■ INSTRUCTIONAL REVIEW: RESEARCH

In vivo models of bone repair

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This review is aimed at clinicians appraising preclinical trauma studies and researchers investigating compromised bone healing or novel treatments for fractures. It categorises the clinical scenarios of poor healing of fractures and attempts to match them with the appropriate animal models in the literature.

We performed an extensive literature search of animal models of long bone fracture repair/nonunion and grouped the resulting studies according to the clinical scenario they were attempting to reflect; we then scrutinised them for their reliability and accuracy in reproducing that clinical scenario.

Models for normal fracture repair (primary and secondary), delayed union, nonunion (atrophic and hypertrophic), segmental defects and fractures at risk of impaired healing were identified. Their accuracy in reflecting the clinical scenario ranged greatly and the reliability of reproducing the scenario ranged from 100% to 40%.

It is vital to know the limitations and success of each model when considering its application.

An experimental model for studying bone repair needs to reflect the biomechanics and the physiology of the particular clinical scenario in humans. However, frequently models are used that do not meet this criterion. Fresh critical-size-defect models are employed to represent a nonunion, despite the fact that most human nonunions do not have a large defect and, by definition, are not fresh.

The clinical scenarios can be considered under the following headings: 1) normal fracture repair – direct and indirect healing; 2) delayed union; 3) established hypertrophic nonunion; 4) established atrophic nonunion – stiff or mobile (pseudarthrosis); 5) fractures with a segmental defect; 6) fractures at risk of delayed or nonunion, i.e. high-energy and open fractures, infected fractures and fractures in compromised patients.

There are many factors relating to the host, local environment, mechanical construct and the biological and infective situation that contribute to delayed bone repair; these need to be taken into account when selecting a model of impaired healing. This review aims to indicate the issues that should be considered with the application of any model, to highlight the range of animal models in the literature for the various clinical scenarios outlined above, and to suggest an algorithm for choosing a model for a given scenario. Our review is based on analysing models used for investigating bone repair in long bones in animals that have been published in the English language; PubMed, OVID and Google Scholar search engines were used.

General model considerations

Age. Table I lists the average time for cessation of bone growth and life expectancy in various animals.[1] Studies show that the age of an animal affects both the quality of bone and time for fracture repair[2,3]: not only is mitosis slower in older animals, but also fewer cells are entering the mitotic cycle and significantly fewer osteogenic precursor cells are produced per mesenchymal stem cell.[3] Therefore, in any study the age of the animal must be carefully controlled.

Gender. Hormonal cycles in the female can have significant influence on bone repair and turnover. Bone mineral density and endochondral growth are greatly suppressed during the reproductive cycle, particularly with the first litter, and if the mother lactates postpartum the deficiency is even greater.[4] Rats have an accelerated catch-up period between cycles, but never reach the same level as nulliparous females.[4,5] Ovariectomised rats, especially older ones, have delayed healing of femoral fractures and reduced bone mineral density (BMD)[6] and are therefore used as a model for osteoporotic fractures. It is important when using female animals to eliminate these

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Table I. Physeal closure, life expectancy and expected time to fracture union in various species[1]

Animal	Physeal closure (tibia) (mths)	Life expectancy (mths)	Expected time to union of simple fractures (wks)
Mouse (Imprinting Control Region)	5	18 to 36	3
Rat (Sprague-Dawley)	11	30 to 48	4 to 6
Rabbit (New Zealand White)	6.8 tibia 5.3 femur	84 to 96	6 to 7
Dog (Greyhound)	7.5 tibia 7.3 femur	108 to 168	10 to 13
Sheep (Suffolk x Dorset)	17	180	10 to 14

variables. Males are more territorial and may require separate cages, making them more expensive to keep.

Choice of animal. The choice of species in orthopaedic research is varied.[7,8] Martini et al[9] analysed 21 500 mammal studies and found that the most common choices were rats (36%), mice (26%), rabbits (13%), dogs (9%), primates (3%), sheep, pigs and cats (2% each).

There is wide variation in the biochemistry, biomechanics and anatomy of normal bone, and healing processes between and within species do not necessarily reflect the properties of human bone.[8,10,11] Sheep have cancellous and cortical bone, undergo bone remodelling and have a similar healing rate, but they also have plexiform bone (akin to woven bone) and fewer Haversian canals, with differences in bone composition and fracture stress levels.[7,8,12] Dogs have similarities to human bone in composition, remodelling and architecture, but have a combination of lamellar and plexiform bone, their remodelling is highly variable and their biomechanical properties differ.[7,11,13] Bone in rabbits remodels quickly[7] and it has a different microstructure from humans.[11] Rats have lamellar bone with good cancellous but less cortical remodelling, and there are significant differences in composition, density and quality.[8]

Mice lack a Haversian canal system,[14] but are attractive owing to their low cost, ease of handling, availability of genetic knockout varieties (breeding in which specific genes within the animal have been deactivated) and the increasing knowledge of their genetic blueprint, but concern has been raised about their size, with issues of relevance to the human situation when testing bone substitute scaffolds on such a small scale.[15] The biomechanical testing of bone in mice requires highly sensitive equipment.

