Video Article

Adjustable Stiffness, External Fixator for the Rat Femur Osteotomy and Segmental Bone Defect Models

Vaida Glatt[1], Romano Matthys[2]

[1]Institute of Health and Biomedical Innovation, Queensland University of Technology

[2]RISystem AG

Correspondence to: Vaida Glatt at vaida.glatt@qut.edu.au

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Abstract

The mechanical environment around the healing of broken bone is very important as it determines the way the fracture will heal. Over the past decade there has been great clinical interest in improving bone healing by altering the mechanical environment through the fixation stability around the lesion. One constraint of preclinical animal research in this area is the lack of experimental control over the local mechanical environment within a large segmental defect as well as osteotomies as they heal. In this paper we report on the design and use of an external fixator to study the healing of large segmental bone defects or osteotomies. This device not only allows for controlled axial stiffness on the bone lesion as it heals, but it also enables the change of stiffness during the healing process *in vivo*. The conducted experiments have shown that the fixators were able to maintain a 5 mm femoral defect gap in rats *in vivo* during unrestricted cage activity for at least 8 weeks. Likewise, we observed no distortion or infections, including pin infections during the entire healing period. These results demonstrate that our newly developed external fixator was able to achieve reproducible and standardized stabilization, and the alteration of the mechanical environment of *in vivo* rat large bone defects and various size osteotomies. This confirms that the external fixation device is well suited for preclinical research investigations using a rat model in the field of bone regeneration and repair.

Video Link

The video component of this article can be found at https://www.jove.com/video/51558/

Introduction

A number of studies have improved our understanding of the biologic mechanisms involved in bone tissue repair[1-6]. The effects of mechanical conditions on bone repair such as axial, shear and interfragmentary movements (IFMs) have been studied extensively[7-15]. In the past several years, more and more studies started to emerge describing the influence of mechanical environment on bone healing using fracture, osteotomy and large segmental bone defect *in vivo* models. Therefore, reliable fixation methods are needed to get reproducible and reliable study outcomes.

The mechanical environment around the healing fracture is very important as it determines the way the fracture will heal. Thus, the choice of fixation device is very important and should be carefully selected depending on the study design, and other factors such as gap size and the type of fracture. The fixation device's mechanical properties are even more important when studying the bony healing of large bone defects to establish a fixation that provides not only a constant gap size throughout the experiment period of full weight bearing, but also an ideal mechanical environment for the healing bone. External fixators are commonly used in fracture and large bone defect experimental healing models because they have an advantage over other fixation devices. The main advantage of external fixators are that they allow for the change of the mechanical environment at the defect site *in vivo* without a secondary intervention, which can be achieved by changing or adjusting the stability bar of the device during the course of the experiment as the bone healing progresses. Moreover, it permits the application of specific local mechanical stimulation to enhance the repair of bone, and also provides the potential to measure the stiffness of callus tissue *in vivo*. Nevertheless, the devices also have a few disadvantages that include: irritation of soft tissue, infections and pin breakage.

Unfortunately, such implants were not available "off the shelf" at the time of the implant development, and investigators were forced to custom design their own fixators for an intended use. Therefore, one constraint of research in this area was the lack of experimental control over the local mechanical environment within a large segmental defect as well as osteotomies as it heals. 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 the distance from the bone surface to the fixator bar (offset). Surprisingly, only a paucity of studies could be found that have investigated the mechanical contributions of the individual components of fixators or whole frame configurations used in rodent studies[16,18,28]. For example, one study's results showed that one of the main contributing factors in determining the total stiffness of the fixation construct was dominated by the flexibility of the pins in relation to their offset, diameter and material properties[28]. The results from the aforementioned studies clearly suggest that knowing the

mechanical environment provided by the fixation device is extremely important, and yet, in many cases is not investigated in detail. The present paper reports the design, specifications, and *in vivo* implantation of an external fixator that addresses this issue. This fixator also allows for the modulation of the mechanical environment as healing progresses, a property that enables the study of the mechano-sensitivity of different stages of the healing process *in vivo*. Additionally, as well as imposing a controlled and reproducible local mechanical environment, its accessibility also allows for the modulation of this environment at different stages of bone healing.

The fixator we designed was based upon external fixation, which is widely used for fracture fixation[16-21] and large defect models in experimental animals[22-27]. The difference between our external fixator and the other existing designs reported in the literature is that their stability bar is secured with screws to have a tight grip with Kirschner wires (K-wires). This type of design requires screws to be retightened biweekly (sometimes even weekly) to make sure that the distance of the offset is maintained as the loading is applied through weight bearing to prevent the loosening of the stability bar. If such loosening occurs, it allows for unwanted additional loading conditions such as angular, transverse and torsional shear movements to the healing bone *(based on personal experience, communication with researchers)*. Knowing this, an external fixator was designed as such that when the stiffness of the fixator needs to be changed, it would be achieved by removing connection elements attached to the main module where the mounting pins are imbedded. The *in vivo* pilot experiment was performed with the new external fixator prototype to make sure that it meets all proposed demands before it is manufactured in larger quantities.

The main aim for this paper is to present a new surgical method for an external fixator used for large bone defects and osteotomies in the rat with the ability to change stiffness *in vivo* during the healing process. This fixation method is applied *in vivo* on the femora of rats.

Protocol

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, Boston, MA. (Protocol Number: 098-2009)

1. Preparation of Surgical Materials and Instruments

1. Sterilize all surgical materials and instruments used to perform surgery prior to use. Pack needed materials, with or without an instrument tray, inside a folded cloth or wrapped paper and seal with autoclave tape for steam sterilization. The temperature of the autoclave should be at 125-135 ºC for 20-25 min of sterilization time, and then 10-15 min of drying time.
2. Make sure that at the time of surgery the rats are 200-250 g. This is very important because if the rats are heavier in size, then a different sized fixator should be used. For rats heavier than 250 g a larger version of the external fixator system should be used.

