DESIGN, CHARACTERISATION AND *IN VIVO* TESTING OF A NEW, ADJUSTABLE STIFFNESS, EXTERNAL FIXATOR FOR THE RAT FEMUR

V. Glatt[1,2,*], C. H. Evans[1,2] and R. Matthys[3]

[1]Center for Advanced Orthopaedic Studies, Harvard Medical School, Boston, MA
[2]Collaborative Research Center, AO Foundation
[3]AO Development, AO Research Institutes, Davos, Switzerland

Abstract

Very little is known about the influence of the mechanical environment on the healing of large segmental defects. This partly reflects the lack of standardised, well characterised technologies to enable such studies. Here we report the design, construction and characterisation of a novel external fixator for use in conjunction with rat femoral defects. This device not only imposes a predetermined axial stiffness on the lesion, but also enables the stiffness to be changed during the healing process. The main frame of the fixator consists of polyethylethylketone with titanium alloy mounting pins. The stiffness of the fixator is determined by interchangeable connection elements of different thicknesses. Fixators were shown to stabilise 5 mm femoral defects in rats *in vivo* for at least 8 weeks during unrestricted cage activity. No distortion or infections, including pin infections, were noted. The healing process was simulated *in vitro* by inserting into a 5 mm femoral defect, materials whose Young's moduli approximated those of the different tissues present in regenerating bone. These studies confirmed that, although the external fixator is the major determinant of axial stiffness during the early phase of healing, the regenerate within the lesion subsequently dominates this property. There is much clinical interest in altering the mechanics of the defect to enhance bone healing. Our data suggest that, if alteration of the mechanical environment is to be used to modulate the healing of large segmental defects, this needs to be performed before the tissue properties become dominant.

Keywords: External fixator, bone healing, small animal model, mechanical stiffness, mechanobiology, dynamisation.

*Address for correspondence:
Vaida Glatt, PhD
Beth Israel Deaconess Medical Center
Center for Advanced Orthopaedic Studies
330 Brookline Avenue, RN115
Boston, MA 02215
USA

Telephone Number: 1-617-667-4621
Fax Number: 1-617-667-7175
E-mail: vglatt@bidmc.harvard.edu

Introduction

A considerable body of research confirms that the healing of osseous fractures is very responsive to the ambient mechanical environment (Huang and Ogawa, 2010; Morgan *et al.*, 2008). It is highly likely that the healing of large segmental defects in bone will be equally responsive, but this possibility has not been investigated in detail. One constraint to research in this area is the lack of experimental control over the local mechanical environment within a large segmental defect as it heals. The present paper reports the design, construction, mechanical evaluation and *in vivo* testing of a novel external fixator that addresses this issue. This fixator also allows modulation of the mechanical environment as healing progresses, a property that enables study of the mechano-sensitivity of different stages of the healing process, and, suitably adapted, may aid healing clinically.

The novel fixator is based upon external fixation, which is widely used for distraction osteogenesis (Pacicca *et al.*, 2002; Radomisli *et al.*, 2001; Richards *et al.*, 1999; Sato *et al.*, 1999; Sato *et al.*, 1998; Seebach *et al.*, 2004; Sojo *et al.*, 2005; Yasui *et al.*, 1997), fracture fixation (Harrison *et al.*, 2003; Kaspar *et al.*, 2007; Mark *et al.*, 2003; Mark *et al.*, 2004; Mark and Rydevik, 2005; McCann *et al.*, 2008; Smith-Adaline *et al.*, 2004) and large defect models in experimental animals (Betz *et al.*, 2006; Cullinane *et al.*, 2002; Cullinane *et al.*, 2003; Dickson *et al.*, 2008; Jager *et al.*, 2005). As well as imposing a controlled and reproducible local mechanical environment, its accessibility allows the modulation of this environment at different stages of healing. The mechanical characteristics of an external fixator are defined by, and can be modulated by, a large number of variables, which include: the distance between the pins, pin diameter, pin material, the number of pins, fixator bar length, fixator bar number, fixator bar material, fixator bar thickness and distance from the bone surface to the fixator bar (offset).

Few studies have investigated the mechanical contributions of the individual components of fixators or whole frame configurations. An *in vitro* study by Mark *et al.* (2003) found that the offset was linearly related to the stiffness in axial loading of rat bone using brass rod constructs. Furthermore, a decrease in pin diameter from 1.2 mm to 1.0 mm changed the axial stiffness of intact rat bone by about 50 %. They also found that if the bone fragments touched, the axial stiffness increased almost 10 times as compared to a gap size of 2 mm. Harrison *et al.* (2003) reported no change in axial stiffness when different materials such as aluminium and titanium were used for the fixator bar, while increasing the osteotomy gap from 1 to 3 mm decreased the mean axial stiffness

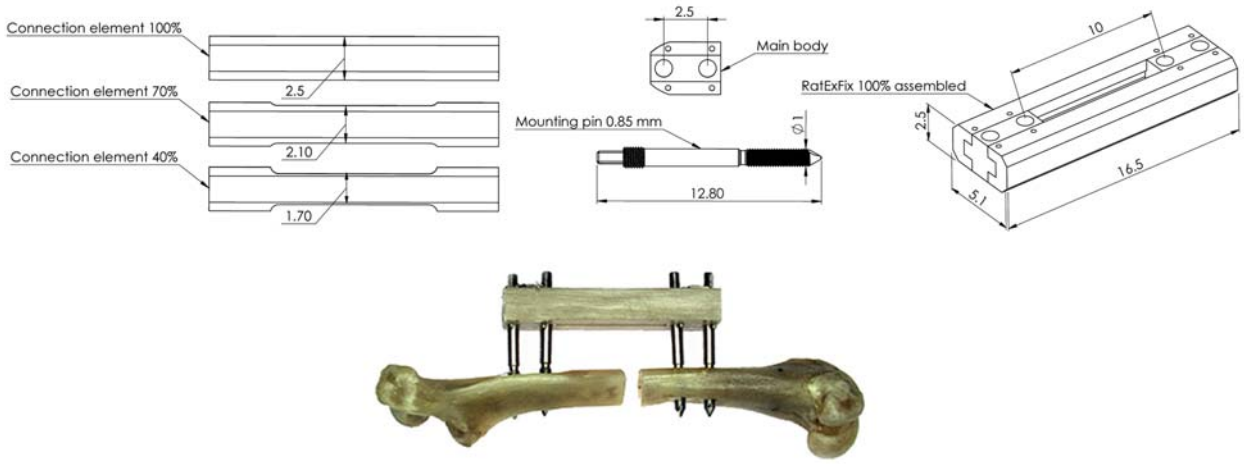

Fig. 1. Components of the novel external fixators. **Top:** Stiffness is determined by connection elements of different thicknesses (left hand side). The fixator is attached to the bone with titanium alloy mounting pins. **Bottom:** Assembled fixator in place on rat femur with 5 mm segmental defect.

only by 6 % for the three fixator frames tested. A study by Willie *et al.* (2009) found that the contribution to the total stiffness of the fixation construct was dominated by the flexibility of the pins in relation to their offset, diameter and material properties. For example, titanium pins produced significantly lower axial stiffness than stainless steel pins of the same design.