Rats are useful for both long bone and calvarial models. They are hygienic and cheap to house, as several females can be kept in one cage. Rabbits have a larger skeleton but are still easily housed; however, there are clear size limitations when assessing implants compared with dogs or sheep.[7] Cats are an uncommon choice. Dogs are expensive and demanding to keep, with additional ethical issues regarding their use. Pigs have been shown to be a useful model for investigating the systemic response to trauma, but are used infrequently for studies of bone healing,[16,17] and their size, proportionately short limbs and housing requirements can be limiting factors, particularly as an adult pig can weigh about 150 kg.

Osteotomy *versus* other fracture technique. By using a manual/guillotine/impact device, a fracture is given more inherent stability from the soft-tissue envelope and from the interdigitating bony fragments, whereas osteotomy creates a cleaner, more controlled break.

An osteotomy model should be used with caution when investigating trauma. Park et al[18] compared the healing process in the rabbit tibia between an open osteotomy and a closed fracture model, and found that there was a significant difference in healing both histologically and biomechanically between the groups. Additionally, the cellular response to an oscillating saw may differ from the response to a burr.

Open *versus* closed fracture technique. A popular closed model uses a guillotine and stabilisation with an intramedullary (IM) nail,[19] which allows containment of the fracture haematoma. The open technique allows direct visualisation of the alignment of the bone, and the precise local introduction of compounds, but this theoretically creates an open fracture and introduces the risks and variables associated with a surgical procedure.

Fracture stabilisation. Fixation with an IM nail may be used in animals of all sizes and allows indirect fracture repair, although there is interference at the fracture site. In larger animal models the proximal cross-screw can be omitted to induce instability.

Plating is used for direct fracture repair; however, as an open technique it will affect the local haematoma formation and hinder radiological assessment. In addition, there will be weakness through the screw holes on removal of the plate that will compromise the testing of biomechanical stress until the holes have filled in.[20-22]

External fixation, both unilateral and circular, has the advantages of distance from the fracture site, ease of removal and lack of interference with histological, radiological or mechanical assessment *post mortem*; the fracture can also be created using a closed technique. Plastic ring fixators have been used to reduce the weight of the frame

Table II. Indirect healing

Authors	% healing achieved	Animal	Bone	Open/closed (method)	Method*	Issues to consider
Gröngröft et al[20]	100	Mouse	Femur	Open (Gigli saw osteotomy)	Flexible bridging plate	Not typical indirect healing on histology; cost of plate
Cheung et al[24]	100	Mouse	Femur	Open (manual fracture)	Ex-fix	Intra-operative haemorrhage and death from popliteal artery damage; pin loosening
Histing et al[25]	100	Mouse	Femur	Open	Locking plate	20% plate failure plate dislocation; plate cost; internal callus; IM healing
Holstein et al[26]	100	Mouse	Femur	Closed (3-point bending)	Locked femoral nail 0.55 mm	Axial instability, implant displacement
Holstein et al[27]	100	Mouse	Femur	Closed (3-point bending)	IM mouse screw 0.5 mm	Cost of implant
Manigrasso and O'Connor[28]	100	Mouse	Femur	Closed (3-point bending)	0.25 mm locked IM nail	9% surgical error
Bonnarens and Einhorn[19]	100	Rat	Femur	Closed (500 guillotine)	0.45 mm IM pin	Good reproducibility; possible bending of IM pin
Reed et al[29]	100	Rat	Tibia	Open (burr osteotomy)	Ex-fix, 1 mm osteotomy	Polyethylene rings
Bak and Andreassen[30]	93	Rat	Tibia	Open (3-point bending)	Forceps, 0.8 mm IM pin	41% excluded, IM pin bending/failure, fracture at incorrect level
Pelker and Friedlaender[31]	100	Rat	Femur	Open (manual fracture)	0.9 mm IM pin, 10 mm periosteal stripping	10% complication rate, variety of reasons
Waters et al[32]	71	Rabbit	Ulna	Open (1 mm saw osteotomy)	No fixator, splintage by radius	19% nonunion rate; extensive callus formation
Hart et al[33]	100	Dog	Tibia	Open (saw osteotomy)	Unilateral ex-fix, 6× titanium pins	
Goodship and Kenwright[34]	100	Sheep	Tibia	Open (osteotomy Gigli saw)	Ex-fix, 3 mm gap +/- micromovement	Micromovement induced, controlled mechanical environment
Schemitsch et al[35]	100	Sheep	Tibia	Open (slap hammer, 3-point bending)	7 mm IM nail, proximal and distal locking	High-energy model

