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Research Article

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

(Part 2: osteoporotic bone and imaging studies)

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Abstract

Improved surgical techniques and applied bioengineering have greatly changed orthopaedic practice over the past 50 years. 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. Many animal models have been developed to investigate bone regeneration and repair. Animal models offer the advantage of greater control over experimental design and particularly randomisation between interventions, treatment compliance and end-points that can be pre-determined. The facility to perform elective necropsy substantially increases our understanding of underlying pathology and the healing process. The objective of this series of papers is to review animal models relevant to contemporary orthopaedic surgery of the appendicular skeleton. To help guide readers to relevant data, we have structured our review by major animal species under four broad topics the first two of which were discussed in detail in the first publication. Namely: Healing, bone defects and non-union; and, Healing in the presence of inter-current disease. In this paper, we examine Osteoporotic bone and Imaging studies.

Keywords: *Translational Research; Bone Healing; Osteoporosis; Animal Models; One Health*

Introduction

As discussed in the first paper of this series, improved surgical techniques and applied bioengineering have changed orthopaedic practice over the past 50 years. 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 [1,2].

The use of animal models as a bridge from *in vitro* to the patient have been use in many situations [3] and these compliment inherent challenges of human-based research. Many animal models have been developed to investigate bone regeneration and repair. The objec-

tive of these papers is to review animal models relevant to contemporary orthopaedic surgery of the appendicular skeleton. To help guide readers to relevant data, we have structured our review by major animal species under four broad topics the first two of which were discussed in detail in the first publication:

1. Healing, bone defects and non-union
2. Healing in the presence of inter-current disease
3. Osteoporotic bone
4. Imaging studies

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 pre-determined. The facility to perform elective necropsy substantially increases our understanding of underlying pathology and the healing process [4].

Bone Regeneration

Ultimately, bone regeneration and strength underpin successful and timely repair. 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.

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 technically 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.* [5] is a useful critique of the advantages and pitfalls of the different fixation techniques in rats and mice. While that by Garcia., *et al.* [6] summarises 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 genetargeted animals to study molecular mechanisms of fracture healing make these very flexible tools.

Osteoporotic bone

Age-related fatty degeneration of the bone marrow contributes to delayed fracture-healing and osteoporosis-related fractures in the elderly. The mechanisms underlying this fatty change are unknown, but they may relate to the level of Wnt signalling within the aged marrow cavity.

Leucht., *et al*. [7] used transgenic mice with a syngeneic bone-graft model to follow the fates of cells involved in the engraftment. Immuno-histochemistry along with quantitative assays were used to evaluate Wn tsignaling and adipogenic and osteogenic gene expression in bone grafts from young and aged mice. Liposomal Wnt3a protein (L-Wnt3a) was tested for its ability to restore osteogenic potential to aged bone grafts in critical-size defect models created in mice and in rabbits. Radiography, microquantitative computed tomography (microCT) reconstruction, histology, and histomorphometric measurements were used to quantify bone-healing resulting from L-Wnt3a or a control substance (liposomal phosphate-buffered saline solution EL-PBS). Expression profiling of cells in the bone graft demonstrated a shift away from an osteogenic gene profile and toward an adipogenic one with age. This agerelated adipogenic shift was accompanied by a significant reduction ($p < 0.05$) in Wnt signaling and a loss in osteogenic potential. In both large and small animal models, osteogenic competence has been restored to aged bone grafts by a brief incubation with the stem-cell factor Wnt3a. In addition, liposomal Wnt3a significantly reduced cell death in the bone graft, resulting in significantly more osseous regenerate in comparison with controls. Liposomal Wnt3a was demonstrated to enhance cell survival and re-establish the osteogenic capacity of bone grafts from aged animals. The clinical relevance is the potential for a clinically applicable, regenerative medicine-based strategy for revitalizing bone grafts from aged patients.

Gentile., *et al.* [8] investigated the impact of inhibiting Cathepsin K (CatK) for treatment of post-menopausal osteoporosis. CatK is a cysteine protease, expressed predominantly in osteoclasts (OC) which degrade demineralized bone matrix. Pharmacological inhibition of CatK reduces OC resorption activity while preserving bone formation in preclinical models. Disruption of the CatK gene in mice also results in high bone mass due to impaired bone resorption and elevated formation. A mid-shaft femoral fracture healing in 8 -10 week old CatK knock-out (KO) versus wild type (WT) mice was assessed. Fracture healing and callus formation were determined *in vivo* weekly via X-ray, and *ex vivo* at days 14,18,28 and 42 post-fracture by radiographic scoring, micro-computed tomography (mu CT), histomorphometry and terminal mechanical four point bend strength testing. Radiological evaluation indicated accelerated bone healing and remodelling for CatK KO animals based on increased total radiographic scores that included callus opacity and bridging at days 28 and 42 post-fracture. Micro-CT based total callus volume was similar in CatK KO and WT mice at day 14. Callus size in CatK KO mice was 25% smaller than that in WT mice at day 18, statistically significant by day 28 and exhibited significantly higher mineralized tissue volume and volumetric BMD as compared to WT by day 18 onward. Osteoclast surface and osteoid surface trended higher in CatK KO calluses at all time-points and osteoblast number was also significantly increased at day 28. Increased CatK KO callus mineral density was reflected in significant increases in peak load and stiffness over WT at day 42 post-fracture. Regression analysis indicated a positive correlation ($r = 0.8671$; $p < 0.001$) between callus BMC and peak load indicating normal mineral properties in CatK KO calluses. Taken together, gene deletion of cathepsin K in mice accelerated callus size resolution, significantly increased callus mineralized mass, and improved mechanical strength as compared to wild type mice.