Although such characterisations are helpful, they fail to take into account the contributions of the regenerate within the osseous lesion to the overall mechanical environment. This is likely to be important, because as bone healing progresses from a haematoma to soft callus then hard callus, woven bone and, finally, lamellar bone, the mechanical contribution of the endogenous tissue within the lesion will change dramatically.

One *in vitro* study (Gardner *et al.*, 1996) looked not only at the local mechanical environment provided by five different clinically used external fixators, both dynamised and non-dynamised, but also at the effect of the resistance to axial interfragmentary motion (IFM) created by four different stiffness materials simulating the main stages of the healing process. Using fixators implanted on glass fibre tubing in axial compression to simulate weight-bearing, Gardner *et al*. (1996) found that when no material was present in the fracture gap, which simulates the conditions immediately post-surgery, the fixator frame was responsible for all stability at the fracture site, and there was no contribution from the gap (based on a weight-bearing of 650 N and an average axial frame stiffness of 60 N/mm). On the contrary, when they interposed material with the lowest stiffness (50 N/mm), which represents early stages of fracture healing, they found that the mechanical properties of the fracture material were as important as those of the fixator in influencing axial IFM at around two to four weeks post-fixation. Furthermore, the five clinical external fixators tested made different mechanical contributions, depending on their stiffness. In contrast, using three stiffer intra-fracture materials, simulating all but the initial stage of fracture healing, axial movement was influenced only by the stiffness of the materials within the fracture site and only slightly affected by the contribution of the fixation devices. Nevertheless, angular, transverse and torsional shear movements were enhanced, depending on the type of fixator. These motions were also enhanced by unlocking the fixator to increase fixator looseness. These data demonstrate the importance of including contributions by the intra-defect tissue when analysing the overall mechanical environment created by an individual fixation device, but no prior research has extensively investigated this interaction.

Therefore, we had four main aims for this study: first to design and manufacture a new external fixator for large femoral defects in the rat with the ability to change stiffness *in vivo* during the healing process; second, to characterise the mechanical environment generated by such a fixator; third to determine how the stiffness created by each of the fixators responded to the intra-lesional insertion of standard materials whose mechanical properties reflected those of tissues present sequentially during the healing process; four, to evaluate the performance of the fixators *in vivo* on the femora of rats.

Materials and Methods

External fixators

The external fixator (RatExFix,™) main frame consists of a polyethylethylketone (PEEK) body. Each external fixator has two connection elements and two main modules (Fig. 1). The connection elements are of different thickness, and hence stiffness, and were developed to achieve fixation stiffness equivalent to 40 % (1.7 mm thick), 70 % (2.1 mm thick) and 100 % (2.5 mm thick). The external fixator stiffness of 100 % was calculated based on the 400 g approximate body weight of a mature rat, and then by doubling the weight (800 g). It is based upon the data of Clark (1995) reporting vertical forces for the hind paw of about 50 % of body weight. This was done to ensure that after creating a 5 mm defect, the fixator was capable of withstanding the weight-bearing of the animal, thereby