* Ex-fix, external fixation; IM, intramedullary

Table III. Direct healing

Authors	Union achieved (%)	Animal	Bone	Method*	Issues
Savaridas et al[36]	100	Rat	Tibia	4-hole compression plate; periosteal stripping	Ensure precise technique with periosteum and osteotomy
Ashhurst et al[37]	100	Rabbit	Tibia	6-hole stainless steel compression DCP OR plastic plate; open saw osteotomy	14% fracture rate through screw holes; direct healing in metal DCP group, indirect with plastic plates
Lewallen et al[38]	100	Dog	Tibia	Open osteotomy; compression 8-hole plate OR unilateral ex-fix	44% mortality rate; 34% pin loosening in ex-fix group
Perren et al[39]	100	Sheep	Tibia	Saw osteotomy; contour plate + lag screw	

* DCP, dynamic compression plate; ex-fix, external fixation

but may be chewed through, which can be overcome by using aluminium three-quarter rings.[23] The unilateral fixator has a tendency towards excessive micromovement and instability in small animal models, which may lead to an unpredictable number of hypertrophic nonunions.

Plaster casts can be applied rapidly and are non-invasive, but have the disadvantage that they can be chewed or soiled.

Fracture models relying solely on the parallel bone (usually radius or tibia) for complete stability have been used in several species. They have the advantage of using no foreign materials, unobscured radiographs and, in the case of a closed fracture model, minimal surgical intervention. However, angular deformity, excessive movement and non-weight-bearing may ensue, resulting in unreliable models of normal fracture healing.

Animal models for different clinical scenarios

The model selected needs to reflect the relevant patient group.

Normal fracture repair. The key feature of this type is that it heals without delay or adjunct. It is often used as a control or to evaluate new agents or interventions. Important issues are deciding between an open or a closed technique, and between a fracture or an osteotomy. Table II lists indirect models of bone healing, differentiating between the formation of an open or closed fracture.[19,20,24-35] All methods except that of Waters et al[32] resulted in good union.

Table IV. Established delayed union

Author/s	Delayed (but eventual) union achieved	Animal	Bone	Method*	Points of consideration
Röntgen et al[40]	Not proven	Mouse	Femur	0.5 mm osteotomy (Gigli saw); flexible external fixator	21-day model only; 40% bone in fracture gap but not bridged
Lu et al[41]	Not proven	Mouse	Tibia	Tibial ex-fix, 3-point bending, femoral artery resection	Acute ischaemia model, 11% mortality and 18% morbidity rate; 21-day model
Aro et al[42]	Not proven	Rat	Tibia	IM pin 0.5 mm or 0.6 mm +/- locking pin; 3-point bending closed fracture technique	Both diameters of pin resulted in delayed union, poor positive control; 21% excluded due to fracture displacement
Park et al[43]	5/6 or 2/5 depending on group	Rabbit	Tibia	4-pin double-bar ex-fix, 13 mm gap; 6 mm periosteal stripping;variable times and number of haematoma excision and washout	Time to union determined by time and number of reoperations. May require some refinement.
Rijal et al[44]	Not proven	Rabbit	Radius	10 mm bone excised, gap curetted at 1 week; no additional splintage	Results of delayed union control group poorly recorded
Paterson et al[45]	Not proven	Dog	Tibia	15 mm resection, silastic spacer, IM pin and plaster cast; silastic removed at 8 weeks, model run for further 4 weeks	Spacer was considered to maintain gap rather than prevent union; 36% complication rate; no results suggestive of any attempt at healing up to 12 weeks
Schell et al[46]	4/8 delayed union at nine weeks, 3/8 at six months	Sheep	Tibia	External fixator and osteotomy; 3 mm gap, delay induced by mechanical instability from reduced stiffness	6-month model; delayed union only achieved 50% of time; remainder developed nonunion

* ex-fix, external fixation; IM, intramedullary

Table V. Established hypertrophic nonunion (HNU)