NELL-1 is a secreted, osteoinductive protein whose expression rheostatically controls skeletal ossification. Overexpression of NELL-1 results in craniosynostosis in humans and mice, whereas lack of Nell-1 expression is associated with skeletal undermineralization. James., *et al.* [9] used a murine model to demonstrate that Nell-1-haploinsufficient mice have normal skeletal development but undergo age-related osteoporosis, characterized by a reduction in osteoblast:osteoclast (OB:OC) ratio and increased bone fragility. Recombinant NELL-1 binds to integrin β1 and consequently induces Wnt/β-catenin signalling, associated with increased OB differentiation and inhibi-

tion of OC-directed bone resorption. Systemic delivery of NELL-1 to mice with gonadectomy-induced osteoporosis resulted in improved bone mineral density. When extended to a large animal model, local delivery of NELL-1 to osteoporotic sheep spine leads to significant increase in bone formation. Their findings suggest that NELL-1 deficiency plays a role in osteoporosis and demonstrate the potential utility of NELL-1 as a combination anabolic/antiosteoclastic therapeutic for bone loss.

Imaging studies

To determine whether magnetic resonance imaging (MRI) could be used to track changes in skeletal morphology during bone healing using high-resolution micro-computed tomography (CT) as a standard. Taha., *et al*. [10] used a mouse model of bone injury to compare CT with MRI. Surgery was performed to induce a burr hole fracture in the mouse tibia. A selection of biomaterials was immediately implanted into the fractures. Imaging sequences were optimised by testing different MRI pulse sequences. Changes in bone morphology over the course of fracture repair were assessed using *in vivo* MRI and CT. Histology was performed to validate the imaging outcomes. The rapid acquisition with relaxation enhancement (RARE) sequence provided sufficient contrast between bone and the surrounding tissues to clearly reveal the fracture. It allowed detection of the fracture clearly 1 and 14 days postsurgery and revealed soft tissue changes that were not clear on CT. In MRI and CT the fracture was seen at day 1 and partial healing was detected at day 14. The RARE sequence was the most suitable for MRI bone imaging. It enabled the detection of hard and even soft tissue changes. These findings suggest that MRI could be an effective imaging modality for assessing changes in bone morphology and patho-biology.

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.* [11] 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.

Osteoporotic bone

Osteoporosis is a systemic metabolic disease characterized by low bone mass with deterioration of the bony microstructure which leads to both bone brittleness and increased risk of fracture. Osteoporosis may cause bone fracture even with minor trauma. Osteoporotic fracture has become a major public health problem. To date, there is no specific guideline to carry out fracture healing studies in animal models for the evaluation of new agents. Ibrahim., *et al.* [12] provide a review of various fracture and fixation methods for experimental osteoporotic fracture healing using rodent models.

Yao., *et al.* [13] examined the role of sclerostin, a protein encoded by the SOST gene which is specifically expressed in osteocytes. Monoclonal antibodies of sclerostin can promote bone formation by antagonizing its inhibitory action. However, the effectiveness of monoclonal antibodies to exert such effects are limited by the large molecular mass and high immunogenicity. They purified a high immune affinity, single-chain antibody of SOST: SOST-single-chain Fv (scFv). Real-time polymerase chain reaction amplification of the variable regions of the heavy- and light-chain gene from a secretory anti-SOST antibody was performed. Their experiments showed that SOST-scFv promoted bone healing in a rat model of osteoporosis.

Discrepancies in bone healing between osteoporotic and non-osteoporotic bone remain uncertain. Thormann., *et al*. [14] evaluated potential healing discrepancies in a metaphyseal defect model in rat femora. Female Sprague-Dawley rats were either ovariectomized (OVX, n = 14) and combined with a calcium-, phosphorus- and vitamin D3-, soy-and phytoestrogenfree diet or received SHAM operation with standard diet rat (SHAM, n = 14). Three months post-ovariectomy, DEXA measurement showed a reduction of bone mineral density reflecting an osteoporotic bone status in OVX rats. Rats then underwent a 3 mm wedgeshaped osteotomy at the distal metaphyseal area of the left femur stabilized with a T-shaped mini-plate and allowed to heal for 6 weeks. Biomechanical competence by means of a nondestructive three-point bending test showed significant lower flexural rigidity in the OVX rats at 3 mm lever span compared to SHAM animals ($p = 0.048$) but no differences at 10 mm lever span. Microcomputer tomography (mu CT) showed bridging cortices and consolidation

of the defect in both groups, however, no measurable differences were found in either total ossified tissue or vascular volume fraction. Furthermore, histology showed healing discrepancies that were characterized by cartilaginous remnant and more unmineralized tissue presence in the OVX rats compared to more mature consolidation appearance in the SHAM group. They conclude that bone defect healing in metaphyseal bone slightly differs between osteoporotic and non-osteoporotic bone in their 3 mm defect model in terms of 3 mm lever span biomechanical testing and histology.

The use of the pharmacological agent strontium ranelate has come to prominence for the treatment of osteoporosis. Wei., *et al*. [15] fabricated strontium-containing scaffolds and show that they enhance bone defect healing in the femurs of rats induced by ovariectomy. Although much emphasis has been placed on using pharmacological agents for the prevention of disease, much less attention has been placed on the construction of biomaterials following osteoporotic-related fracture. The aim of their study was to incorporate bioactive strontium (Sr) trace element into mesoporous bioactive glass (MBG) scaffolds and to investigate *in vivo* efficacy for bone defect healing in the femurs of rats induced by ovariectomy. Thirty animals were divided into five groups: empty defect (control); empty defects with estrogen replacement therapy; defects filled with MBG scaffolds alone; defects filled with MBG + estrogen replacement therapy; and, defects filled with strontium-incorporated mesoporebioglass (Sr-MBG) scaffolds. The two groups demonstrating the highest levels of new bone formation were the defects treated with MBG + estrogen replacement therapy and the defects receiving Sr-MBG scaffolds as assessed by mu-CT and histological analysis. Furthermore, Sr scaffolds had a reduced number of tartrate-resistant acid phosphatase-positive cells when compared to other modalities. The results demonstrate that the local release of Sr from bone scaffolds may improve fracture repair.