2. Surgical Procedure and Application of the External Fixator

1. Purchase Sprague-Dawley (or any other strain) rats (male or female, 200-250 g) from any certified animal supplier. Follow the appropriate animal care and experimental protocols in accordance with national guidelines that is approved by the investigator's Institutional Animal Care and Use Committee. Allow a minimum of 48 hr acclimatization period before the procedure.
2. For surgery, transport the rat to a dedicated surgical procedure room.
3. Anesthetize the rat with isoflurane first via induction chamber, and then continue with face mask connected to an anesthesia machine at a rate of 1.5-2% in 1-1.5 L of O_2/min. At the start of surgery make sure the animal is under deep anesthesia. To do this, use pedal reflex technique by extend the limb and pinching the web between the toes with the fingers (**not the toe itself!**). The animal is not sufficiently anesthetized if the limb is withdrawn, muscle twitch occurs or if the animal makes noise.
4. After the rat is under deep general anesthesia for surgery, inject the antibiotic (cefazolin, 20 mg/kg) and the analgesic buprenorphine (dose 0.08 mg/kg) intramuscularly in the right leg. To avoid large amount of fluid loss during the surgery administer warm sterile saline subcutaneously at 3-5% of the body weight prior to the surgery, and if it's necessary at the end. Apply sterile eye ointment to the eyes to keep them hydrated to prevent corneal injury.
5. Following the drug injections, shave and clean the entire right hind leg of the rat with chlorohezadine or other disinfecting solution and transfer the animal to the surgical table. (The leg operated upon must be the same as the one that was injected.)
6. Place the animal on a heated surface in the prone position (**Figure 1A**). Make sure the facemask remains on the nose and mouth after the transfer to the surgical table, and maintain the anesthesia regimen mentioned in 2.3). Drape the area of the surgical procedure with a sterile fenestrated drape so that only the leg intended for surgery is exposed.
7. Make an approximate incision of 3-4 cm (**Figure 1B**) through the skin running craniolateral on the surface of the right femur from the greater trochanter to the supracondylar region of the knee using a scalpel (**Figure 1C**). Expose the shaft of the femur by gentle dissection separating the fascia lata, and making sure that the muscle tissue is not cut. After that, separate apart the M. vastus lateralis and M. biceps femoris and lift the M. tensor fasciae latae to expose the full length of the femur (making sure that the sciatic nerve is preserved; **Figure 1D**).
8. In the planned area of osteotomy, prepare the femur along the midway area of the diaphysis by releasing the surrounding muscle tissue from the femur. First, start by putting the Henahan elevator perpendicular to the exposed surface of the femur, and then using a scalpel, release the muscle in the adjacent area.
    1. Continue by advancing forward and go around the femur, staying close to the bone surface, until all of the surrounding muscle tissue is released from the entire middle section of the bone (where the defect will be created), and the muscle tissue is completely cleaned from the bone. While doing this, it is very important to stay close to the bone surface to avoid cutting any major vessels.
9. For a 5 mm large bone defect, loop 2 pieces of Gigli wire saw (0.22 mm) around the bone in medio-lateral orientation (**Figure 1E,F**). After looping the wire saw, position one piece on the distal side of the femur, close to the knee joint, and a second piece on the proximal side close to the hip joint. Clamp the Gigli wire saw pieces on each side using S-shape curved dissecting and ligature forceps, so that it stays in the intended place. If a single cut osteotomy is planned, then only use one piece of wire saw.
10. Use the external fixator plate as a template to determine the exact position of the implant. The position of the external fixator has to be as close as possible to the center of the femur.

1. Position the external fixator plate on the anterolateral surface of the bone. This is achieved by externally rotating the femur. In that position the soft tissue layer is at its thinnest, which prevents excessive soft tissue tension under the fixator plate after the wound is closed.
2. Then slightly lift the external fixator plate off the bone surface to make sure that the holes of the plate are centered to the bone surface. Hold the external fixator with a small clamp for it to stay parallel to the longitudinal axis of the bone, and then use a power tool or a hand drill to predrill the first hole on the proximal side of the femur with the 0.79 mm drill bit. Before advancing forward, make sure that the tip of the drill bit is still centered on the bone surface .
3. If the tip of the drill keeps slipping, use the 1.00 mm counter sinker (**Figure 8F**) to center the position of the first hole. The counter sinker should be used to position all remaining mounting pins. This will ensure a perfect alignment of the drill holes and the fixator plate relative to the bone surface.

3. External Fixator Implantation Method Using Saw Guide

1. Make sure that the plate of the external fixator is not mounted upside down before clipping it on the saw guide. Determine this by comparing the size of the holes on the plate. The correct side is with the larger hole's diameter facing up. If the difference between the size of the holes within the fixator is not apparent, use the counter sinker.
    1. Insert the tip of the counter sinker into one of the holes on the fixator plate, if the counter sinker easily fits into the hole then this is the upside of the fixator, however, if the tip of the counter sinker doesn't fit then this is a bottom side of the fixator, and has to be flipped for the implantation accordingly.
    (**Important:** Make sure to drill perpendicular to the longitudinal axis of the bone as this will ensure a perfect orientation of the fixator to the bone surface. The direction of the first drill hole determines the final orientation of the fixator on the bone. Remember the mounting pins are the same length, and if the fixator is not parallel to the longitudinal axis of the bone the distance between the fixator and the bone will vary too much and might prevent the ability of all four mounting pins to penetrate both cortices.)
    2. After the orientation is confirmed, clip the plate on the saw guide (**Figure 2A,B**) and then clip the unit on the bone so that the first predrilled hole is aligned with the first hole on the plate (**Figure 2C**). Use the 0.70 mm square box wrench inserted into the hand drill to drive the first mounting pin into the hole. Doing this will permit reproducible positioning for the remaining mounting pins.
    3. After the first mounting pin is in place, then drill the most distant hole from the first mounting pin on the distal side, and drive the second mounting pin into the hole. The implantation order of the two middle mounting pins is not important.

4. External Fixator Implantation Method Without the Saw Guide:

The application of the external fixator can be also performed without using the saw guide. The beginning steps of the external fixator implantation are the same up until the unit with the saw guide is clipped on the bone (step 3.1). If the saw guide is not used, it is very crucial to keep the fixator plate in the correct orientation during the entire application procedure. The femur needs to be externally rotated in the anterolateral direction.