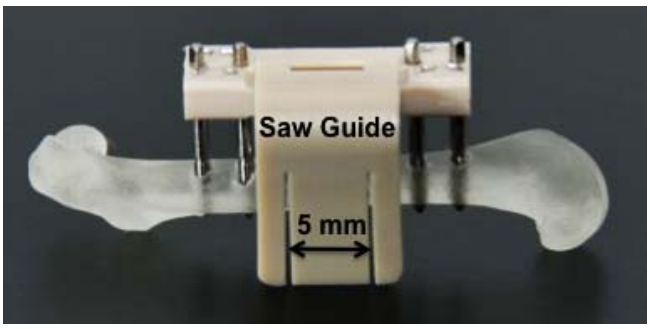

Fig. 2. The saw guide for creating a 5 mm defect, in place on a rat femur

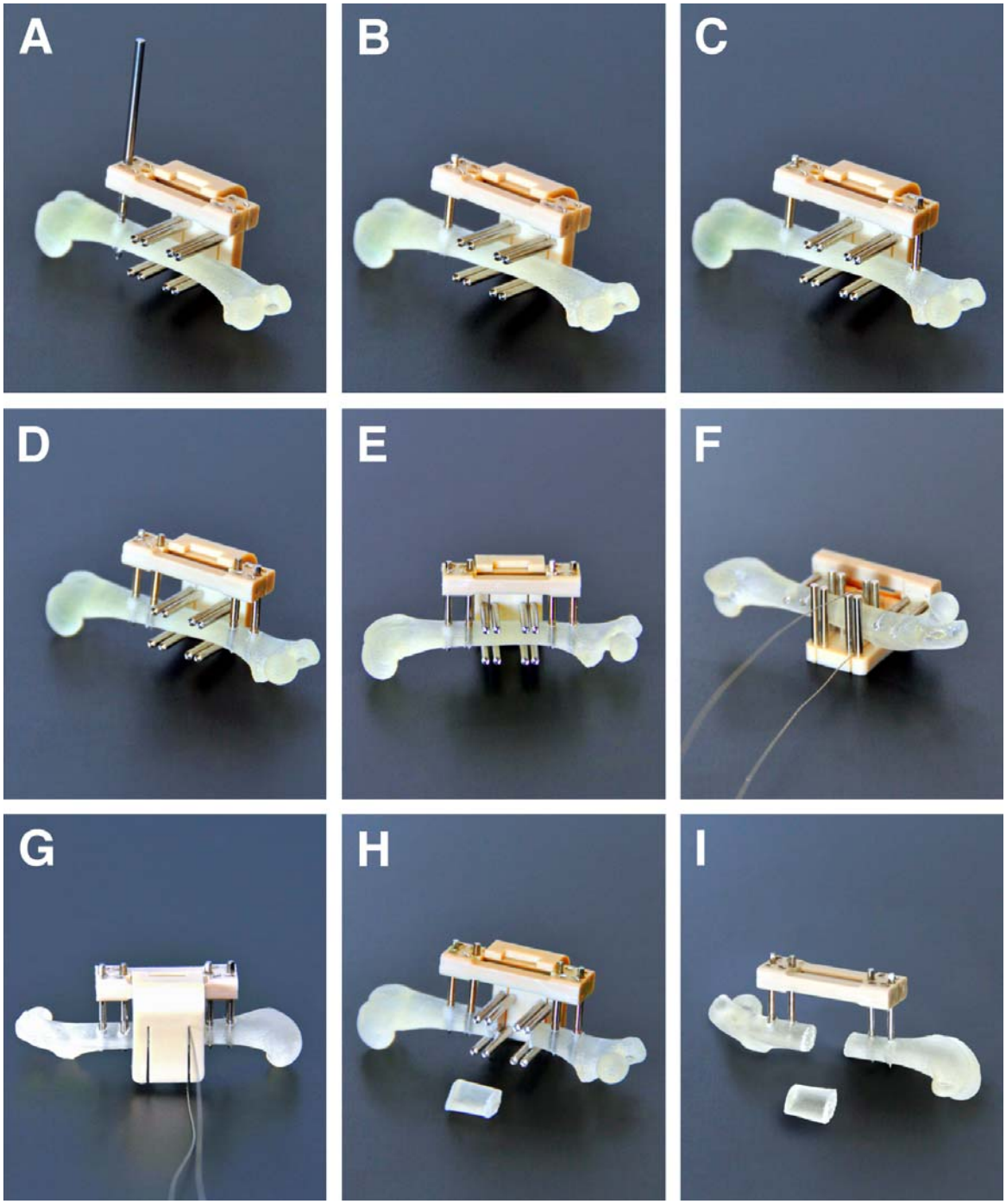

Fig. 3. Application of external fixators. **A)** ExFix stability bar clipped on the Gigli saw guide attached the femur with the first drilled hole and drill bit in place; **B)** Insertion of the first titanium screw in the most distal position; **C)** Insertion of the second titanium screw in the most distal position on the other side; **D) and E)** Insertion of two inner titanium screws - side and front views; **F)** Gigli saw application through the saw guide; **G)** Gigli saw passed through the saw guide grooves; **H)** Creation of 5 mm defect with saw guide in place; **I)** Completion of the procedure, saw guide removed.

maintaining alignment and preventing the dislocation of defect fragments.

The remaining two fixator stiffnesses were decreased by 30 % respectively from the highest (100 %) to have a variety of stiffnesses for the study. For convenience, these are referred to as ExFixLow, ExFixMed and ExFixHigh.

Each main module has two holes where the screws are inserted. The fixator stiffness can be changed while it is still attached to a living animal by changing connection elements with a wire applicator. TAN (titanium alloy) was used to make the mounting pins shown in Fig. 1. Four mounting pinsare used to secure the stability bar to the femur (Fig. 1). The fixator comes in four pieces and needs to be assembled prior to use. The distance between the outer screws is 16 mm and the distance between the middle screws is 11 mm. All holes are predrilled using a 0.76 mm drill bit. The screws are locked in corresponding holes in the main fixator frame, which is parallel to the bone surface and set at the distance of 6 mm from the bone (Fig. 1).

A saw guide was developed to enable the creation of a 5 mm segmental defect in the femur (Fig. 2); it also serves as a positioning guide for the installation of the external fixator. The main frame of the external fixator is clipped on to the saw guide and then the whole system is clipped onto the bone as shown in Fig. 3. The 5 mm gap is generated with a 0.22 mm micro Gigli saw (AO Research Institute, Davos, Switzerland) (Fig. 3).

To minimise a slip-stick effect, two elements of this saw are constructed from wires of different diameters. The thicker wire, diameter 0.07 mm, serves as an axial core, while the second wire, diameter 0.04 mm forms a cutting spiral with adjustable pitch around the core wire. Both the saw guide and the micro Gigli saw can be autoclaved at 134 ºC.

Due to the miniature size of the external fixator, a special set of implantation instruments was designed and acquired; a customised screwdriver was also developed. A drill bit with a diameter of 0.76 mm was used to create holes in the bone. The core diameter of each screw is 0.02 mm bigger than the drill bit to guarantee proper fitting of the screw into the bone. When used together with a self-cutting screw tip, this has been shown to prevent loosening due to bone surface resorption at the bone-scrûı interface (Hess *et al.*, 1991). The drill bit is operated by a miniature, electrical, pen drill producing 2500 rpm with a power of 500 mW.

Three-point-bending of stability bars

Non-destructive, 3-point-bending mechanical tests with 6 samples per group were used to determine the mechanical properties of the stability bars for all external fixators. The load was applied midway between two supports 14.09 mm apart; the diameter of the supports was 1.95 mm. Load-displacement curves were recorded at a crosshead speed of 1 mm/min to a 2 % strain rate or a maximum force of 250 N using a Mechanical Testing System Synergie 200 (MTS, MN, USA). Samples were preloaded to 8 N before each test. The force/displacement curves were acquired at 100 Hz. Three-point-bending stiffness was calculated from the linear region of the load-deflection curve.

Axial compression testing

In this test four materials were chosen to simulate the Young's moduli of different types of tissue present in the lesion at the various stages of the bone healing process. Their selection was based upon the mechanical data of Morgan *et al*. (2008). A rubber insert was chosen to simulate the early stage of healing, at the time when the haematoma within the defect is being replaced by cartilage. According to the literature, at this stage of healing the tissue elastic modulus is about 0.01-0.1 GPa (Morgan *et al.*, 2008), which is in the same range as rubber. For the subsequent stage, at which calcified cartilage is being replaced by woven bone, low-density polyethylene (LDPE) was chosen to simulate tissue stiffness, which has been reported to be in the range of 0.2-0.9 GPa (Morgan *et al.*, 2008). For the final stage of bone healing, during which woven bone is maturing into lamellar bone, two different woods were evaluated. The elastic modulus of cortical bone has been reported to lie in the range of 10 GPa, similar to values reported for maple and oak (Morgan *et al.*, 2008). Axial compression testing was performed to confirm that their Young's moduli, as measured under our experimental circumstances, corresponded to those reported in the literature.

Rubber and LDPE were purchased (McMaster-Carr, Elmhurst, IL, USA) in sheets of 36x36 cm, 5.5 mm thick. They were cut into 25x25 mm squares for testing. Maple and oak were purchased (McMaster-Carr, Elmhurst, IL, USA) as rods 0.9144 m long and 9.54 mm in diameter and cut into 5.5 mm thickness samples for testing. Six samples per group were used to determine the Young's moduli of these materials in axial compression testing using an Instron 8550 mechanical testing system. Load-displacement curves were recorded at a crosshead speed of 0.5 mm/min to a 5 % strain rate using a 2 kN load cell. The force/displacement curves were acquired at 100 Hz.

After stability bar and material testing was completed, axial compression testing was used to determine the mechanical environment created when the 3 different stiffness external fixators were assembled onto rat femora with and without the 5 mm defect. For this experiment the external fixators were assembled in position on the right femora of 8 freshly euthanised Sprague Dawley rats. After the external fixators were in place, a 5 mm segmental defect was created in one of the femora in each group and then excised from the body. The procedure was performed in this manner to avoid drying out and cracking of the bones. Also, 6 intact contralateral femora were used as controls. Both ends of each specimen were embedded in polymethylmethacrylate to provide a reproducible, flat interface with the testing fixture. The four different test materials were put into defects to simulate different stages of fracture healing. The thickness of all materials was cut to 5.5 mm to fit tightly the defect; the diameter for the wood was 9.54 mm, rubber and LDPE was cut into 6x6 mm squares.

Six samples from each material were placed in the segmental defect for each of the different fixators to determine axial stiffness. Samples were tested in an unconfined compression setup (Fig. 4). Preloading (4-6 N) was undertaken to ensure that there was no sliding within the jig.