Fracture repair occurs by two broad mechanisms: direct healing, and indirect healing with callus formation. Savaridas., *et al.* [16] investigated the effects of bisphosphonates on fracture repair by direct fracture healing. A rodent model of rigid compression plate fixation of a standardised tibial osteotomy was used. Ten skeletally mature Sprague-Dawley rats received daily subcutaneous injections of 1 µu g/kg ibandronate (IBAN) and ten control rats received saline (control). Three weeks later a tibial osteotomy was rigidly fixed with compression plating. Six weeks later, at necropsy, fracture repair was assessed with mechanical testing, radiographs and histology. The mean stress at failure in a four-point bending test was significantly lower in the IBAN group compared with controls (8.69 Nmm 2) (SD 7.63) vs 24.65 Nmm(-2) (SD 6.15); p = 0.017). Radiographs of the extricated tibiae indicated the mean bone density assessment at the osteotomy site was lower in the IBAN group than in controls (3.7 mmAl (SD 0.75) vs 4.6 mmAl (SD 0.57); p = 0.01). In addition, histological analysis revealed progression to fracture union in the controls but impaired fracture healing in the IBAN group, with predominantly cartilage-like and undifferentiated mesenchymal tissue (p = 0.007). Bisphosphonate treatment in a therapeutic dose, as used for risk reduction in fragility fractures, had an inhibitory effect on direct fracture healing. They concluded that bisphosphonate therapy should not be commenced until after the fracture has united if the fracture has been rigidly fixed and is undergoing direct osteonal healing.

Fu., *et al.* [17] investigated long-term effects of alendronate (Am), a widely used oral bisphosphonate, on fracture healing and bone remodelling in ovariectomized rats. Adult female SD rats underwent ovariectomy, and then bilateral femoral osteotomy at 12 weeks post-ovariectomy. From d 2 post-ovariectomy, the animals were divided into 3 groups, and treated with Aln (3 mg.kg(-1).d(-1), po) for 28 weeks (Aln/Aln), Aln for 12 weeks and saline for 16 weeks (Aln/Saline) or saline for 28 weeks (Saline/Saline). At 6 and 16 weeks postfracture, the fracture calluses were examined with X-ray radiography, and biomechanical testing and histological analysis were performed. The calluses were labelled with tetracycline and calcein to evaluate the mineral apposition rate (MAR). The fracture line was less distinct in the 2 Aln-treated groups at 6 weeks post-fracture, and disappeared in all the 3 groups at 16 weeks post-fracture. The size of the callus and radiographic density of the femora in the Aln/Aln group were the highest among the 3 groups at 6 and 16 weeks post-fracture. Similar results were observed in the ultimate load at failure and energy absorption. However, the treatment with Aln delayed endochondral ossification of the callus, and significantly increased the total sagittal-sectional area, percentage callus area and callus thickness, and decreased the MAR at 6 and 16 weeks post-fracture. In this ovariectomized rat model Aln was beneficial for to mechanical properties of the callus, but delayed callus remodelling of woven bone into lamellar bone.

Delayed bone healing noted in osteoporotic humans patients has been demonstrated in ovariectomized (OVX) rat models of estrogen-depletion osteopenia. Pulsed electromagnetic field (PEMF) devices are clinically approved as an adjunct to cervical fusion surgery in

humans at high risk for non-fusion and for the treatment of fracture non-unions. Androjna., *et al*. [18] state that these bone growth stimulating devices accelerate the healing of fresh fracture repair in skeletally mature normal rats but have not been tested for efficacy to accelerate and/or enhance the delayed bone repair process in OVX rats. Their study tested the hypothesis that daily PEMF treatments would improve the fracture healing response in skeletally mature OVX rats. By 6 weeks of healing, PEMF treatments resulted in improved hard callus elastic modulus across fibula fractures normalizing the healing process in OVX rats with respect to this mechanical property. Radiographic evidence showed an improved hard callus bridging across fibula fractures in OVX rats treated with PEMF as compared to sham treatments.

Imaging studies

Precise assessment of fracture-healing is vital in both clinical and research settings, where therapies are typically assessed using animal models with union as the study end point. Radiographic scoring systems have been developed for clinical use; however, have not been validated in pre-clinical models. Tawonsawatruk., *et al*. (2014) reviewed thirty sets of radiographs of rat tibial shaft fractures, treated with external fixation. Six observers used the Radiographic Union in Tibia (RUST) scale, the Lane & Sandhu score, and an overall impression of union. Fleiss's kappa and Intra-class Correlation Coefficients (ICC) were used to determine reliability. Inter-observer and intra-observer agreement using the general impression score were moderate [kappa; 0.58; 95%CI (0.49 - 0.65) and 0.66 (0.43 - 0.89), respectively]. Inter-observer and intra-observer agreement were excellent using both the RUST score [ICC; 0.81 (0.72 - 0.89) and 0.86 (0.74 - 0.93), respectively], and Lane & Sandhu score [ICC; 0.88 (0.81 - 0.93) and 0.90 (0.81 - 0.95), respectively]. Employing a defined scoring system enhances both the reproducibility and repeatability of bone healing assessment in a rat model. They conclude that routine reporting of fracture scoring methodology should be encouraged to enrich results and facilitate data synthesis across studies.

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.