Author/s	HNU % rate	Animal	Bone and fixation method*	Method	Points of consideration
Aro et al[42]	40%	Rat	Fibula; no stabilisation	Proprioceptive receptor and sciatic nerve denervation by stripping 8 mm periosteum; fibula fracture with scissors	HNU possibly due to periosteal stripping not denervation, non-weight-bearing bone, poor HNU rate
Hietaniemi et al[49]	100%	Rat	Femur; IM nail	Open osteotomy; 11 mm reaming, 7 mm 'nail'; 4 mm cauterisation	4 of 52 had proximal nail migration; hypertrophic callus ceases at 15/52 and changes from a hypertrophic to an atrophic nonunion
Altner et al[50]	70% (20% atrophic nonunion)	Dog	Ulna; no stabilisation	Osteotomy 3 mm to 5 mm bone excision, muscle interposition	Non-weight-bearing bone, variable nonunion type
Heckman et al[51]	100% at 12 weeks	Dog	Ulna; fibreglass plaster cast	3 mm osteotomy, periosteal strip, removal of gap tissue at 12 weeks	Reoperation; may represent delayed union
dos Santos Neto and Volpon[52]	85% HNU, 15% 'oligotrophic'	Dog	Radius; no stabilisation	3 mm resection osteotomy, 10 mm periosteal strip, bone wax interposed	Use of bone wax, no complications noted
Volpon[53]	54% HNU, 46% atrophic nonunion	Dog	Radius; no stabilisation	5 mm osteotomy, 40 mm periosteal resection	32% complication rate including 5% union rate; inconsistent nonunion type created

* IM, intramedullary

Table III provides examples of direct healing by open reduction and internal fixation, with good results; this technique is more commonly described in larger animals.[36-39]

Established delayed union. Delayed union includes bone repair after fracture and osteotomy, where time to union is prolonged but eventually occurs, with return of structural integrity and function. It is a clinical diagnosis and relies on establishing the expected time of healing. This results in wide inter-observer variation (Table IV).[40-46] Bhandari et al[47] questioned 444 orthopaedic surgeons and found a huge variation in the definitions of delayed union and nonunion of the tibia. The expected time to union of a simple fracture in an animal model (Table I) will depend on many factors, including the fracture technique. Models of delayed union require a positive control group that demonstrates that the model does eventually unite.

Many different methods have been used to recreate this scenario: instability, reduced vascularity, foreign materials, reoperation and distraction osteogenesis (Table IV). These models illustrate lack of healing, but few confirm eventual delayed union, and several have flaws in their design.

Park et al[43] created a reproducible delayed union in the rabbit by repeated wound irrigation, which delayed the mean time to bridging from 6.2 weeks to 7.6 weeks with confirmed delayed union at 10 weeks. In Choi et al's[48] murine distraction

osteogenesis model for atrophic nonunion (ANU), one control group showed consistently delayed union.

It remains a challenge to find a clinically relevant, reliable and reproducible technique that results in delayed but eventual full bone bridging.

Established hypertrophic nonunion (HNU). HNU is characterised by abundant callus formation, visible radiologically, that does not bridge the fracture (Table V).[42,49-53] The gap is not freely mobile, being filled with fibrocartilage. Clinically, HNU arises as a consequence of excess movement at the fracture site, achieved by using an IM nail without the locking screws or by relying on the parallel bone alone for stabilisation.

The ability of bone wax (traditionally beeswax with almond oil and salicylic acid) to stem bleeding from bone was first described by Horsely in 1892.[54] Howard and Kelley[55] found that it prevented osteogenesis, induced a thin fibrotic membrane and depressed the inflammatory response.

The studies in Table V show that the ability to achieve consistent hypertrophic nonunion was poor: many had cases of stiff atrophic rather than hypertrophic nonunion and nonunions among the positive controls. Heckman et al[51] reported a rate of HNU of 100% simply by creating a 3 mm osteotomy gap, but the model was only given 12 weeks to unite. Hietaniemi et al[49] reported a high rate of HNU in their model, but by one year the abundant callus formation had become atrophic, and the model has been used as a 'pseudarthrosis' model in other studies.

Models of atrophic non-union (ANU). Atrophic nonunion is a well-accepted concept in orthopaedics, but defining it with clarity is difficult. Human ANU is broadly defined as when a fracture shows no attempt at healing and no progression of healing or callus formation after an acceptable period of time, usually judged radiologically with lack of callus and rounding-off of the fracture ends. What constitutes an acceptable period of time is highly inconsistent between orthopaedic surgeons.[47] In animal models of nonunion it has been defined as being a fracture that will not heal in the lifetime of that animal. In many small animal studies 16 weeks is accepted as a reasonable period of observation, as it is well beyond the expected timeframe for union.