After each test, constructs from all groups were removed, and one of the material samples inserted into the defect and repositioned in the mechanical testing machine. Load-displacement curves were recorded at a crosshead speed of 0.5 mm/min to a 1.5 % strain using an Instron 8550 mechanical testing system (Instron, MA, USA). The force/displacement curves were acquired at 100 Hz. The stiffness of the construct was automatically calculated from the linear region of the load-deflection curve.

Animals

Sprague Dawley rats (male, 350-400 g) were purchased from Charles River Laboratories International, Inc. (Wilmington, MA USA). Animal care and experimental protocols were followed in accordance with NIH guidelines and approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee. Following a minimum 48 h acclimatisation period, the animals were transported to a dedicated surgical procedure room.

A total of 12 rats were used for the *ex vivo* mechanical testing experiments, 4 animals per group. Animals were euthanised with CO_2 and cardiac puncture and the fixators surgically applied as described in the text. Femora with the fixators attached were excised and loaded as described in the text.

For *in vivo* experiments, rats were anaesthetised with isoflurane (2 % at 2 L/min by air mask). Before surgery rats were given antibiotic (cefazolin, 20 mg/kg) and the analgesic buprenorphine (dose 0.08 mg/kg) intramuscularly in the left leg. The entire right hind leg of the rat was shaved, cleaned with 70 % ethanol and 10 % iodine and draped with sterile fenestrated drape so only the right leg was exposed. An incision of approximately 3.5-4 cm was made through the skin running craniolateral on the surface of the right femur from the greater trochanter to the supracondylar region of the knee. The shaft of the femur was exposed by gentle dissection between the quadriceps and hamstring muscles.

The external fixator bar was used as a positioning guide, first clipped on to the wire saw guide and then placed on to the craniolateral aspect of the femur, to guide the drill and permit reproducible positioning of four drill holes with a diameter of 0.76 mm. The miniature electrical pen drill was used to drill all the holes for the screws one at the time, starting either with the distal or proximal side. Once the first hole was made the screw was driven into the femur on the distal/proximal side, first through the external fixator bar and then into the bone, making sure that it entered perpendicularly into the femur. After the first screw was in place, the most distant hole from the first screw was then drilled, and the second screw was driven into the hole. The implantation order of the two middle screws is not important as long as they are perfectly perpendicular to the craniolateral femoral surface. After the fixator was in place, the saw guide was used to make the segmental defect.

Fig. 4. Mechanical testing rig showing ExFixHigh assembled on a rat femur with a 5 mm defect.

For this, the 0.22 mm Gigli saw was passed through the 2 grooves underneath the femur to create a 5 mm segmental defect by reciprocal motion back and forth. After the defect was created, the saw guide was removed and the wound was closed in layers. On the first three postoperative days, the rat was given analgesic every 12 h and antibiotic every 24 h.

Defects were examined radiologically immediately after surgery and then once per week for 8 weeks.

Statistical analysis

For the stability bar and the material mechanical testing, comparisons of continuous variables between the groups were performed using analysis of variance (One Way-ANOVA). If the difference between the groups was significant, a *post hoc* test (Tukey) was performed. To compare tested materials (rubber, LDPE, maple and oak), an unpaired *t*-test was performed, with differences considered significant at $p < 0.05$. For the axial compression test, statistical analyses were performed using either analysis of variance (Two Way-ANOVA) or a two-tailed *t*-test to determine if there was a significant correlation between the stiffnesses of the different fixators and the materials used. A power analysis after the study was calculated to determine if we had sufficient numbers of samples per group to detect a significant difference. The power level for all the data was

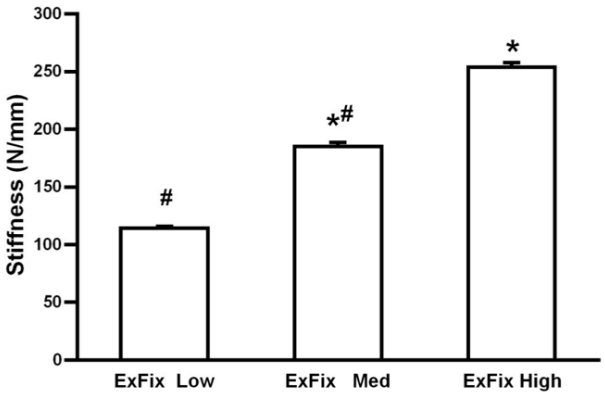

Fig. 5. Connection element stiffness in 3-point-bending. ExFixLow, ExFixMed and ExFixHigh connection elements were tested. Values given are means ± SEM; asterisk indicates statistically significant difference from ExFixLow, and hash signs indicate statistically significant difference from ExFixHigh ($p < 0.05$, $n = 6$ per group).

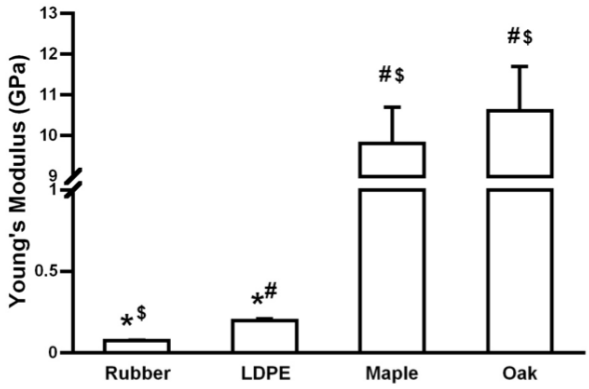

Fig. 6. Young's moduli calculated from axial compression of test materials. Values given are means ± SEM; dollar signs indicate statistically significant difference from low density polyethylene (LDPE), hash signs indicate statistically significant difference from rubber, and asterisks signs indicate statistically significant difference from oak ($p < 0.05$, $n = 6$ per group).

found to be from 0.9 to 1. Thus the numbers of samples per group used for this study were sufficient to determine a 5 % difference between the test groups. All tests were two-tailed, with differences considered significant at $p < 0.05$. Data are presented as mean ± SE, unless otherwise noted.

Results

Three-point-bending of stability bars

Three-point-bending tests performed on the stability bars recorded the bending stiffnesses of ExFixLow, ExFixMed and ExFixHigh as 114, 185 and 254 N/mm, respectively (Fig. 5). These values were significantly different from each other ($p < 0.001$). ExFixHigh was 27 % stiffer than the ExFixMed ($p < 0.0011$) and ExFixMed was 38 % stiffer than ExFixLow ($p < 0.0008$).

Axial compression validation testing

Axial compression testing was performed to confirm Young's moduli for rubber, LDPE, maple and oak. These tests showed that Young's moduli for all four materials (Fig. 6) fell within the range reported in the literature (Morgan *et al.* 2008). There was no significant difference between maple and oak. Therefore, for further *in vitro* testing, maple was chosen due to its smaller inter-sample variability. There was a statistically significant difference when rubber was compared to LDPE and both woods ($p < 0.0001$). Statistical significance was also seen when LDPE was compared to all other materials tested ($p < 0.0001$).