Osteoporotic bone

Wang., *et al*. [19] investigated the potential of increasing bone mass and preventing fractures in osteoporosis using stem cell therapy using critical-sized segmental bone defects in a rabbit model of osteoporosis and an allogenic stem cell-based tissue engineering (TE) approach. Rabbit fetal bone marrow mesenchymal stem cells (BMSCs) were harvested and expanded *in vitro*. Decalcified bone matrix (DBM) scaffolds were then seeded with allogenic fetal BMSCs and cultivated in osteogenic media to engineer BMSC/DBM constructs. Critical-sized radial defects were created in ovariectomized (OVX) rabbits and the defects were repaired either by insertion of BMSC/DBM constructs or by DBM scaffolds alone. Also, nonovariectomized age-matched (non-OVX) rabbits were served as control. At 3 months post-treatment under the osteoporotic condition (OVX rabbits), the BMSC/DBM constructs inserted within the defect generated significantly more bone tissue when compared to the DBM scaffold as demonstrated by the X-ray, microcomputed tomography, and histological analyses. In addition, when compared to a normal nonosteoporotic condition (age-matched non-OVX rabbits), the defect treatment efficacy was adversely affected by the osteoporotic condition with significantly less bone regeneration. This study demonstrated the potential of allogenic fetal BMSC-based TE strategy for repairing bone defects in an osteoporotic condition. However, the treatment efficacy could be considerably compromised in the OVX animals.

Pazzaglia., *et al.* [20] studied bone aging in rabbit femurs in three populations aged 0.5, 1.5, and 7.5 years. Cortical bone histology was compared with a data set from a 1.5-month-old population of an earlier published paper. From 0.5-year-old onward, the mean femur length did not increase further. Thereafter, the mean marrow area increased and the cortical area decreased significantly with aging. This was associated with a structural pattern transformation from plexiform to laminar and then Haversian-like type. The distal metaepiphysis bone trabecular density of the oldest populations also was significantly lower in specific regions of interest (ROI). Percentage sealed primary vascular canals in laminar bone significantly increased with aging without variation of percentage sealed secondary osteons.

Remodeling rate reflected by the density of cutting cones did not significantly change among the age populations. These data suggest that laminar bone vascular pattern is more functional in the fast diaphyseal expansion but not much streamlined with the renewal of blood flow during secondary remodeling. Bone aging was characterized by: secondary remodelling subendosteally; increment of sealed primary vascular canals number; increased calcium content of the cortex; and, cortical and trabecular bone mass loss in specific ROIs. Taken together, the data may give a morphological and morphometric basis to perform comparative studies on experimental models of osteoporosis in the rabbit.

Osteoporosis (OP) is characterized by a reduction in bone quality, which is associated with inadequacies in bone marrow mesenchymal stromal cells (BMSCs). As an alternative cell source to BMSCs, adipose-derived stem cells (ASCs) were investigated by Ye., *et al.* [21] because of their osteogenic potential and self-renewal capability. The use of autologous ASCs can promote bone regeneration under osteoporotic conditions has not been elucidated.

The OP rabbit model was established by means of bilateral ovariectomy (OVX). Both BMSCs and ASCs were harvested from OVX rabbits and expanded *in vitro*. The effects of osteogenic-induced ASCs on the *in vitro* adipogenic and osteogenic capabilities of BMSCs were evaluated. Autologous ASCs were then encapsulated by calcium alginate gel and transplanted into the distal femurs of OVX rabbits (n = 12). Hydrogel without loading cells was injected into the contralateral femurs as a control. Animals were killed for investigation at 12 weeks after transplantation. Osteogenic-induced ASCs were able to promote osteogenesis and inhibit adipogenesis of osteoporotic BMSCs through activation of the bone morphogenetic protein 2/bone morphogenetic protein receptor type IB signal pathway. Local bone mineral density began to increase at 8 weeks after ASC transplantation ($P < 0.05$). At 12 weeks, micro-computed tomography and histological evaluation revealed more new bone formation in the cell-treated femurs than in the controlgroup ($P < 0.05$). The study demonstrated that ASCs could stimulate proliferation and osteogenic differentiation of BMSCs *in vitro* and enhance bone regeneration *in vivo*, which suggests that autologous osteogenic-induced ASCs might be useful to alleviate OP temporally.

Qiu., *et al*. [22] assessed the effect of oxytocin on bone and bone fat masses using microCT, *in vivo* magnetic resonance spectroscopy (MRS), and histopathological adipocyte quantification. Early *in vivo* oxytocin (OT) treatment to the osteoporosis (OP) rabbit model may reliably inhibit bone degeneration and reduce bone marrow fat accumulation by decreasing marrow adipocyte size and density. Sixty 20-week-old female rabbits were randomly assigned into three groups. The control and OP groups were subjected to either sham surgery or bilateral ovariectomy (OVX). The OT group was subcutaneously injected with OT daily from the second week after OVX for 8 weeks. The left proximal femurs of the rabbits were evaluated through MRS, micro-CT, and histopathological examination at 0, 4, 8, 10, and 12 weeks after operation. Differences in fat fraction (FF) values, micro-CT parameters, and calculated pathological marrow adipocytes among three groups were analyzed. The FF values of the OP group significantly increased ($p = 0.019$), but the tissue mineral density (TMD) decreased ($p = 0.037$) from eighth week compared with those of the control group. The FF values of the OT group significantly decreased ($p = 0.044$), but the TMD values increased ($p = 0.042$) from eighth week compared with those of the OP group. The adipocyte diameter of the OT group significantly decreased ($p = 0.041$) from eighth week and then adipocyte density decreased from the tenth week, compared with those of the OP group at the same time point. No difference in adipocyte calculation was found between the OT and control groups until the 12th week after operation. Early *in vivo* oxytocin treatment slowed bone deterioration and reduced bone marrow adiposity accumulation in a rabbit osteoporosis model, which is consistent with pathologic findings.