There are two types of ANU, stiff and mobile, which require different approaches to patient management. The stiff ANU shows no attempt at healing radiologically, but histologically there is tissue across the fracture site and a certain amount of mechanical stiffness. The second type again has no radiological signs of healing, but histologically there is a mobile cystic cavity that offers no mechanical stability. Mobile ANU is less common and more typically referred to in animal models as pseudoarthrosis; however, this term is used with great variability and often interchangeably with ANU, without clarity. We would recommend that ANUs are described as mobile or stiff, and that the term pseudarthrosis be used with caution.

All the models employ an insult to the tissues to establish an ANU without creating a critical size defect (CSD). What is important is how closely they reflect the clinical scenario, what the insult is, and how reproducible and reliable the model is.

Table VI details some of the models of stiff ANU that have been used in the literature (some are modifications of the work of others): 100% nonunion was achieved in most models, obtained by a variety of techniques, but all have certain issues that must be considered.[29,43,48,56-66]

Several authors have used foreign materials to isolate the fracture from the surrounding soft tissues: this does not mimic the clinical setting but does result in a rate of ANU of 100%.[56] Muscle interposition has been noted to contribute to human nonunion since the 1800s[50] and has been used to create ANU,[59] but does not always provide a consistent result. Others have employed a thermal or a chemical insult.[60,64,65] Reoperation has also been used. Boyan et al[66] adapted Müller, Schenk and Willenegger's[67] original canine nonunion model of 1968 but also reoperated, excising the repair tissue from the gap. Brownlow and Simpson[63] and Reed et al[29] describe similar models in rabbit and rat, respectively, by stripping endosteum and periosteum from around the osteotomy site, as might occur in a high-energy injury.

Three models of mobile ANU (pseudarthrosis) are also described in four studies shown in Table VI that use either movement, distraction or instability.[68-71] In 1995, Hietaniemi et al[49] described a model which they termed a hypertrophic nonunion; in 1998[68] they used a similar model without cautery that led to non-bridging callus and a 100% rate of nonunion, with a gap filled with cartilage.

Segmental/critical-size-defect (CSD) model. The definition of CSD is the minimum amount of bone loss that will not heal by bone formation in the lifetime of that animal.[72] Hollinger and Kleinschmidt[73] defined it as a defect with < 10% bony regeneration. The CSD model was first proposed in 1934 by Key (Key's hypothesis),[74] who stated that segmental bone loss 1.5 times the diaphyseal diameter would lead to nonunion; Toombs et al[75] suggested this to be an overestimation. Einhorn et al[76] found that removing 20% (6 mm) was adequate for nonunion in the rat femur. Table VII illustrates the species-related variation in size of the critical gap.[76-87]

In CSD the gap created is too wide to be bridged; in a model of nonunion bridging is not achieved because of problems other than the size of the gap, such as poor vascularity or stability. The advantages of the CSD are that it is a reproducible, single cause for lack of repair, with no need for insults such as foreign body insertion or thermal damage.

A long bone CSD of 25 mm to 30 mm in sheep[88,89] and of 21 mm to 30 mm in dogs[90,91] has been found to be effective, whereas in the rabbit a gap of 15 mm in the radius/ulna/tibia is reliable.[83,92] There is a paucity of data for the cat and mouse.

The CSD model is commonly used in investigating bone regeneration as it is a simple way of developing bony nonunion. Recently the model's primary application has been to test the osseo-inductive and osteoconductive capabilities

Table VI. Atrophic nonunion (ANU; stiff and mobile)

