteosynthesis of Large Segmental Femoral Diaphyseal Defects in a Miniature Pig Model". *Acta Veterinaria Brno* 79.4 (2010): 599-606.

60. Necas A., *et al*. "Mathematical Modelling of Crack Fractography after Implant Failure of Titanium 4.5 LCP Used for Flexible Bridging Osteosynthesis in a Miniature Pig". *Acta Veterinaria Brno* 79.4 (2010): 621-626.

61. Heino TJ., *et al*. "Comparison of the osteogenic capacity of minipig and human bone marrow-derived mesenchymal stem cells". *Journal of Orthopaedic Research* 30.7 (2012): 1019-1025.

62. Royal JM., *et al*. "Assessment of Postoperative Analgesia after Application of Ultrasound-Guided Regional Anesthesia for Surgery in a Swine Femoral Fracture Model". *Journal of the American Association for Laboratory Animal Science* 52.3 (2013): 265-276.

63. Johansen LK., *et al*. "Therapy of haematogenous osteomyelitis--a comparative study in a porcine model and Angolan children". *In Vivo* 27.3 (2013): 305-312.

64. Johansen LK., *et al*. "A new technique for modeling of hematogenous osteomyelitis in pigs: inoculation into femoral artery". *Journal of Investigative Surgery* 26.3 (2013): 149-153.

65. Kubacki MR., *et al.* "Angle Stable Nails Provide Improved Healing for a Complex Fracture Model in the Femur". *Clinical Orthopaedics and Related Research* 472.4 (2014): 1300-1309.

66. Volpon JB., *et al*. "Low-internsity ultrasound application in distal radius metaphyseal bone defects of dogs". *Ultrasound in Medicine and Biology* 36.11 (2010): 1849-1855.

67. Heo S., *et al*. "Effect of Xenogenic cortical bone on fracture healing in a canine fracture model". *Acta Veterinaria Hungarica* 61.2 (2013): 149-159.

68. Dejardin LM., *et al*. "In Vivo Biomechanical Evaluation of a Novel Angle-Stable Interlocking Nail Design in a Canine Tibial Fracture Model". *Veterinary Surgery* 43.3 (2014): 271-281.

69. Varshney AC., *et al*. "Experimental model of staphylococcal osteomyelitis in dogs". *Indian Journal of Experimental Biology* 27.9 (1989): 816-819.

70. Southwood LL, *et al.* "Evaluation of Direct In Vivo Gene Transfer in an Equine Metacarpal IV Ostectomy Model Using an Adenoviral Vector Encoding the Bone Morphogenetic Protein-2 and Protein-7 Gene". *Veterinary Surgery* 41.3 (2012): 345-354.

71. McDuffee LA., *et al*. "Osteoprogenitor Cell Therapy in an Equine Fracture Model". *Veterinary Surgery* 41.7 (2012): 773-783.

72. Seltzer KL., et al. "The effect of hole diameter on the torsional mechanical properties of the equine third metacarpal bone". *Veterinary Surgery* 25.5 (1996): 371-5.

73. Pollock S., et al. "A musculoskeletal model of the equine forelimb for determining surface stresses and strains in the humerus--part I.

Volume 4 Issue 2 October 2016