1. Hold the external fixator plate with a small clamp or S-shape curved dissecting and ligature forceps so that it is parallel to the longitudinal axis of the bone (**Figure 3A**). The application of the first mounting pin will determine the alignment of the fixator, therefore, the rotation of bone has to be retained until the first pin is inserted (**Figure 3B**). After the first pin is in place, carefully use the forceps to hold the fixator plate that is acting as a drill guide.
2. Insert the drill bit into the second hole – this is the most distal hole to the planned osteotomy gap (**Figure 3C**). Before drilling, check to make sure that the second hole has the same orientation as the first hole; also make sure that after the drilling is complete, both cortices are penetrated.
3. Insert the 0.70 mm square box wrench to the hand drill and then insert the mounting pin into the tip. Carefully insert it into the plate of the external fixator without losing the alignment of the first predrilled hole.
4. As soon as the tip is in contact with the bone, start turning the wrench under continuous axial loading applied to the proximal end of the hand drill. After about 5 full turns, make sure that the thread at the proximal end of the mounting pin catches the external fixator plate's body. This thread locks the system. Stop turning when the end of the bone thread is close to the top surface of the bone (**Figure 3D**).
5. After the pins on the most distal and proximal side are in place, predrill the remaining two middle holes. The implantation order of the two middle pins is not important (**Figures 3C**).
6. After the external fixator is in place, use the 0.22 mm Gigli wire saw directed by the saw guide to make the segmental defect (**Figure 4A**). If the latter method is chosen, the saw guide is clipped before making a defect.
    1. For this, pass a 0.22 mm Gigli wire saw through the 2 grooves underneath the femur (**Figure 5A**) to create a 5 mm segmental defect by reciprocal motion back and forth (**Figure 5B**) using sufficient irrigation (use 5 ml syringe to dispense saline at the time of the defect creation). To avoid damage to the soft tissue, cut the saw wire close to the bone on one side after completing the osteotomy. Remove the saw guide (**Figure 4B**).

7. After the defect or osteotomy is created, remove the saw guide and close the wound in layers, the muscle first (**Figure 4C**), and then the skin (**Figure 4D**). Before the wound is closed, treat the defect as planned in the study protocol. Close the muscular layer and the fascia lata using Ethibond vicryl suture 4-0, and the skin using Ethicon Monocryl 3-0 suture. Avoid dragging suture material over non-sterile surfaces while suturing wounds. Note: To avoid wound biting, the suture must not end distal to the lower implant. Likewise, skin glue can be used instead of a suture.
8. On the first three postoperative days, give the rat analgesic every 12 hr and antibiotic every 24 hr. Of course, the post-operative regimen of drugs will vary depending upon the make and brand of the drugs used by each investigator (refer to drug specification instructions).
9. Monitor animals frequently after the procedure to make sure they recover from anesthesia and only then return them to the housing facility. Provide solitary housing for the first few days after the surgery to make sure that there are no complications.

10. Monitor water, food intake and body weight after the surgery to make sure the animal is not in pain and distress. If the animal shows a decreased activity level, difficulty moving around (possible implant failure), ataxia, unkempt greasy fur, porphyrin staining around eyes and nostrils, hunched posture, respiratory distress, reduced intake of food and water, etc. consult a veterinarian.

5. Change of External Fixator Stiffness *In vivo*

1. If the study protocol requires the change of fixator stiffness during the healing process *in vivo* this is achieved by changing the connection elements secured with the special interlocking screws using 0.5 mm square wrench box attached to the hand drill. For this procedure, anesthetize the rat (refer to the 2.3 in the protocol) and give analgesia (refer to the 2.4 in the protocol) only once at the time of the procedure (**Figure 6A**).
    1. Sedate the rat, and then insert the tip of the 0.50 mm square box wrench into the interlocking screw attached to the side of the assembled fixator, and start carefully turning it counter clockwise until the pin is half way out (**Figure 6B**). After this part is complete, repeat the procedure for the second pin that is on the same side of the external fixator plate (**Figure 6C**).
    2. When both pins on the same side are half way out, use forceps or a clamp to remove the connection element on the opposite side with a gentle motion (**Figure 6D**). The connection element should come off easy, if it doesn't, then do an additional couple of turns on both interlocking screws to make sure that the tip of the interlocking screw is not imbedded in the connection element.
    3. After the connection element is removed, slide the desired stiffness connection element in place of the removed one (**Figure 6E**), and from the opposite side using the square box wrench start turning until the interlocking screw is half way out on the opposite side (**Figure 6F**). Repeat the same procedure for the second interlocking screw (**Figure 6G**). Important: this will require switching to the opposite side of the plate to make sure that both interlocking screws are half way out on the side where the connection element was replaced **(Figure 6H,I)**.
    4. After this part is successfully completed, remove the second connection element (**Figure 6J**) and replace it with the same stiffness connection element as the one replaced on the opposite side (**Figure 6K**). After the second connection element is in place, start driving the interlocking screw until the interlocking screw end exits the opposite side of the plate, and the interlocking screw tip has exited the same amount on each side (**Figure 6L**). Repeat the same procedure for the second interlocking screw (**Figure 6M,N**). This procedure takes about 15 min to complete.

Representative Results

Design specifications

Stabilization of the rat femur with the external fixation system enables the creation of osteotomies from 0.5 to 5 mm. The external fixator system is a locked external fixator made of polyether ether ketone (PEEK - [the main body]) and titanium-aluminium-niobium alloy (TAN - [the mounting pins]), which offers a simple, reproducible and adjustable design, and is available in four different stiffnesses: 10, 40, 70 and 100% (100% being the standard, most rigid fixator (**Figure 7**) . Depending on each investigator's study requirements, whether they will have to do implant stiffness adjustment *in vivo* as the bone healing progresses, the external fixator plate comes either as one solid piece (**Figure 8**) or with two connection elements (**Figure 9A**) and two main modules (**Figure 9B**) secured with two interlocking screws (**Figure 9C**) that have to be assembled prior to surgery (**Figure 10A-F**). The connection elements are of different thickness, and hence stiffness, and were developed to achieve fixation stiffness equivalent to 10% (0.75 mm thick), 40% (1.70 mm thick), 70% (2.10 mm thick) and 100% (2.50 mm thick; **Figure 7**). The external fixator stiffness of 100% was calculated based upon the 200 g approximate body weight of a mature rat, and then multiplied by a factor of 4, to a mass equal of 800 g. This was done to make sure that after creating a 5 mm defect, the fixator is capable of withstanding the weight bearing of the animal, thereby maintaining alignment and preventing the dislocation of defect fragments. The remaining three fixator stiffnesses were decreased by 30% respectively from the highest (100%) to have a variety of stiffnesses for studies with various purposes.