Authors	% ANU achieved	Animal	Bone	Method*	Issues to consider
Stiff ANU					
Choi et al[48]	Variable, 60	Mouse	Tibia	External fixator without latency period and varying speeds of distraction	Variable bone bridging seen depending on speed of distraction with a few delayed unions by day 27
Oetgen et al[56]	100	Mouse	Femur	Part osteotomy/fracture, retrograde IM pin, partial diathermy	1/3 mortality rate; use of diathermy
Garcia et al[57]	100	Mouse	Femur	Osteotomy, 1.8 mm gap and 4 mm periosteal stripping; IM pin and internal clip fixation	Less consistent result without stripping or smaller gap, intramedullary and extramedullary tissue stripping
Dickson et al[58]	97	Rat	Femur	Osteotomy, periosteal diathermy, endosteal stripping, stabilised with ex-fix	Multiple insults to osteotomy site including diathermy
Fujita et al[59]	Unclear	Rat	Tibia	Closed 3-point bending fracture, IM nail, muscle interposition	Lack of quantitative data, initial HNU followed by ANU
Kokubu et al[60]	100	Rat	Femur	IM pin, closed 3-point bending fracture, 4 mm cautery around fracture site	15% complication rate; use of diathermy
Reed et al[29]	100	Rat	Tibia	Ex-fix, 1 mm osteotomy, stripped periosteum and curetted canal distance equal to 1× tibial diameter	Chewing of nylon device; no complications
Latterman et al[61] and Oni[62]	100	Rabbit	Tibia	2 mm osteotomy, 30 mm periosteal stripping, IM reaming and nailing, silastic tubing round fracture (removed at 4 weeks)	Extensive IM and periosteal insult with foreign body in situ; repeat operation at 4/52
Brownlow and Simpson[63]	100	Rabbit	Tibia	Unilateral ex-fix, osteotomy, 2 mm gap, stripped periosteum and curetted canal distance equal to 1× tibial diameter	No complications
Park et al[43]	100	Rabbit	Tibia	4-pin double-bar ex-fix, 13 mm gap, 6 mm periosteal strip, repeat re-operation to debride	Repeat reoperation at day 1 and 2, excision haematoma and irrigation
Markel et al[64]	100	Dog	Tibia	5 mm bone resection and gap, 15 mm double application of liquid nitrogen, unilateral ex-fix	Use of chemical insult
Tiedeman et al[65]	100	Dog	Tibia	Ex-fix, 6 mm bone resection, endosteum and periosteum cauterised	Mixed gender and breed
Boyan et al[66]	100	Dog	Radius	3 mm bone excised, reoperation at 1/52 to remove gap tissue, fibreglass splintage	Repeat operative intervention, mechanical instability for ANU model
Mobile ANU					
Hietaniemi et al[68,69]	100	Rat	Femur	Partial osteotomy, partial manual fracture; reamed 7 mm, loose unlocked 4 mm or 7 mm IM nail; +/-endosteum cauterised	Most animals run to 9 weeks, a few for several months; gross mechanical instability used +/- cautery; no complications cited
Cullinane et al[70]	66	Rat	Femur	Ex-fix, 3 mm osteotomy, custom fixator with interfragmentary bending strain and micromovement	Inconsistent healing in control group; Type II cartilage filled gap; 5-week duration
Harrison et al[71]	100	Rat	Femur	Ex-fix, osteotomy, 3 mm distraction	Bone ends capped; gap mostly void or fibrous tissue; 5-week duration

* ex-fix, external fixation; IM, intramedullary; HNU, hypertrophic nonunion

of growth factors and proteins in association with bone scaffolds and grafts.

Clinically, a CSD model mimics situations where there has been substantial bone loss, either due to trauma or through surgery for tumour or infection. However, a CSD does not reflect the circumstances where the pathway to osseous regeneration has been arrested in some way, such as due to instability or metabolic disturbance.

High-energy, comminuted and open injury models. High-energy and comminuted injuries are associated with greater trauma to soft tissues and higher risks of delayed or nonunion. In investigating these situations it is important to have a model that reflects such soft-tissue and periosteal injury. High-energy injuries can be mimicked in models by stripping or excising periosteum from the fracture site, crushing or removing muscle, ligating arteries and dividing nerves. Many of the models that reflect periosteal stripping have been reported as models of delayed union or nonunion (Table VIII).[18,43,93-98]

Utvag et al[94] studied the effect of muscle injury on fracture healing in the rat tibia and found that muscle loss but not crushing significantly affected healing time. Claes et al[98] studied various forms of fixation on a three-part 'fracture' (osteotomy) in sheep: the external fixator resulted in the fewest complications, and the compression plate produced the worst outcome. Richards and Schemitsch[97] used a segmental canine model and either muscle flap or skin flap cover, with rates of nonunion of 25% and 75%, respec-

Table VIII. High-energy/comminuted/open fracture models

Authors	Animal	Bone	Model and aim*	Model considerations
Schindeler et al[93]	Mouse	Tibia	Comparison of distal and midshaft open tibial fracture. IM nail, open 3-point bending fracture	Fast capacity of murine healing. Technical challenge of model. No muscle or skin trauma
Utvag et al[94]	Rat	Tibia	Osteotomy and IM pin with ST insult. Comparison of IM muscle crushing with excision on fracture healing	Fibula N resected to create drop foot. Skin coverage was complete and primary closure. Low-energy method
Claes et al[95]	Rat	Tibia	Effect of ST trauma on fracture repair. IM nail fixation, 3-point bending +/- ST crushing (impaction device)	Closed injury model. High-energy ST injury but no ST/periosteal stripping
Utvag et al[96]	Rat	Femur	3-part segmental fracture; reamed, IM pin +/- periosteal stripping. Study of periosteal stripping on healing and vascularity in segmental fracture	Soft tissues and skin kept intact, low-energy fracture model
Park et al[43]	Rabbit	Tibia	Osteotomy, 3 mm gap, ex-fix. Effects of repeated irrigation and haematoma debridement	Pin site fractures. Gap plus debridement
Park et al[18]	Rabbit	Tibia	Open osteotomy with irrigation *vs* closed fracture, ex-fix stabilisation for both	Many variables; open *vs* closed, osteotomy *vs* 3-point bending, +/- irrigation
Richards and Schemitsch[97]	Dog	Tibia	2.5 cm devascularised segment, plate fixation, flap coverage. Muscle flap *vs* skin flap for revascularising segmental bone	Low energy osteotomy. Controlled, non-traumatic technique
Claes et al[98]	Sheep	Tibia	Segmental osteotomy with DCP/IM nail/bridge plate/ex-fix	Comminuted fracture model but controlled low-energy osteotomy technique with minimal ST disruption