Axial compression testing

Axial compression mechanical t stingwas used to determine the local mechanical properties within the critical size defects imposed by the three different stiffness fixators. First, the influence of the fixators on the stiffness of intact rat femur was determined, and then the three different materials validated in the previous section were inserted in turn into defects in the rat femur.

ExFixLow and ExFixMed had no statistically significant effect on the stiffness of the intact femur. ExFixHigh, in contrast, increased stiffness by 20 % (Fig. 7). Having tested intact bone, 5 mm defects were created in the femur with three different stiffness fixators and tested without any inserts (Fig. 7B). The stiffness of the empty defects increased progressively with the application of ExFixLow, ExFixMed and ExFixHigh. A similar, statistically significant progression was seen with the interposition of a rubber insert to simulate early stages of healing (Fig. 7C). When LDPE was used to simulate a later stage of fracture healing (Fig. 7D), significant differences were seen with the ExFixHigh construct as compared to the other external fixators ($p < 0.001$), but stiffnesses produced by the ExFixLow and ExFixMed were not different from each other (Fig. 7D). When maple was used in the defect (Fig. 7E), there was no difference in stiffness between any of the fixators. Two Way Anova revealed that, overall, there is significant interaction between the materials and external fixators used ($p < 0.0001$).

In vivo experiments

Radiological examination confirmed that fixators of all stiffnesses maintained the 5 mm femoral defect during the entire 8 weeks of the experiment (Fig. 8). Because these are critical size defects, there was no spontaneous healing. No distortion or infections, including pin infections, were observed and pin loosening was absent.

Discussion

The main goals of this study were to design, manufacture and characterise a new, variable stiffness external fixator for the rat femur, and to use this fixator to interrogate the interplay between fixator stiffness and the regenerate in determining the overall mechanical environment of the defect during healing. Although the fixators were designed to bear double the load of a 400 g rat, Wehner *et al.* (2010)

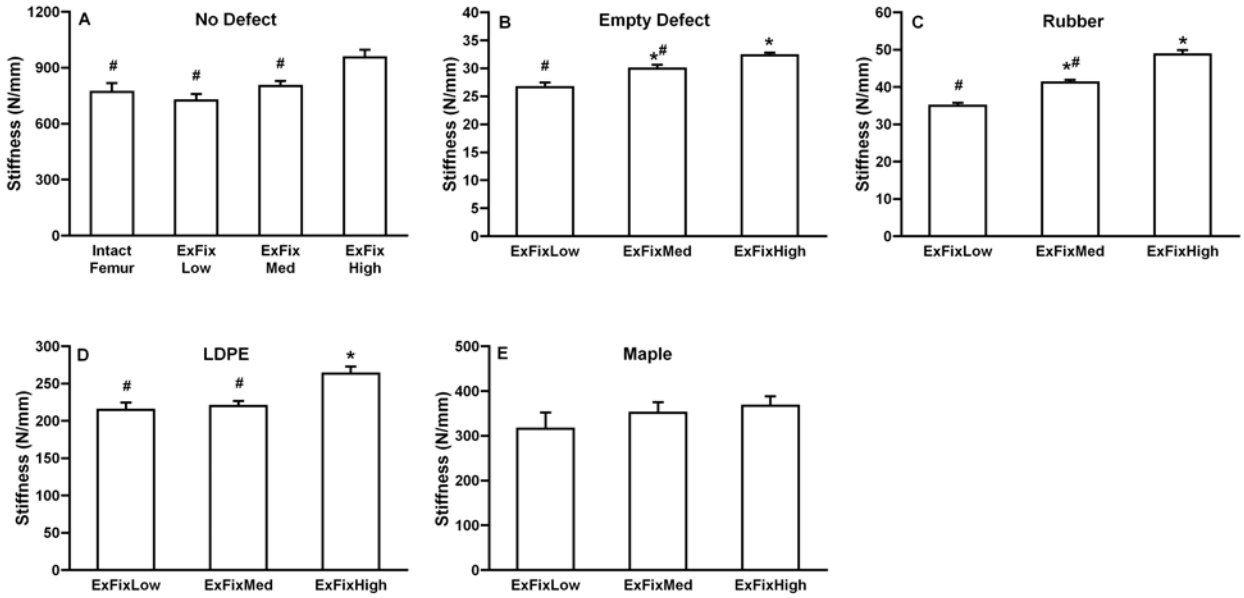

Fig. 7. Stiffnesses created by the different fixators applied to intact rat femur, femur with empty 5 mm defect, and defect with different materials inserted. ExFixLow, ExFixMed and ExFixHigh were assembled on isolated rat femora and stiffnesses measured in axial compression. **(A)** Intact femora, **(B)** Femora with 5 mm empty defect, **(C)** Femora with 5 mm defects containing rubber, **(D)** Femora with 5 mm defects containing LDPE, **(E)** Femora with 5 mm defects containing maple. Values given are means ± SEM; asterisks indicate statistical significance from ExFixLow, and hash signs indicate statistical significant difference from ExFixHigh ($p < 0.05$, $n = 6$ per group).