Each main module has two holes where the Mounting Pins are inserted. The fixator stiffness can be changed while it is still attached to the living animal by changing the connection elements secured with special interlocking screws (**Figure 9C**) using 0.5 mm square wrench box (**Figure 9H**) attached to the hand drill (**Figure 9K**). TAN (Titanium alloy) was used to make for mounting pins (**Figure 9D**) to secure the stability bar to the femur (**Figure 7**). The fixator comes in four pieces and needs to be assembled prior to use if a stiffness change is intended for the study (**Figure 10A-F**), if not, a single solid pieced fixator should be used. 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 0.79 mm drill bit (**Figure 9E**). The screws are locked in corresponding holes in the main fixator frame, which is parallel to the bone surface and set at a distance of 6 mm from the bone (**Figure 7**).

A saw guide was developed to enable the creation of an accurate, reproducible, 5 mm segmental defect in the femur (**Figure 9I**); 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 **Figure 2B,C**. The 5 mm gap is generated with a 0.22 mm Gigli wire saw (**Figure 9J**). Both the saw guide and the Gigli wire saw can be autoclaved at 134 ºC. If a different sized osteotomy is intended for the study, a custom designed saw guide is available. Due to the miniature size of the external fixator, a special set of implantation instruments was designed and acquired; a customized 0.79 mm drill bit (**Figure 9E**), 1.00 mm counter sinker for the predrilling of the holes (**Figure 9F**), 0.7 mm square wrench box for application of the mounting pins attached to the hand drill (**Figure 9G**), 0.5 mm square wrench box for application of the interlocking screws (**Figure 9H**), hand drill (**Figure 9K**). An Accu Pen drill (**Figure 9L**) was also developed. The core diameter of each mounting pin is 0.02 mm bigger than the drill bit to guarantee proper fitting of the mounting pins 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-screw interface[29]. The drill bit (**Figure 9E**) is operated by a miniature electrical Accu Pen drill that produces 2,500 rpm with a power of 500 mW (**Figure 9L**).

In vivo experiments

Radiological examination confirmed that fixators of all stiffnesses maintained a 1 mm (not shown) or a 5 mm femoral defect during the entire 8 weeks of the experiment (**Figure 11**). This was especially important for the 5mm critical size defects, where spontaneous healing does not

occur. No distortion or infections, including pin infections, were observed and pin loosening was absent if the instructions of the application were followed[30]. A complication of using the external fixator was seen if the weight of the rat at the time of surgery has exceeded 250 g, and a smaller size plate was used. In some of those instances, the loading on the mounting pins increased to a critical level so that the pin pullouts were occurring on the distal side of the femur anywhere from one week to two weeks after the surgery (**Figure 12**). In addition to that, if a larger sized animal is used, the muscle tissue surrounding the femur is relatively thick, which creates skin tension in the vicinity of the implant after the skin closes. Due to swelling tension, when the skin starts to heal it creates an itching sensation to the animal making some of the rats bite the fixator. Since the fixator is created from PEEK material, which is basically high density plastic, on rare occasions, some rats were known to chew through it. Again, in order to avoid this, it is very important to select the recommended body weight for animal studies or switch to the larger version of external fixator.

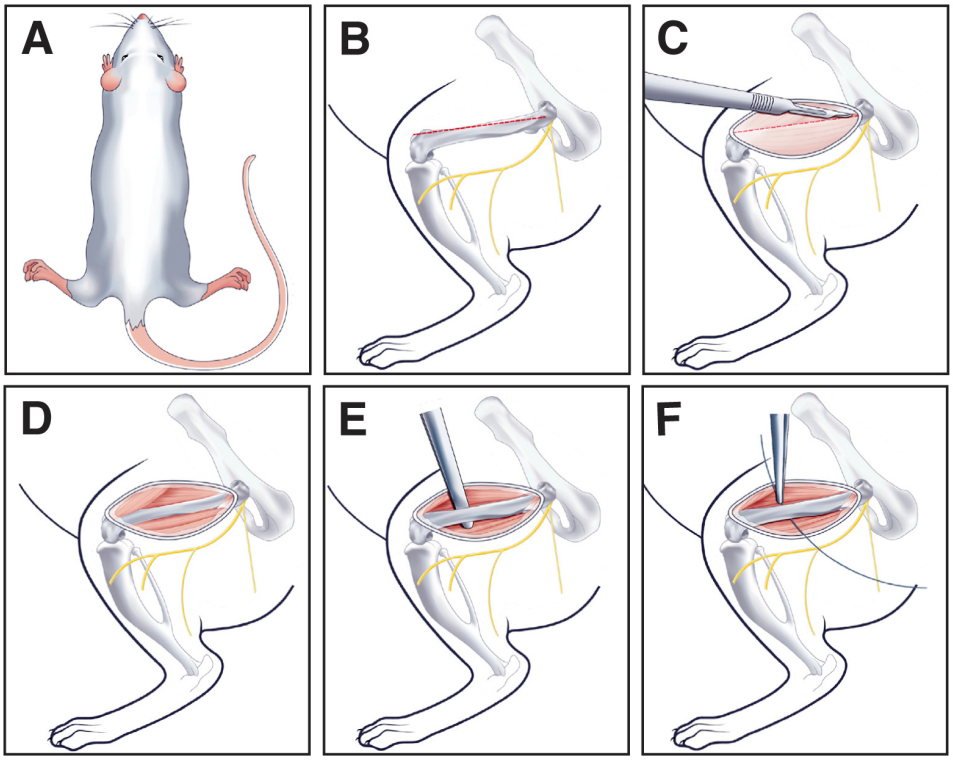

Figure 1. Surgical preparation of the rat femur. (A) Rat positioned in the prone position. **(B)** Shows the direction of the incision on the femur. **(C)** Shows incision made in the skin to expose muscle. **(D)** Shows incision made through the muscle to expose femur. **(E)** Shows a small clamp positioned under the bone to pass Gigli wire. **(F)** Shows Gigli wire passed underneath of bone.