* IM, intramedullary; ST, soft-tissue; ex-fix, external fixation; DCP, dynamic compression plate

Table VII. Segmental/critical size defect

Authors	Union rate	Animal	Bone	Defect size; stabilisation technique*	Considerations
Drosse et al[77]	0	Mouse	Femur	6 mm; plate or ex-fix	
Wingerter et al[78]	Not given	Rat	Femur	5 mm bone excised; 5-hole plate or IM K-wire	Qualitative study; different histology depending on fixation type; 4-week run model
Yasko et al[79]	0%	Rat	Femur	5 mm burr defect; polyethylene plate fixation	9/45 were excluded from study; 10% fixation failure
Einhorn et al[76]	0	Rat	Femur	6 mm (20% of the femur); 4-pin unilateral ex-fix	Easy to construct and low cost PMMA ex-fix
Ibiwoye et al[80]	0	Rat	Fibula	6 mm segment excised; no splintage/fixation	Non-weight-bearing bone
Oakes et al[81]	0	Rat	Femur	8 mm defect by burr; polyethylene plate	Athymic model; 16-week run model
Ma et al[82]	16%	Rabbit	Tibia	14 mm defect "created"; unilateral fixator	1/6 united; 8-week run model
Cook et al[83]	0	Rabbit	Ulna	15 mm segment excised; no splinting/fixation	Non-weight-bearing bone; 12-week run model
Bolander and Balian[84]	0	Rabbit	Ulna	20 mm; no splinting/ fixation	Proximal callus fused to radius; non-weight-bearing bone
Johnson et al[85]	11%	Dog	Radius	20 mm segment excised; ex-fix stabilisation	1/9 healed early, others no union at 24 weeks
Pluhar et al[86]	0	Sheep	Tibia	50 mm; IM nail, locked	Partial bone ingrowth but resolved later by complete periosteal excision
Rozen et al[87]	50% nil, 50% minimal	Sheep	Tibia	32 mm segment excised	Plate fixation; small quantity of bone found within the gap

* ex-fix, external fixation; IM, intramedullary; PMMA, polymethylmethacrylate

tively. These studies reproduce the endpoint well, but the models do not always reflect the high-energy transfer associated with such an injury.

Park et al[18] compared an osteotomy technique to a closed fracture model in the rabbit and found delayed healing, with smaller haematomas and greater periosteal damage. They also reported that repeated irrigation and debridement led to delayed healing.[18,43] It is clear that damage and interference to the periosteal and muscle envelope will have measurable effects on the degree of callus formation, vascularity and inflammatory cascade of that model.

Bone repair with infection models. Models of bone infection have been reviewed[99,100]: there are many models of osteomyelitis and septic arthritis, but relatively few incorporate fracture repair in the presence of infection. Essentially, models for early infection in the presence of trauma to bone are very similar to the simple fracture models (Table IX).[101-105] Few studies focus on late infection during fracture repair.

Compromised host models. Multiple host and clinical factors are known to impair fracture healing, including diabetes, hypothyroidism, malnutrition, alcohol, smoking and drugs such as non-steroidal anti-inflammatory drugs (NSAIDs). For each of these situations animal models

Table IX. Bone repair with infection

Author/s	Animal	Bone	Method*
Chen et al[101,102]	Rat	Femur	6 mm defect, ex-fix, *Staphylococcus aureus*
Andriole et al[103]	Guinea pig	Tibia	Closed fracture, IM nail, *Staph. aureus* inoculum
Worlock et al[104]	Rabbit	Tibia	Open fracture, IM nail, *Staph. aureus* into fracture site
Southwood et al[105]	Rabbit	Femur	10 mm defect, plate fixation, *Staph. aureus*; issues with mortality and plate bending