Imaging studies

A study conducted by Pinheiro., *et al.* [23] compared the effect of infrared laser (lambda 780 nm, 50 mW, 4 x 4 J/cm(2) = 16 J/cm(2), I center dot = 0.5 cm(2), CW) and the use of hydroxyapatite on osteosynthesis in surgical fractures fixed with wire, using Raman spectroscopy and laser fluorescence. Surgical tibial fractures were created under general anesthesia in 15 rabbits. Two groups were grafted with hydroxyapatite (HA) and guided bone regeneration (GBR) technique used. Animals in two groups were irradiated every other day for 2 weeks (4 x 4 J/cm(2), 16 J/cm(2) = 112 J/cm(2)). Observation time was that of 30 days. At necropsy, specimens were stored in liquid nitrogen and used for Raman spectroscopy. There were significant differences between groups ($p < 0.05$). Pearson correlation was negative

and significant (R (2) = -0.60; $p < 0.001$), and indicative that, when the Raman peaks of calcium hydroxyapatite (CHA) are increased, the level of fluorescence is reduced. It was concluded that the use of near-infrared lasertherapy associated to HA graft and GBR was effective in improving bone healing on fractured bones as a result of the increasing deposition of CHA measured by Raman spectroscopy and decrease of the organic components as shown by the fluorescence readings.

Southwood., *et al*. [24] evaluated use of technetium Tc 99m disodium hydroxymethylene diphosphonate (99m-Tc-HDP) for assessing fracture healing and 99m-Tc-HDP and technetium Tc 99m ciprofloxacin (99m-Tc-CIPRO) for early diagnosis of osteomyelitis in rabbits. A femoral fracture defect was created in 32 skeletally mature New Zealand White rabbits and stabilized with bone plates and cortical screws. Scintigraphy was performed 4, 8, 12, and 16 weeks after surgery. The 99m-Tc-CIPRO scan was performed 48 hours after the 99m-Tc-HDP scan. The uptake ratio of the experimental limb to the normal limb was calculated by use of multiple regions of interest. Results of radiography performed to determine external callus and lysis grade and percentage defect ossification at 16 weeks were compared with scintigraphy results. Infected fractures had a higher uptake ratio for 99m-TcHDP and 99m-Tc-CIPRO than non-infected fractures. Infected fractures could be differentiated from noninfected fractures late in healing by use of 99m-Tc-HDP. Although 99m-Tc-CIPRO was better than 99m-Tc-HDP for identifying infection, there was a high incidence of false positive and negative results with 99m-Tc-CIPRO. There was an association between 99m-Tc-HDP uptake ratio and callus formation and a good correlation between 99m-Tc-HDP uptake ratio and defect ossification after 4 weeks. It was concluded that 99m-Tc-HDP and 99m-Tc-CIPRO may be useful for diagnosing osteomyelitis late in fracture healing; however, false positive and false negative results occur.

Mead., *et al*. [25] investigated the specificity of indium-111 leukocyte scans for osteomyelitis when fractures are present. Midshaft tibial osteotomies were performed in 14 New Zealand white rabbits, seven of which were infected postoperatively with *Staphylococcus aureus* per Norden's protocol. All 14 rabbits were scanned following injection with 75 microCi of indium 111 at 72 h after osteotomy and at weekly intervals for 4 weeks. Before the rabbits were killed, the fracture sites were cultured to document the presence or absence of infection. The results of all infected osteotomy sites were positive, whereas no positive scans were found in the non-infected osteotomies.

Hartshorne., *et al*. [26] used simultaneous digital acquisition of [67Ga] and [99mTc]MDP images, and subsequent division of the first by the second to produce a parametric ratio image (G/T) to characterize the relative localization of the two radiopharmaceuticals in early rabbit tibia *Staphloccocus aureus* osteomyelitis and fracture repair. Images obtained during the first 48 hr of each condition show preferential 67Ga accumulation probably reflecting an initial inflammatory response while G/T images at 5 - 7 days show predominance of the boneseeking scan agent, which may indicate that the dominant process is osteoblastic repair.

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 hindlimbs, 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.

Osteoporotic bone

Several groups around the world have used ovariectomized sheep as a model because of the ease of housing and handling, low expense compared to other large animals, availability and acceptance in society as a research animal. They have been used to study the response to new therapies for post-menopausal osteoporosis, low-magnitude mechanical stimulation, orthopedic implants in osteoporotic bone and bioactive ceramics to strengthen vertebral bodies. Many ovine models have been utilised to mimic osteoporotic conditions including a combination of estrogen deficiency following ovariectomy and calcium-deficient diets.

Bindl., *et al.* [27] established a model of osteoporosis in sheep using hypothalamic-pituitary disconnection (HPD). As central regulation is important for bone metabolism, HPD-sheep develop severe osteoporosis because of low bone turnover. This study investigated metaphyseal fracture healing in HPD-sheep. To elucidate potential pathomechanisms, we included a treatment group receiving thyroxine T4 and 17-estradiol. Because clinically osteoporotic fractures often occur in the bone metaphysis, HPD-sheep and healthy controls received

an osteotomy in the distal femoral condyle. Half of the HPD-sheep were systemically treated with thyroxine T4 and 17-estradiol during the healing period. Fracture healing was evaluated after 8 weeks using pQCT, mu CT, and histomorphometrical analysis. Bone mineral density (BMD) and bone volume/total volume (BV/TV) were considerably reduced by 30% and 36%, respectively, in the osteotomy gap of the HPD-sheep compared to healthy sheep. Histomorphometry also revealed a decreased amount of newly formed bone (29%) and some remaining cartilage in the HPD-group, suggesting that HPD disturbed fracture healing. Thyroxine T4 and 17-estradiol substitution considerably improved bone healing in the HPD-sheep. The results illustrate that fracture healing requires central regulation and that thyroxine T4 and 17-estradiol contribute to pathological mechanisms of delayed metaphyseal bone healing in HPD-sheep.