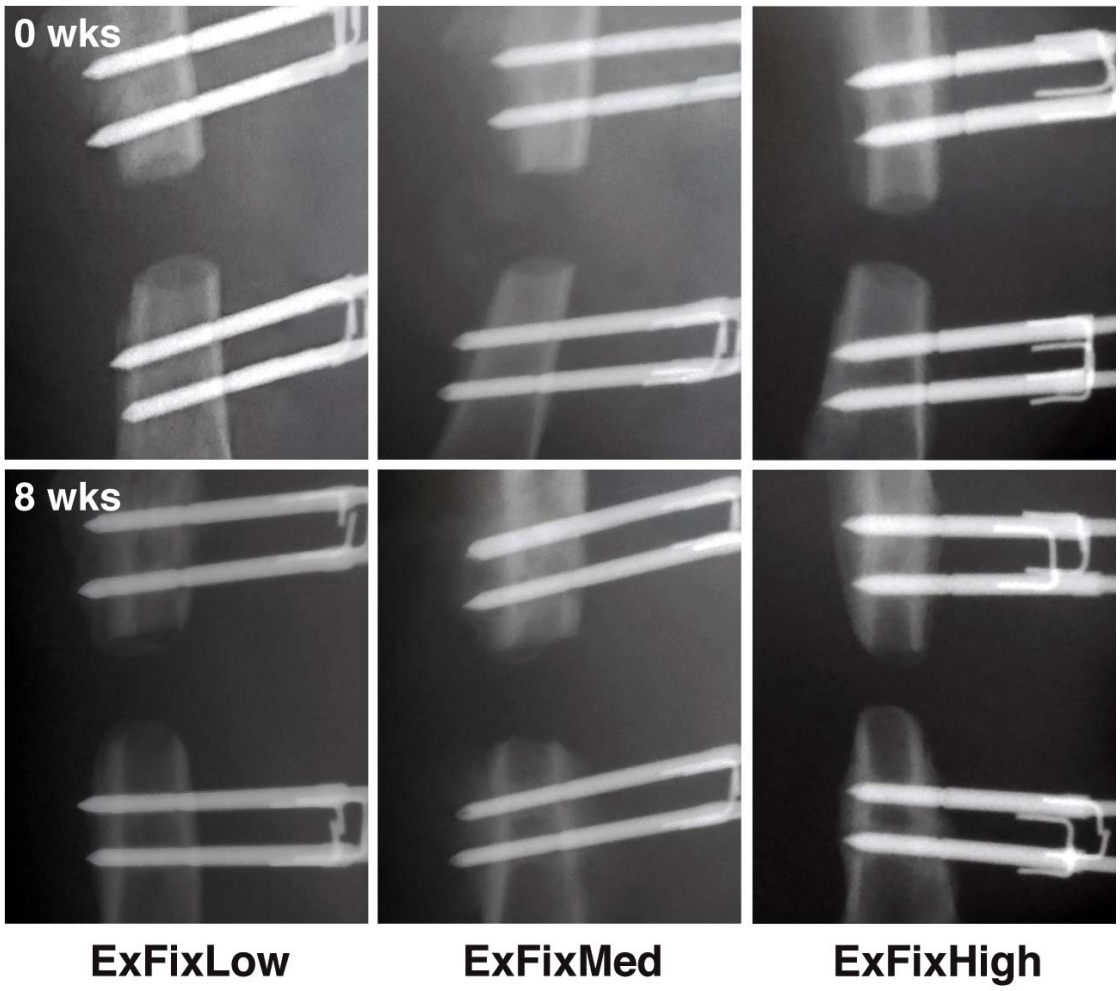

Fig. 8. *In vivo* X-ray images of defects in rats immediately post-surgery and 8 weeks later. External fixators of all 3 stiffnesses were surgically implanted on rat femora and 5 mm segmental defects created. The defects were X-rayed immediately after surgery (0 weeks) and at weekly intervals until 8 weeks when the experiment was terminated.

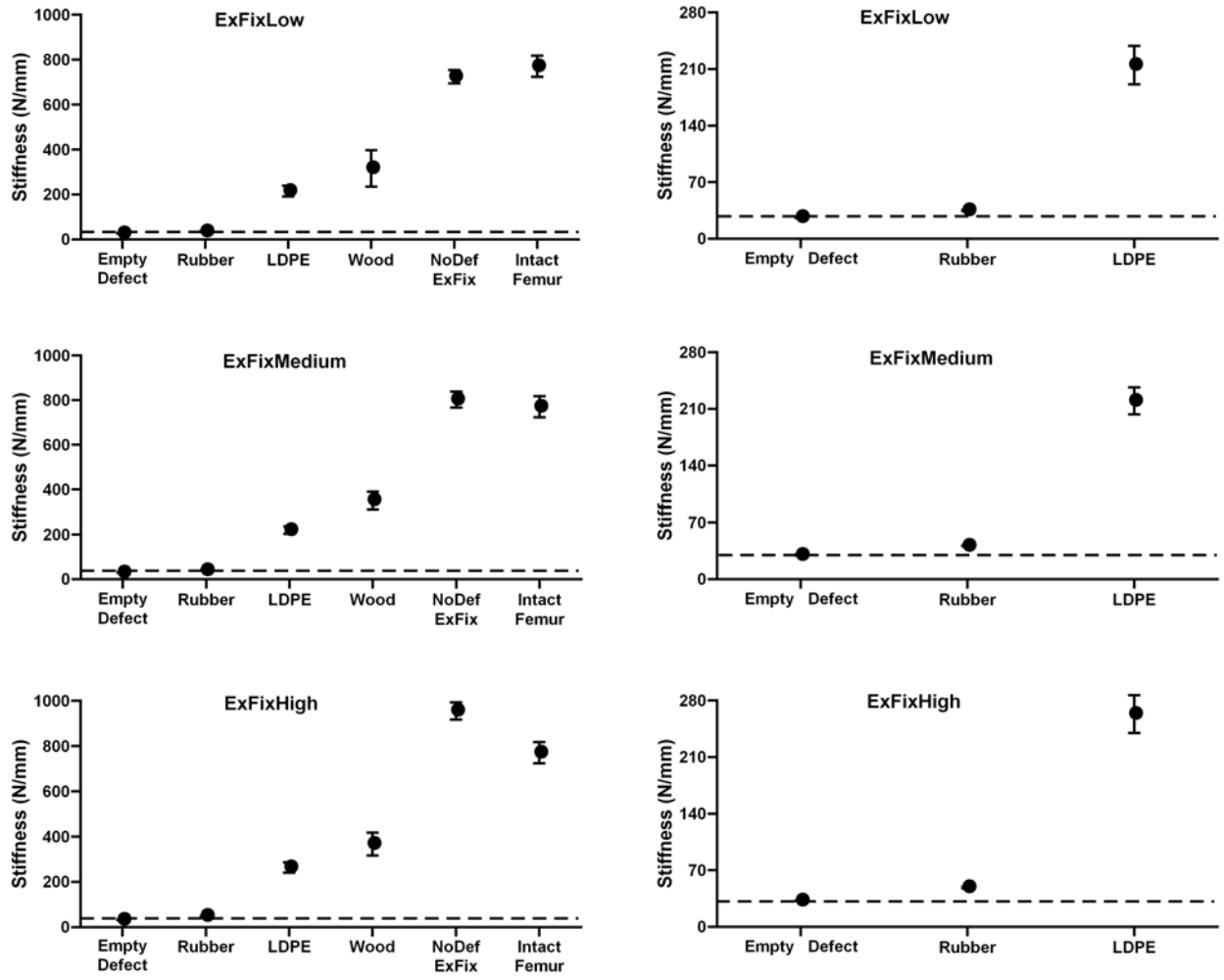

Fig. 9. Contribution of external fixators to overall axial stiffness as a function of the material inserted into the defect. The external fixator makes the major contribution to overall axial stiffness in the empty defect (dashed line) and when rubber is placed in the defect to simulate early healing when the defect is transitioning from haematoma to soft callus. When LDPE is inserted to simulate calcified cartilage/woven bone, the contribution of the fixator to overall stiffness is substantially diminished. At the woven-lamellar bone stage (wood insert), the fixators have minimal effect on overall stiffness. **Left Hand Side:** Full range of conditions; empty defects, inserts and intact femur. **Right Hand Side:** Expanded view from empty defects to LDPE only.

found loads of up to 6-7 times body weight acting on the rat femur during normal gait. This study emerged after we had designed and constructed the fixators described here. Nevertheless, as described in the results section, these fixators maintained the 5 mm defect for the 8 weeks of the *in vivo* experiment. Moreover, it would be a straightforward matter to produce customised connection elements with increased stiffness to accommodate higher loads based on these recent findings.