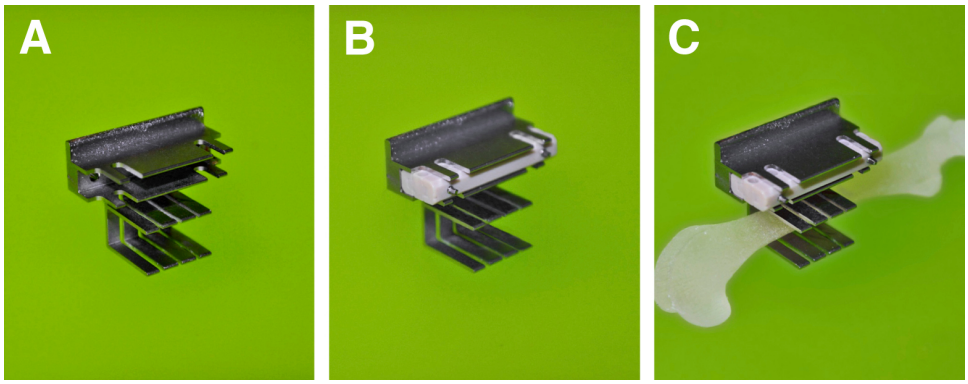

Figure 2. (A) Saw guide. **(B)** External fixator clipped on the Saw guide. **(C)** Saw guide with the external fixator clipped onto femur.

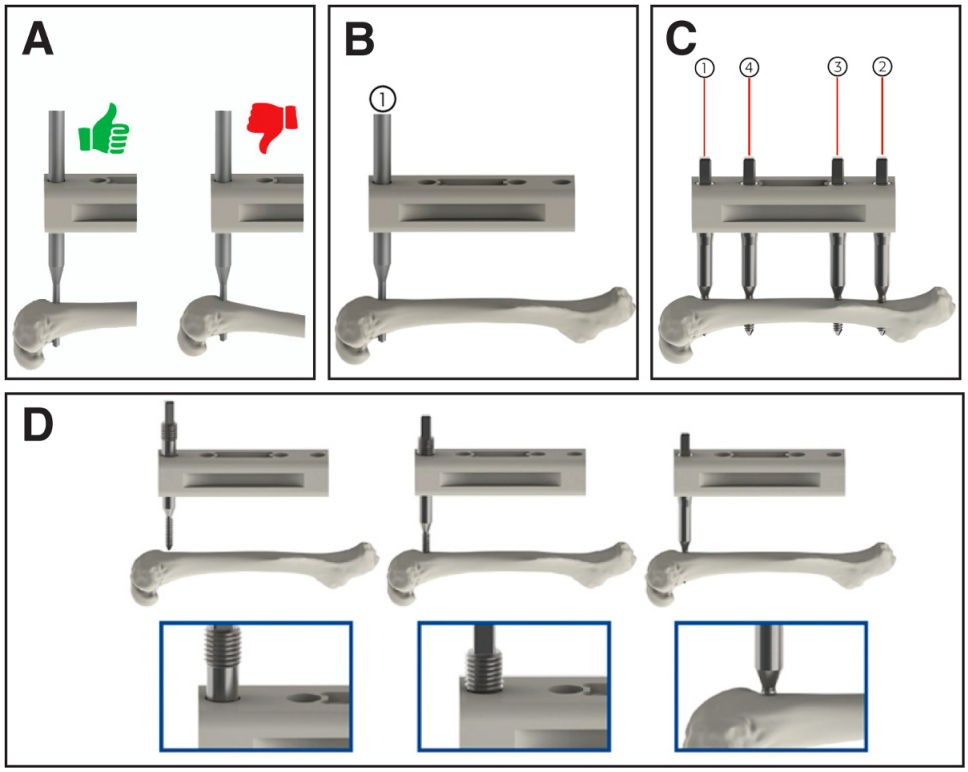

Figure 3. Application of external fixator. (A) Shows correct application of the first mounting pin with the plate reclining antero-laterally and parallel to the bone - green hand, and the incorrect application – red hand. **(B)** Shows insertion of the first Mounting Pin in the outer distal position. **(C)** Shows insertion of the remaining Mounting Pins starting with the most proximal position, followed by the two middle mounting pins. **(D)** Shows insertion of Mounting Pin – more detailed description in the protocol section 4.4.