Andreasen., *et al*. [28] used an ovine model to determine how glucocorticoid treatment of ovariectomised sheep affects the cancellous bone, determining the cellular events within the bone remodelling process that contributes to their bone loss. Twenty female sheep were assigned for two groups; an untreated control group and an ovariectomised group treated with glucocorticoids (0.6 mg/kg/day, 5 times weekly) for 7 months. At 7 months the glucocorticoid-treated ovariectomised sheep showed a significant change in the bone microstructure revealed by a decreased trabecular bone volume and thickness compared to the control sheep. The treatment led to a temporary elevation of the bone resorption marker CTX (c-terminal collagen telopeptide), while the bone formation marker osteocalcin remained suppressed all 7 months. Histomorphometrically, the treated sheep had a complete absence of osteoid surfaces, and a 5-fold increase in the extent of eroded/reversal surfaces after 7 months. Most of these reversal surfaces were actually arrested reversal surfaces, defined as reversal surfaces without the presence of neighbouring osteoid surfaces or osteoclasts, which is classically observed next to active reversal surfaces. As in humans, these arrested reversal surfaces had compared to active reversal surfaces a reduced canopy coverage, a significantly decreased cell density, and a decreased immunoreactivity for the osteoblastic markers osterix, runx2 and smooth muscle actin in the mononuclear reversal cells colonising the surfaces. In conclusion, glucocorticoid treatment of ovariectomised sheep induced a significant bone loss, caused by an arrest of the reversal phase, resulting in an uncoupling of the bone formation and resorption during the reversal phase, as recently demonstrated in postmenopausal women with glucocorticoid-induced osteoporosis. They conclude that this supports the relevance of the sheep model to the pathophysiology of glucocorticoid-induced osteoporosis in postmenopausal women, making it a relevant preclinical model for orthopaedic implant and biomaterial research.

Bisphosphonates are commonly prescribed for treatment of osteoporosis. Long-term use of bisphosphonates has been correlated to atypical femoral fractures (AFF). AFFs arise from fatigue damage to bone tissue that cannot be repaired due to pharmacologic treatments. Despite fatigue being the primary damage mechanism of AFFs, the effects of osteoporosis treatments on fatigue properties of cortical bone are unknown. To examine if fatigue-like differences occur in bone tissue after different pharmacologic treatments for osteoporosis, Brock., *et al*. [29] tested bone tissue from the femurs of sheep given a metabolic acidosis diet to induce osteoporosis, followed by treatment with a selective estrogen reception modulator (raloxifene), a bisphosphonate (alendronate or zoledronate), or parathyroid hormone (teriparatide, PTH). Beams of cortical bone tissue were created and tested in fourpoint bending fatigue to failure. Tissues treated with alendronate had reduced fatigue life and less modulus loss at failure compared to other treatments, while tissue treated with PTH had a prolonged fatigue life. No loss of fatigue life occurred with zoledronate treatment despite its greater binding affinity and potency compared to alendronate. Tissue mineralization measured by microCT did not explain the differences seen in fatigue behavior. Increased fatigue life with PTH suggests that current treatment methods for AFF could have beneficial effects for restoring fatigue life. These results suggest that fatigue life differs with each type of osteoporosis treatment.

Chronic environmental fluoride exposure under calcium stress causes fragility fractures due to osteoporosis and bone quality deterioration, at least in sheep. Proof of skeletal fluorosis, presenting without increased bone density, calls for a review of fracture incidence in areas with fluoridated groundwater, including an analysis of patients with low bone mass. Simon., *et al.* [30] studied the skeletal phenotype of sheep chronically exposed to highly fluorinated water in the Kalahari Desert, where livestock is known to present with fragility fractures. Dorper ewes from two flocks in Namibia were studied. Chemical analyses of water, blood and urine were analysed for both cohorts. Skeletal phenotyping comprised micro-computer tomography (μCT), histological, histomorphometric, biomechanical, quantita-

tive backscattered electron imaging (qBEI) and energy-dispersive X-ray (EDX) analysis. Analysis was performed in direct comparison with undecalcified human iliac crest bone biopsies of patients with fluoride-induced osteopathy. The fluoride content of water, blood and urine was significantly elevated in the Kalahari group compared to the control. A significant decrease in both cortical and trabecular bones was found in sheep chronically exposed to fluoride. Furthermore, osteoid parameters and the degree and heterogeneity of mineralization were increased. The latter findings are reminiscent of those found in osteoporotic patients with treatment-induced fluorosis. Mechanical testing revealed a significant decrease in the bending strength, concurrent with the clinical observation of fragility fractures in sheep within an area of environmental fluoride exposure. Their data suggested that fluoride exposure with concomitant calcium deficit may aggravate bone loss via reductions in mineralized trabecular and cortical bone mass and can cause fragility fractures and that the prevalence of skeletal fluorosis especially due to groundwater exposure should be reviewed in many areas of the world as low bone mass alone does not exclude fluorosis.