The mechanical properties of the new fixators were examined at three levels. First, *in vitro* three-point-bending tests were carried out to determine the rigidity of the stability bars. Fixators were then installed on excised rat femora, with and without 5 mm defects, and tested in axial compression to imitate weight-bearing. To approximate the alterations in the mechanical properties of the defect that occur as it heals, rubber, LDPE and maple were inserted into the defects to simulate progressive phases of healing. Finally, fixators were applied to rat femora and their *in vivo* performance monitored radiographically for 8 weeks.

The results of these studies show convincingly that the mechanical environment cannot be explained solely in terms of the properties of the components used to construct the fixator. It is essential to measure the overall mechanical properties of the entire fixator construct attached to bone, and to take into account mechanical contributions from the defect tissue itself as it heals. A future, further refinement of this analysis will take into account the mechanical contributions of muscle and other surrounding soft tissues.

The primary innovation of this fixator is the ability to exchange the stability bar connection elements to select different, standardisedûstiffnesses. Because the stability bar's connection elements can be exchanged while the device is attached to the animal, the stiffness can be adjusted at different stages during the healing process. The connection elements are exchanged one at

a time to prevent misalignment of the defect edges and the destruction of newly formed tissue. Although three different stiffnesses were generated for the present work, additional stiffnesses can be achieved simply by fabricating additional connection elements of different thicknesses.

Previous authors have designed alternative external fixators with adjustable stiffnesses. The device of Claes *et al.* (2009, 2011), for instance, uses two crossbars and stiffness is reduced by removing the inner bar. This is a novel design, but allows less opportunity for precise, multiple manipulations of the mechanical environment during healing, especially as the bars are secured by K-wires. The fixator also weighs about 5 g, and may impose uncontrolled loading due to inertia. Using a different approach, Strube *et al.* (2008) developed a fixator where stiffness was changed by altering the offset. This has the advantage of providing a large, continuous range of stiffnesses, but is also a large, heavy device secured by K-wires.

In our device, mounting pins and main frame were made from TAN and PEEK, respectively, because these materials are already used for orthopaedic implants in humans and their biocompatibility is well established. These materials also allow *in vivo* imaging in the early stages of fracture repair with minimal distortion, and reduce the incidence of infections. *In vivo* experiments confirmed that the fixators allowed clear imaging and maintained a 5 mm segmental gap for at least 8 weeks without infection or pin loosening.

As an additional design feature, the fixator has a preset offset of 6 mm from the bone surface to the stability bar no matter which stiffness connection elements are used. This feature makes implantation of the fixator very reproducible. Another major advantage over alternative designs described in the literature (Einhorn *et al.*, 1984; Mark *et al.*, 2003; Strube *et al.*, 2008; Claes *et al*., 2009) including the one we have used in the past (Glatt *et al.*, 2009), is that the new external fixator was designed to have a minimal mass (0.32 g) to avoid uncontrolled loading due to inertia. The new fixators were made taking into account the rat body weight and the size of femur; this relationship was scaled as with human fixation devices. Furthermore, after the implantation and suturing of the skin, the clearance between the implant cross bar and the skin is only about 4 mm. Such close proximity to the skin surface minimises the moment force, which prevents the possibility of an additional loading within the defect other than the one intended from the external fixator.

In addition, to keep the surgical trauma low, conventional and rotating saws were not considered as tools for creating 5 mm defects. Such saws either cut into adjacent tissue or strip the periosteum when the tissues are retracted. In the past we have used a 4.5 mm dental burr saw to create 5 mm defects and found that it was impossible to create exact and reproducibly sized defects with parallel ends (Betz *et al.*, 2006; Glatt *et al.*, 2009). To avoid all these problems we took an advantage of the Gigli saw. A conventional Gigli saw has substantially different static and kinetic friction and two intertwined diamond cords create minimal damage to the bone while sawing. The saw guide was developed to create precise defects with the parallel ends.

Having designed, built and evaluated this, the fixator was subject to further, detailed, quantitative analysis to determine its precise mechanical properties and certain aspects of the mechanical environment it generates within a segmental defect *in vitro*. When a 5 mm defect was created and tested in axial compression, the highest stiffness of the system was achieved with the most rigid external fixator, and it decreased linearly as the stiffness of the fixator bar decreased. This is to be expected, because the load is solely carried by the fixation device when the fracture gap is empty.

When rubber was inserted to mimic the initial phase of healing, the total stiffness of the system increased, with the external fixators still making a substantial contribution to the overall stiffness; the magnitude of this contribution increased with the stiffness of the external fixator. With LPDE in the defect, there was a very large increase in axial stiffness, but the contributions of the external fixators were small. This indicates that once healing reaches the stage of calcified cartilage, which LPDE mimics, the external fixators have only a minor influence on the axial stiffness of the defect. When maple was inserted into the defect, stiffness increased considerably, but the contributions of the external fixators were minimal and there was no effect of fixator stiffness.

When the change in overall axial stiffness is considered with respect to the material within the lesion (Fig. 9), it is clear that the axial stiffness within a large, segmental, osseous lesion is dominated by the material properties of the regenerate once healing has progressed beyond the initial soft callus stage. Gardner *et al*. (1996) also found that the amount of axial movement was mainly controlled by the material in the fracture gap rather than by the fixator stiffness. Collectively, these data suggest that if healing within a large osseous defect is to be manipulated mechanically by an external fixator of the type studied in the present paper, this should be implemented before the mechanical environment becomes dominated by the mechanical properties of the regenerate.

Acknowledgements

This work was supported by the AO foundation (S-08-42G). We thank Ben Roberts for assistance with mechanical testing and John Muller for writing MatLab code to analyse mechanical testing data. The authors have no conflict of interest and the study was not supported by a commercial entity.

References

Betz OB, Betz VM, Nazarian A, Pilapil CG, Vrahas MS, Bouxsein ML, Gerstenfeld LC, Einhorn TA, Evans CH (2006) Direct percutaneous gene delivery to enhance healing of segmental bone defects. J Bone Joint Surg Am **88**: 355-365.

Claes L, Blakytny R, Gockelmann M, Schoen M, Ignatius A, Willie B (2009) Early dynamization by reduced

fixation stiffness does not improve fracture healing in a rat femoral osteotomy model. J Orthop Res **27**: 22-27.

Claes L, Blakytny R, Besse J, Bausewein C, Ignatius A, Willie B (2011) Late dynamization by reduced fixation stiffness enhances fracture healing in a rat femoral osteotomy model. J Orthop Trauma **25**: 169-174.

Clarke KA (1995) Differential fore- and hindpaw force transmission in the walking rat. Physiol Behav **58**: 415-419.

Cullinane DM, Lietman SA, Inoue N, Deitz LW, Chao EY (2002) The effect of recombinant human osteogenic protein-1 (bone morphogenetic protein-7) impregnation on allografts in a canine intercalary bone defect. J Orthop Res **20**: 1240-1245.

Cullinane DM, Salisbury KT, Alkhiary Y, Eisenberg S, Gerstenfeld L, Einhorn TA (2003) Effects of the local mechanical environment on vertebrate tissue differentiation during repair: does repair recapitulate development? J Exp Biol **206**: 2459-2471.