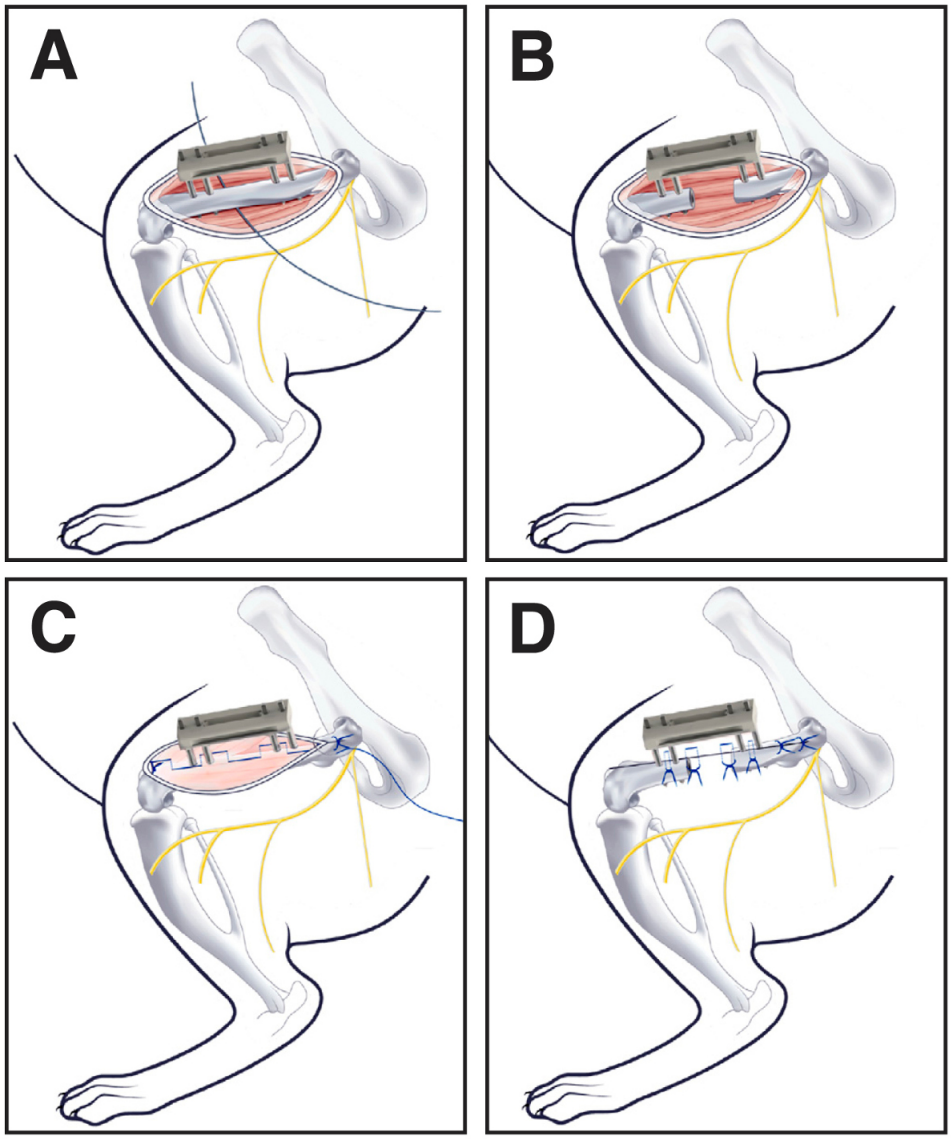

Figure 4. Surgical implantation of the external fixator on the rat femur. (A) Demonstrates completion of the surgical procedure with external fixator in place with the Gigli wire. **(B)** Demonstrates created 5 mm segmental defect. **(C)** Demonstrates sutured muscle layer with exposed external fixator stability bar. **(D)** Demonstrates sutured skin with exposed external fixator stability bar.

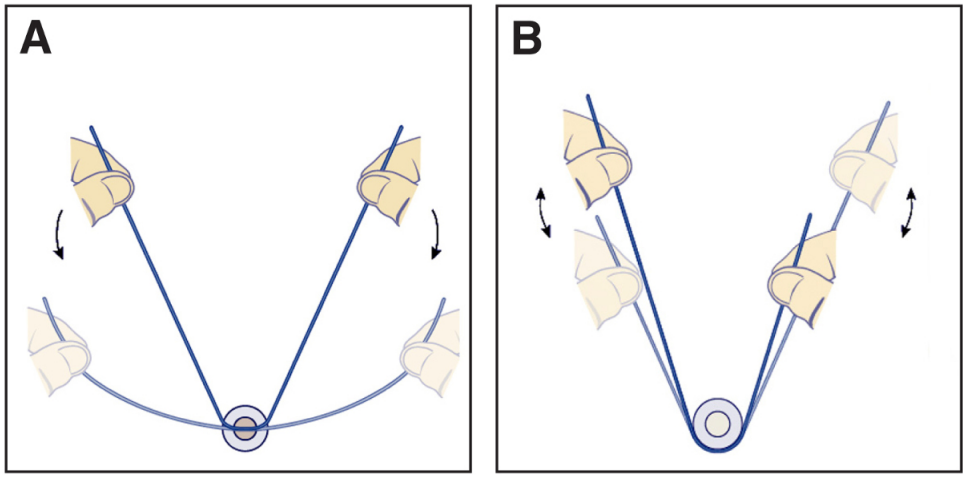

Figure 5. (A) Initial position of Gigli wire for defect creation. **(B)** An image showing reciprocal motion of Gigli wire.