While much research has been dedicated to understanding osteoporosis, the nature of mineral distribution and the mechanical property variation in diseased bone is poorly understood. Brennan., *et al.* [31] investigated the effect of estrogen deficiency and bisphosphonate therapy on bone tissue properties using an ovine model of osteoporosis. Skeletally mature animals (4 + years) were divided into an ovariectomy group (ovx, n = 20) and a non treatment control group (control, n = 20). A zoledronic acid treated group was also included in which animals were estrogen deficient for 20 months prior to receiving treatment (Zol, n = 4). Half of the control and ovx groups were euthanized 12 or 31 months post-operatively and all Zol animals were euthanised at 31 months. Individual trabeculae were removed from the proximal femur and were analysed at specific locations across the width of the trabeculae. The mineral content was measured using quantitative backscatter electron imaging and the modulus was measured using nanoindentation. The spatial distribution of tissue modulus and mineral content in bone from ovariectomised animals was similar to control. However, ovariectomy significantly reduced the overall mineral content and tissue modulus relative to the control group after 12 months. Interestingly, significant differences were not maintained 31 months post-OVX. Treatment with zoledronic acid increased the mineral content and tissue modulus relative to both the ovariectomised and control groups. Zoledronic acid was also found to alter the mineral and modulus gradients normally associated with healthy bone tissue. Both estrogen deficiency and zoledronic acid therapy significantly altered mineral content and the mechanical properties of trabecular tissue. Brennan., *et al.* [32] also found that estrogen deficiency resulted in significant increases in the levels of osteocyte apoptosis while zoledronic acid significantly reduced the level of apoptosis in osteocytes. Zoledronic acid treatment resulted in the formation of more microcracks. However, these cracks were shorter than in control or OVX groups which may provide one explanation as to why increased damage levels following bisphosphonate treatment have not lead to increased fractures. This study also provides additional evidence of the importance of estrogen in preserving the osteocyte network.

Healy., *et al.* [33] examined the effect of estrogen deficiency on compact bone turnover and associated geometrical structural adaptation over a 31-month period. Twenty-seven skeletally mature sheep were divided into control (n = 16) and ovariectomy group (OVX, n = 11). Animals were administered five different fluorochrome dyes to label intracortical bone turnover, and necropsied at 31 months. Compact bone samples were analyzed for cortical geometry, intracortical turnover at five time points, resorption cavities, porosity, and compressive strength. Intracortical bone turnover was significantly increased in OVX, which demonstrated seasonal variation. Cross-sectional area in OVX was significantly greater than control and was associated with an increased section modulus. Intracortical porosity was significantly increased in OVX, however, there was no significant difference in ultimate compressive strength between the groups. The results demonstrated increased intracortical bone turnover, resportion spaces, and porosity in OVX, without adversely affecting compressive strength and supported the hypothesis of geometrical adaptation of compact bone in response to estrogen deficiency. These results suggest an early structural compensatory response in compact bone, despite increased intracortical turnover.

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.

Osteoporotic bone

Osteoporotic hip fracture in humans is common in the elderly with high morbidity and mortality. Ambulation and quality of life are significantly affected by the fracture femoroplasty, the injection of bone cement into the proximal femur to augment femoral strength and to prevent fracture is an option which until recently has remained at the stage of biomechanical testing. Luo., *et al.* [34] have developed a proximal femur fracture goat model that consistently fractures at the proximal femur when subject to vertical load, simulating osteoporotic hip fractures in human. Six pairs of fresh frozen mature Chinese goats' femora were obtained and randomly assigned into two groups. For the experimental group, a cylindrical bone defect was created at the proximal femur, while the control was left untreated. In addition, a configuration to mimic the mechanical axis of the goat femur was developed. When subjected to load along the mechanical axis, all the specimens from the bone defect group experienced femoral neck fractures, while fractures occurred at the femoral neck or other sites of the proximal femur in the control group. The biomechanical property (failure load) of the bone defect specimens was significantly lower than that of the control specimens ($p < 0.05$). Osteoporotic hip fractures of humans were simulated by a goat fracture model, which may serve as a reference for future femoroplasty studies *in vivo*.

Imaging studies

Blokhuis., *et al.* [35] evaluated the reliability of radiographs in the evaluation of healing of closed fractures. A closed mid-shaft tibial fracture was created in 40 goats and stabilized with an external fixator. The animals were assigned to four groups: no injection, injection of 1 mg osteogenic protein-1 (OP-1), 1 mg OP-1 with collagenous carrier, or carrier alone.

Radiographs were performed weekly until the animals were killed after 2 and 4 weeks. Healing was evaluated using radiographs, biomechanical testing, and histological examination. All radiographs were examined by two independent observers. Interobserver agreement was calculated and radiographic scores were compared with mechanical and histological scores using regression analysis. Regression analysis showed poor correlation between radiographic scores and biomechanical and histological data. Correlation coefficients varied between 0.39 and 0.63. Good agreement between the observers was seen in only three parameters: visibility of the fracture line, weight-bearing ability, and a combined healing parameter. They conclude that plain radiography provides poor parameters for monitoring the fracture healing process.

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.

Osteoporotic bone

Kim., *et al.* [36] developed a model of osteoporosis using micropigs, which differ from other miniature pigs in the genetic background. Female micropigs were used for the induction of a moderate osteoporosis model by bilateral ovariectomy (OVX) and compared with sham operated animals. For osteoporosis evaluation, clinical biomarkers such as blood osteocalcin (OSC) and parathyroid hormone (PTH) levels were measured, as well as bone mineral density (BMD) using micro-computed tomography (micro-CT). Compared to sham, OVX animals had decreased blood OSC level, while the blood PTH level increased in blood sera. In addition, they observed significantly decreased BMDs in the tibia region in OVX animals.