* ex-fix, external fixation; IM, intramedullary

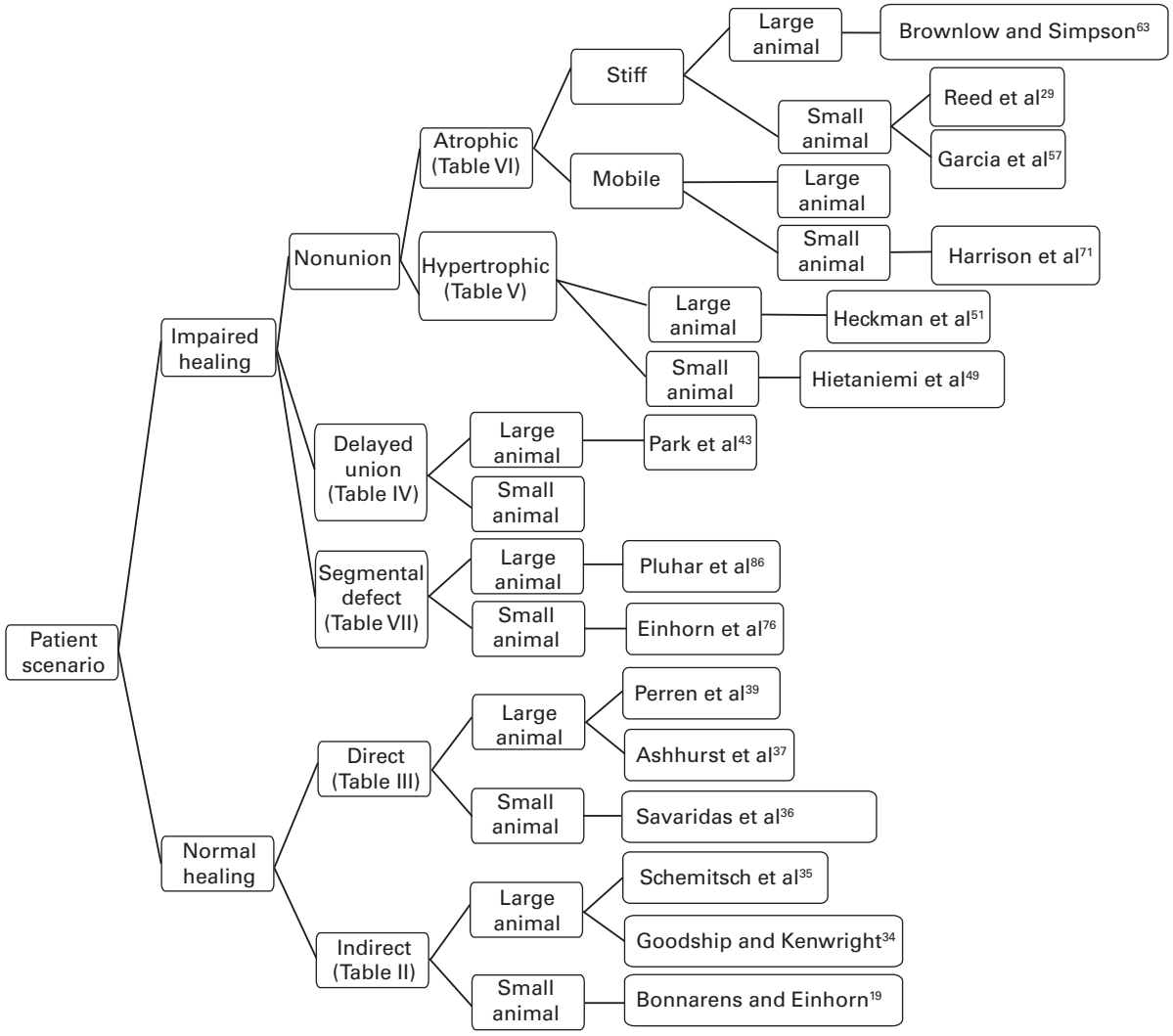

Fig. 1

Flow chart showing suitable animal models for each clinical scenario.

of repair have been described and reviewed by Gaston and Simpson.[106]

For studies evaluating the effect of the host genotype on fracture repair, strains of mice in which specific genes are suppressed are valuable. Mice with specific genes knocked out are available for diseases such as diabetes, for example, as are ones with deficiencies in the immune system (nude mice/rats, severe combined immunodeficiency (SCID) mice) or animals that enable certain cells to be tracked (green fluorescent protein (GFP) mice).

Conclusions

In conclusion, a variety of animal models for repair of long bone fractures are available to the researcher and can be classified according to the range of scenarios that are encountered clinically. The success achieved with each model varies, and this has implications for power calculations performed in the design of experimental studies. Figure 1 suggests suitable models of bone repair in animals that could be used to represent different clinical scenarios of human bone healing.

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