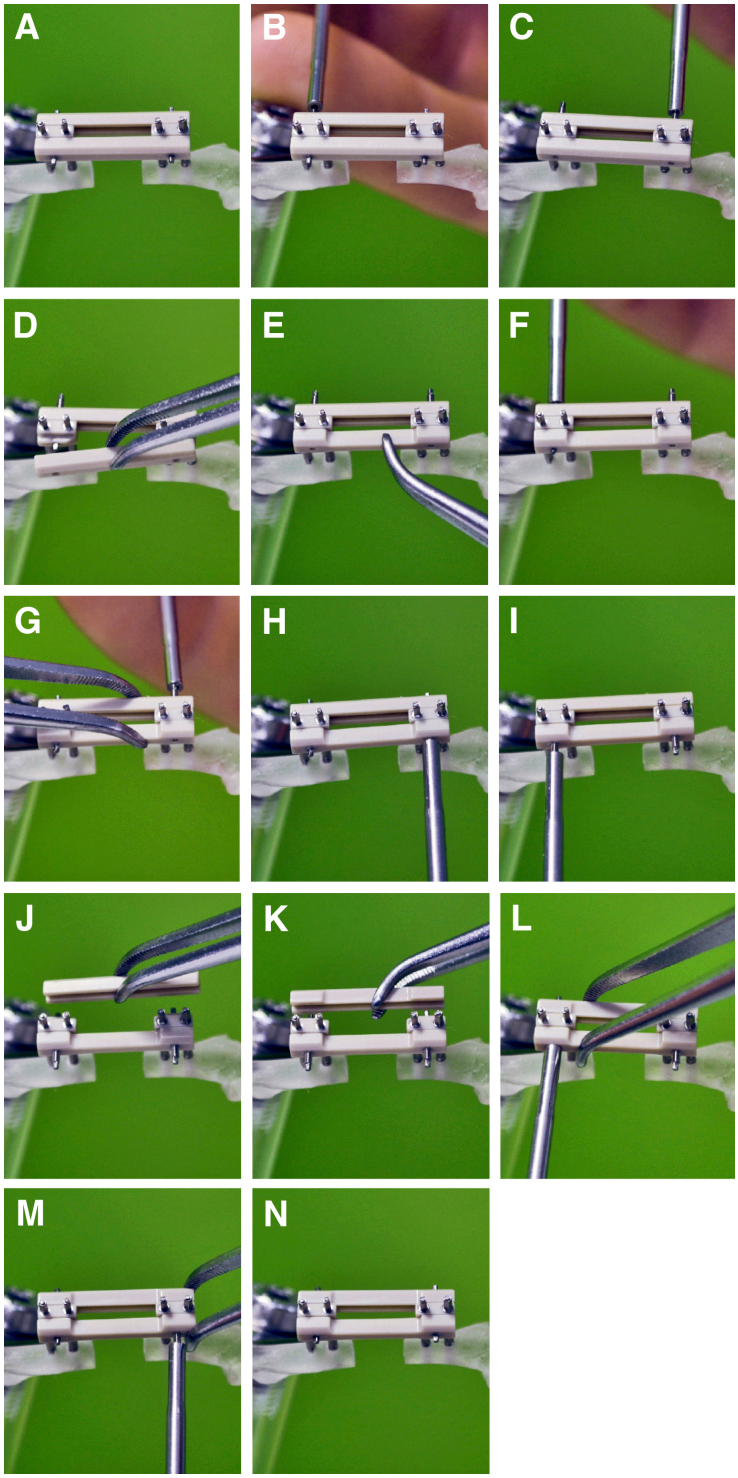

Figure 6. Change of External Fixator stiffness *in vivo.* **(A)** External fixator implanted on the femur. **(B)** Shows removal of the first interlocking screw by carefully turning it counter clockwise until the pin is half way out. **(C)** Shows removal of the second interlocking screw by carefully turning it counter clockwise until the pin is half way out. **(D)** Demonstrates removal of the connection element on the opposite side. **(E)** Demonstrates replacement of desired stiffness connection element in place of the removed one. **(F)** Demonstrates how to secure the first replaced connection element from the opposite side by turning the square box wrench until the interlocking screw is half way out of the opposite side. **(G)** Demonstrates how to secure the second replaced connection element from the opposite side by turning the square box wrench until the interlocking screw is half way out of the opposite side. **(H, I)** Demonstrates switching to the opposite side of the plate to make sure that both interlocking screws are half way out on the side where the connection element was replaced. **(J)** Demonstrates removal of the second connection element. **(K)** Demonstrates replacement of the second stiffness connection element in place of the removed one. **(L, M)** Demonstrates driving of both interlocking screws until the interlocking screw end exits the opposite side of the plate. **(N)** Demonstrates completed procedure.

Figure 7. Components of the external fixators. Left: Stiffness is determined by connection elements of different thicknesses. The fixator is attached to the bone with titanium alloy mounting pins. Right: Assembled fixator in place on rat femur with 5 mm segmental defect.

Figure 8. External fixator as a one unit.

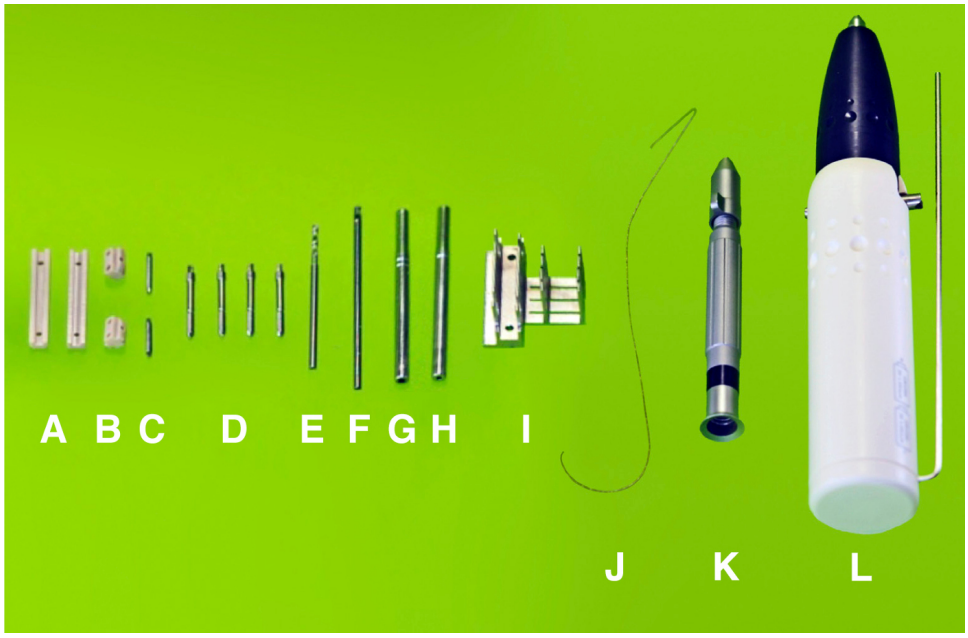

Figure 9. Parts and instruments designed for use with the external fixator. (A) Two connection elements. **(B)** Two main modules. **(C)** Two interlocking screws. **(D)** Four mounting pins. **(E)** A 0.79 mm drill bit. **(F)** A 1.00 mm counter sinker for the predrilling of the holes. **(G)** A 0.7 mm square box wrench for the application of mounting pins. **(H)** A 0.5 mm square box wrench for the application of interlocking screws. **(I)** A 5 mm saw guide. **(J)** A 0.22 mm Gigli wire saw for creation of defect. **(K)** Hand drill for the attachment of drill bits, 0.70 and 0.50 mm square box wrench. **(L)** AccuPen 6V+ (Miniature electrical pen drill) used to drive the drill bits.

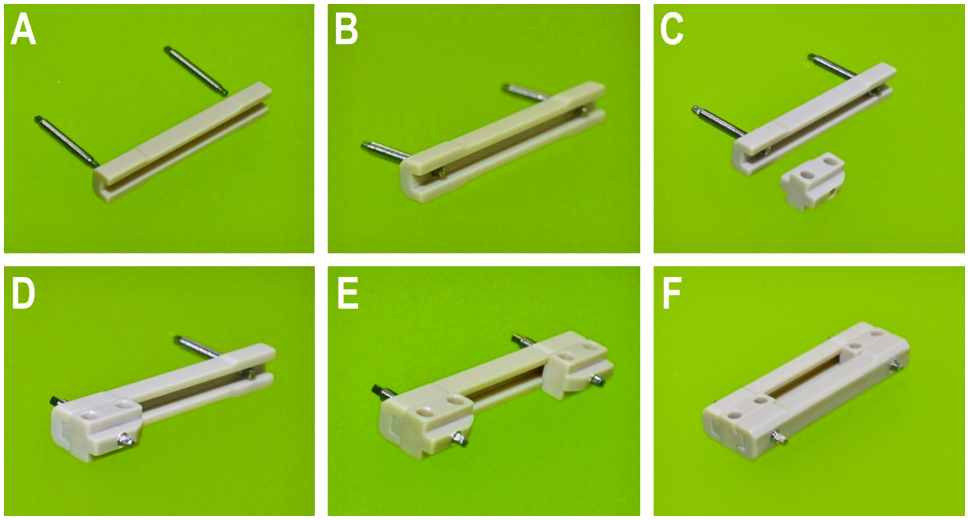

Figure 10. Assembly of the external fixator. (A) 70% stiffness connection element. **(B)** The connection element and one of the main modules. **(C)** demonstrates how one of the main modules slides inside of the connection element. **(D)** Demonstrates how both of the main modules slide inside of the connection element. **(E)** Demonstrates both of the main modules and both connection elements in place. **(F)** demonstrates fully assembled stability bar – main modules and connection elements secured with interlocking screws.