Scholz-Ahrens., *et al*. [37] studied the long and short-term effect of glucocorticoid on bone in 50 healthy adult (30-mo-old) primiparous Göttingen minipigs. Calcium absorption decreased from baseline by -2,488 +/- 688 mg/7 days ($P < 0.001$) compared with -1,380 +/- 1,297 mg/7 days (NS) in the control group. Plasma bone alkaline phosphatase (BAP) decreased from baseline by -17.8 +/- 2.2 U/l ($P < 0.000$) and was significantly different ($P < 0.05$) from the value of the control group of -1.43 +/- 4.8 U/l. In the long term, the loss of BMD became more pronounced and bone mineral content (BMC), trabecular thickness, mechanical stability, calcium absorption, 25-hydroxyvitamin D(3), 1,25-dihydroxyvitamin D(3), and parathyroid hormone tended to be lower compared with the control group. There

was a negative association between the cumulative dose of GC and BMD, which was associated with impaired osteoblastogenesis. The main outcomes after GC treatment were comparable to symptoms of GC-induced osteoporosis in human subjects and they postulate that the adult Göttingen miniature pig appears to be a valuable animal model for GC-induced osteoporosis.

Imaging studies

Riegger., *et al.* [38] evaluated multi-detector CT volumetry as a means to the assessment of bone defect healing in comparison to histopathological findings in an animal model. In 16 mini-pigs, a circumscribed tibial bone defect was created. Multi-detector CT (MDCT) of the tibia was performed on a 64-row scanner 42 days after the operation. The extent of bone healing was estimated quantitatively by MDCT volumetry using a commercially available software programme (syngo Volume, Siemens, Germany). The volume of the entire defect (including all pixels from -100 to 3,000 HU), the nonconsolidated areas (-100 to 500 HU), and areas of osseous consolidation (500 to 3,000 HU) were assessed and the extent of consolidation was calculated. Histomorphometry served as the reference standard. The extent of osseous consolidation in MDCT volumetry ranged from 19 to 92% (mean 65.4 +/- 18.5%). There was a significant correlation between histologically visible newly formed bone and the extent of osseous consolidation on MDCT volumetry ($r = 0.82$, $P < 0.0001$). A significant negative correlation was detected between osseous consolidation on MDCT and histological areas of persisting defect ($r = -0.9$, $P < 0.0001$). They concluded that MDCT volumetry was a promising tool for non-invasive monitoring of bone healing, showing excellent correlation with histomorphometry.

Canine Models

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

Osteoporotic bone

Bisphosphonate treatment used to prevent bone loss in postmenopausal osteoporosis has been implicated in an apparent increase in subtrochanteric femoral fractures. Previous work showed that bisphosphonates can reduce the energy to fracture of cancellous bone, but limited data exist on material-level mechanical properties of compact bone from the long bones. Burr., *et al.* [39] examined intrinsic mechanical properties of the femoral diaphysis of a canine model treated for 1 or 3 years with alendronate at two different doses. Seventy-two dogs were treated orally with 0.2 mg/kg/day alendronate or 1.0 mg/kg/day alendronate; a control group was administered saline. Prismatic beam specimens were tested in four-point bending under displacement control, and the intrinsic mechanical properties were calculated. No significant differences were found among groups in any mechanical property at either 1 or 3 years of treatment. They concluded that the material properties of the femoral diaphysis are not degraded following 1 to 3 years' treatment with alendronate, even at high doses. Longer periods of treatment have not been studied using clinical doses of alendronate, but such studies need to be carried out to confirm a lack of effect of alendronate on mechanical properties of cortical bone in the subtrochanteric region of the femur.

Martin., *et al*. [40] investigated the effects of ovariectomy on beagle dogs aged 3-7 years old and followed for 48 weeks with measurements of body weight, tibial shaft bone mineral content (BMC), and serum biochemistry. Necropsy, measurements were made of bone strength and histomorphometry. Ovariectomy(OX) significantly reduced serum estrone and estradiol concentrations and their variability from month to month. There was a transient decrease in cortical BMC of the OX dogs during the first 12 postoperative weeks but no difference between the groups after 48 weeks. Serum osteocalcin was elevated, but there was little effect on serum alkaline phosphatase, Ca, P, or calcitonin. OX increased the number of tetracycline-labeled osteons in cortical bone but reduced the percent trabecular surface labeled with tetracycline. OX produced no significant changes in the composition of the bones or loss of cortical area, but a statistically significant 15% trabecular bone loss occurred in the spine. However, bone strength had not been significantly affected at the time of sacrifice.

Uhthoff and Jaworski [41] conducted early studies on the response to long-term "nontraumatic" immobilisation in young adult Beagle dogs by means of radiomorphometry and histomorphometry, the right forelimb being encased in plaster and the left forelimb serving as

a control. The dogs were necropsied at 2, 4, 6, 8, 12, 16, 20, 24, 32, 40 weeks and the third metacarpal, radius, ulna and humerus removed for analysis of the contributions of the periosteal, haversian and endosteal envelopes to the bone loss at the mid-diaphysis. The bone mass responded to long-term immobilisation in three stages. First there was a rapid initial loss of bone, reaching its maximum (some 16 per cent of original mass) at six weeks, to which all three bone envelopes contributed. A rapid reversal followed, the bone mass approaching the control values between eight and twelve weeks after immobilisation. A second stage of slower but longer lasting bone loss ended twenty-four to thirty-two weeks after immobilisation; the periosteal envelope was the main contributor (80 to 90 per cent of the total loss). The third stage was characterised by maintenance of the bone mass which had been reduced by some 30 to 50 per cent of original values. This pattern was qualitatively similar in all four bones but the distal bones lost more bone than the proximal bones. The extent of resorption surface and the total histologically active periosteal envelope increased parallel to the phases of bone loss. The linear mineralisation rate did not differ significantly between the experimental and control sides.

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 been 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 out-

comes 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 categorised 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, inter-current disease, osteoporotic bone and imaging. In a subsequent review papers we take the same approach to consider the axial skeleton and joints and cartilage repair.

The objective of these two papers has been 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. This review has therefore been organised 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.

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