Dickson GR, Geddis C, Fazzalari N, Marsh D, Parkinson I (2008) Microcomputed tomography imaging in a rat model of delayed union/non-union fracture. J Orthop Res **26**: 729-736.

Einhorn TA, Lane JM, Burstein AH, Kopman CR, Vigorita VJ (1984) The healing of segmental bone defects induced by demineralized bone matrix. A radiographic and biomechanical study. J Bone Joint Surg Am **66**: 274-279.

Gardner TN, Evans M, Kenwright J (1996) The influence of external fixators on fracture motion during simulated walking. Med Eng Phys **18**: 305-313.

Glatt V, Kwong FN, Park K, Parry N, Griffin D, Vrahas M, Evans CH, Harris M (2009) Ability of recombinant human bone morphogenetic protein 2 to enhance bone healing in the presence of tobramycin: evaluation in a rat segmental defect model. J Orthop Trauma **23**: 693-701.

Harrison LJ, Cunningham JL, Stromberg L, Goodship AE (2003) Controlled induction of a pseudarthrosis: a study using a rodent model. J Orthop Trauma **17**: 11-21.

Hess T, Hopf T, Fritsch E, Mittelmeier H (1991) [Comparative biomechanical studies of conventional and self-tapping cortical bone screws.] Z Orthop Grenz **129**: 278-282.

Huang C, Ogawa R (2010) Mechanotransduction in bone repair and regeneration. FASEB J **24**: 3625-3632.

Jager M, Sager M, Lensing-Hohn S, Krauspe R (2005) The critical size bony defect in a small animal for bone healing studies (II): implant evolution and surgical technique on a rat's femur. Biomed Tech (Berl) **50**: 137-142.

Kaspar K, Schell H, Toben D, Matziolis G, Bail HJ (2007) An easily reproducible and biomechanically standardized model to investigate bone heûıing in rats, using external fixation. Biomed Tech (Berl) **52**: 383-390.

Mark H, Bergholm J, Nilsson A, Rydevik B, Stromberg L (2003) An external fixation method and device to study fracture healing in rats. Acta Orthop Scand **74**: 476-482.

Mark H, Nilsson A, Nannmark U, Rydevik B (2004) Effects of fracture fixation stability on ossification in healing fractures. Clin Orthop Relat Res **419**: 245-250.

Mark H, Rydevik B (2005) Torsional stiffness in healing fractures: influence of ossification: an experimental study in rats. Acta Orthop **76**: 428-433.

McCann RM, Colleary G, Geddis C, Clarke SA, Jordan GR, Dickson GR, Marsh D (2008) Effect of osteoporosis on bone mineral density and fracture repair in a rat femoral fracture model. J Orthop Res **26**: 384-393.

Morgan EF, Gleason RE, Hayward LN, Leong PL, Palomares KT (2008) Mechanotransduction and fracture repair. J Bone Joint Surg Am **90 Suppl 1**: 25-30.

Pacicca DM, Moore DC, Ehrlich MG (2002) Physiologic weight-bearing and consolidation of new bone in a rat model of distraction osteogenesis. J Pediatr Orthop **22**: 652-659.

Radomisli TE, Moore DC, Barrach HJ, Keeping HS, Ehrlich MG (2001) Weight-bearing alters the expression of collagen types I and II, BMP 2/4 and osteocalcin in the early stages of distraction osteogenesis. J Orthop Res **19**: 1049-1056.

Richards M, Huibregtse BA, Caplan AI, Goulet JA, Goldstein SA (1999) Marrow-derived progenitor cell injections enhance new bone formation during distraction. J Orthop Res **17**: 900-908.

Sato M, Yasui N, Nakase T, Kawahata H, Sugimoto M, Hirota S, Kitamura Y, Nomura S, Ochi T (1998) Expression of bone matrix proteins mRNA during distraction osteogenesis. J Bone Miner Res **13**: 1221-1231.

Sato M, Ochi T, Nakase T, Hirota S, Kitamura Y, Nomura S, Yasui N (1999) Mechanical tension-stress induces expression of bone morphogenetic protein (BMP)-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. J Bone Miner Res **14**: 1084-1095.

Seebach C, Skripitz R, Andreassen TT, Aspenberg P (2004) Intermittent parathyroid hormone (1-34) enhances mechanical strength and density of new bone after distraction osteogenesis in rats. J Orthop Res **22**: 472-478.

Smith-Adaline EA, Volkman SK, Ignelzi MA Jr, Slade J, Platte S, Goldstein SA (2004) Mechanical environment alters tissue formation patterns during fracture repair. J Orthop Res **22**: 1079-1085.

Sojo K, Sawaki Y, Hattori H, Mizutani H, Ueda M (2005) Immunohistochemical study of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2, -4 (BMP-2, -4) on lengthened rat femurs. J Craniomaxillofac Surg **33**: 238-245.

Strube P, Mehta M, Putzier M, Matziolis G, Perka C, Duda GN (2008) A new device to control mechanical environment in bone defect healing in rats. J Biomech **41**: 2696-2702.

Wehner T, Wolfram U, Henzler T, Niemeyer F, Claes L, Simon U (2010) Internal forces and moments in the femur of the rat during gait. J Biomech **43**: 2473-2479.

Willie B, Adkins K, Zheng X, Simon U, Claes L (2009) Mechanical characterization of external fixator stiffness for a rat femoral fracture model. J Orthop Res **27**: 687-693.

Yasui N, Sato M, Ochi T, Kimura T, Kawahata H, Kitamura Y, Nomura S (1997) Three modes of ossification during distraction osteogenesis in the rat. J Bone Joint Surg Br **79**: 824-830.

Discussion with Reviewers

Reviewer I: The developed fixator had an axial stiffness range between 25-30 N/mm. Previous studies have used much larger fixator stiffness ranges, such as Claes *et al.* (10 *versus* 74 N/mm) and Strube *et al.* (between 41 and 7 N/mm). The fact that the authors' fixator covered only a small axial stiffness range may be a limitation for future dynamisation studies. Does the fixator design allow for an easy adaptation, so that stiffness ranges similar to these previous studies could be covered?
Authors: Yes, it is a straightforward matter to expand the range of stiffnesses by altering the thicknesses of the connection elements. In principle, any relevant stiffness could be obtained in this way.

Reviewer II: It is known that bones are subjected to complex and varying loads during different activities, and based on the result of experiments with different fixator designs in sheep the influence of compressive and shear loading on fracture healing has been discussed controversially. You have tested your implants only in bending (connection bars only) and axial compression (bone/implant constructs). Can you comment on the effect of the different fixator stiffnesses on shear loading with your model, which could be caused by torsional loads acting on the bone during gait?
Authors: We have no data on torsional loading with these fixators. However, using a different type of external fixation system it was found that a difference in torsional loading was only seen when the difference of axial stiffness was at least 60 % (VG, unpublished data). In the work presented here, a 60 % difference in axial stiffness was seen between ExFixHigh and ExFixLow, so there might be difference in torsional loading with these 2 fixators.