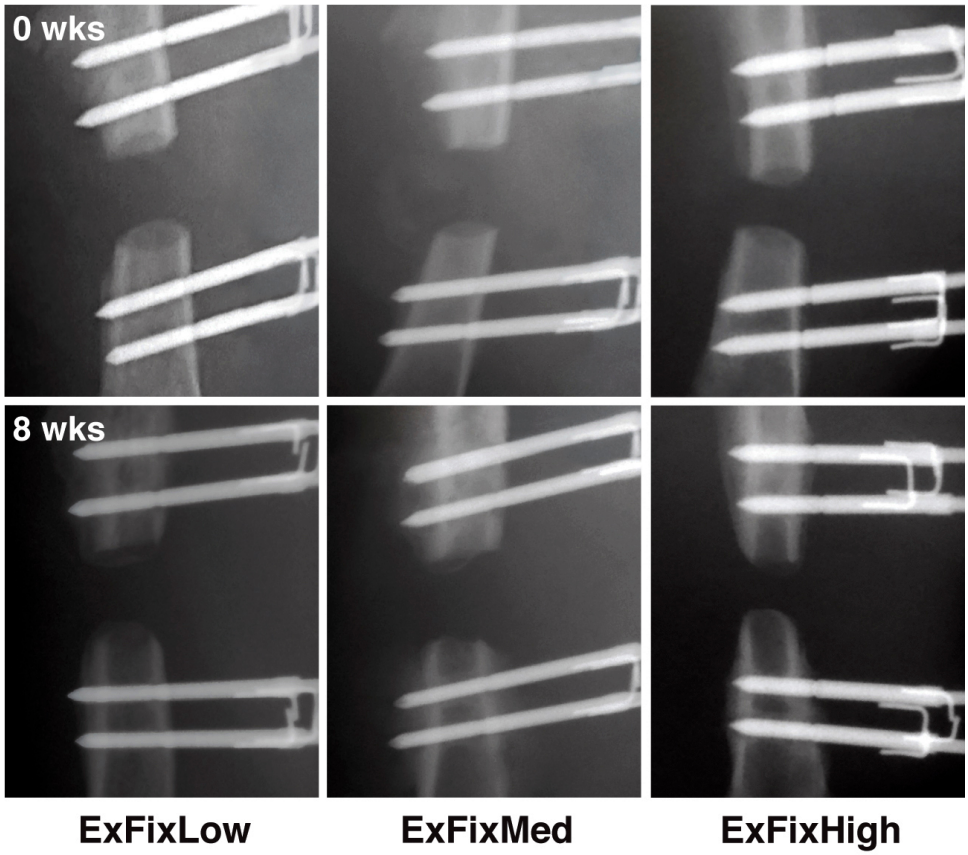

Figure 11. *In vivo* X-ray images of defects in rats immediately post-surgery and 8 weeks later. External fixators of all 3 stiffnesses were surgically implanted on rat femora and 5 mm segmental defects created. The defects were X-rayed immediately after surgery (t = 0) and at weekly intervals until 8 weeks (t = 8 weeks) when the experiment was terminated. Reproduced with kind permission from eCM journal (http://www.ecmjournal.org). Please click here to view a larger version of this figure.

Figure 12. *In vivo* X-ray image of the defect in rat 9 days post-surgery with the distal pins pulled out (at the time of the surgery the body weight of the rat was 340 g). Please click here to view a larger version of this figure.

Discussion

The most critical steps of a surgical procedure to create a large bone defect are: 1) choosing the appropriate body weight of the rat to match the size of the external fixator; 2) maintaining a sterile environment during the procedure; and 3) following the surgical procedure protocol.

The main goals of this study were to design, manufacture and characterize a new, variable stiffness external fixator for the rat femoral large defect model, and to use this fixator in determining the interplay between biological and mechanical factors during the healing process. The mechanical properties of the new fixators were examined at three levels and the characterization of the fixators is published in a different manuscript[30]. The fixators were also applied to rat femora and their *in vivo* performance monitored radiographically for 8 weeks with and without the treatment[30,31].

The primary innovation of this fixator is its ability to exchange the stability bar connection elements to select different, standardized stiffnesses. 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 as described in the protocol. Currently, four different stiffnesses are available, but additional stiffnesses can be achieved simply by ordering different connection elements of different thicknesses through the producer of the implant system.

The 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 a reduced 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 pre-set 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[1,18,26,27], is that the new external fixator was designed to have a minimal mass (0.32 g) to avoid uncontrolled loading due to inertia. Furthermore, after the implantation and suturing of the skin, the clearance between the implant cross bar and the skin is only about 2 mm. Such close proximity to the skin surface minimizes 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 large or small osteotomies. 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[22,26,27]. To avoid all these problems we took an advantage of the Gigli wire saw of 0.22 mm. The saw guide was developed for reproducibly creating precise defects with parallel ends.

There are a few limitations when using this technique. One of the main concerns when using this external fixator is the possibility mentioned in the results section, that the rats might chew through the external fixator plate, which is made of PEEK. However, a special metal cover has recently been developed by the producer of the fixator to prevent this from happening. Likewise, an Elizabethan Collar can be used for the first couple of weeks after surgery to prevent the animal from chewing. An additional concern is that if an empty bone defect is used for the study, there is a chance that the mounting pins can pull out from the bone several weeks after the surgery. Furthermore, it is crucial that the fixator is implanted in the exact orientation that is outlined in the protocol. If the instructions are not carefully followed, there is a great risk that the mechanical environment provided by the specific stiffness fixator will not be as was intended, and will introduce an error, giving false results.

The fixators described in this paper enable investigators to undertake the experiments that are necessary to determine empirically the effects of various mechanical environments and/or mechanical (stiffness) modulation on bone healing in large defects or osteotomies[30,31]. Furthermore, the external fixator technology can be used in various studies where different pharmaceuticals and biomaterials are tested to discover new therapies not only for complex fractures, but also for the treatment of standard fractures in order to accelerate the healing process.

Disclosures

The author Romano Matthys is an employee of RISystem AG Davos, Switzerland that produces the implants, implant specific instruments & consumables used in this article. The author Vaida Glatt has no competing financial interests.

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