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BIOMECHANICAL ASSESSMENT OF FRACTURE HEALING IN A PIGEON ULNA

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ABSTRACT

A study was conducted on pigeon to evaluate the biomechanical assessment of fracture healing in ulna model. Four adult birds were included in this study, each pigeon was anesthetized with Isoflurane anesthesia. A simple fracture was created at the mid-point of ulna (left) and fixed with four external skeleton fixations (ESF) pins. After 12 weeks fracture healing, all birds were sacrificed for bones biomechanical testing. On post-mortem examination, all healed ulna bones showed complete clinical union on palpation. Each ulna bones were placed on fixture machine with two supports. Three-point bending (flexural force) was applied vertically at mid-point of ulna bone. The flexure stress at break (MPa) of right intact (normal) and left-healed ulna fracture data were compared. There was no any significant difference in three-point bending force (p>0.064) between left and right ulna breaking. The results of this experiment indicate that ESF pins provided good bending strength at 12 weeks of ulna fracture healing. The ESF technique seems to be suitable for fracture stabilization in pigeons.

Keywords: Bone, biomechanics, external skeletal fixation, fracture, pigeon.

INTRODUCTION

Fracture fixation in birds is important for survival of injured birds domestic as well as wild (Tunio *et al*., 2014). External skeletal fixation (ESF) pins provide mechanical support for fracture healing in birds (Bennett, 1997). In veterinary practice, a large number of fractured birds need mechanical stabilization to restore the skeletal function quickly (Redig and Cruz, 2008).While, biomechanical examination of healed fracture is an important technique for assessment of fracture healing (Koivukangas, 2002). Yet biomechanical assessment of healed fracture in avian is not fully studied, where mechanical assessment is done by manual palpation. Several studies have been conducted to assess biomechanical efficacy of different devices used for fracture treatment in human medicine and small animal surgery (Aron *et al.*, 1991; Kroese-Deutman, 2009). Meanwhile,

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very limited information is available about biomechanical assessment of fracture healing in bird model. In addition, external skeletal fixation (ESF) pins are the best choice for fracture fixation technique in birds, because ESF pins along with acrylic column fixation have ability to resist the strength, torsion and compression (Redig, 2000; Sanaei *et al*., 2011). Furthermore, biomechanical study is needed to assess the usefulness of fracture stabilization technique with ESF in birds (Jalila *et al*., 2004). Various biomechanical testing methods are used, such as torsion, tension, compression and bending test are performed on animal models (An *et al*., 1999; Koivukangas, 2002; Kroese-Deutman, 2009). Three point bending test is mostly used procedure on experimental animals using an Instron machine (Kokubo *et al*., 1982). The specimens are tested by flexural and fixator force at mid-point till failure. It is used to find out the strength of a healed bone and has proved useful in animal models, such as rat (Molster *et al*., 1983) and mouse (Hiltunen *et al*., 1993). The use of three point bending test had been developed on the bones of laboratory animals (Koivukangas, 2002), but this is a first time study to evaluate the biomechanical assessment of the healing property of ulna fracture in bird model and fixed with ESF pins. Hence, it could be questioned that, will ESF provide mechanical support for fracture healing in birds and why ESF pins are used for fracture fixation in birds. We hypothesized that ESF type would provide mechanical support for fracture healing in the pigeon ulna model. Therefore, the objective of this study was to determine the three point-bending strength of healed ulna fracture and stabilized with ESF pins in a pigeon.

MATERIALS AND METHODS

Domestic pigeon (*Columba livia*) was selected as a bird model. Four adult healthy pigeons with 291.25±8.53g average body weight were used in this experiment. This study was approved by Animal Care and Use Committee of Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM/FPV/10 R118/Feb 2011-Jan 2012). Birds were kept at Animal house of Faculty of Veterinary Medicine, Universiti Putra Malaysia. All birds were off fed six hours prior to surgery and the operation was performed under isoflurane anesthesia at 5% with face mask and mixed with oxygen (1-1.5L/min). At that time birds were maintained at 1.5-2.5% isoflurane anesthesia with oxygen (1-1.5L/min) using modified Jackson Rees (MJR) non-rebreathing anaesthesia circuit system. The left ulna was scrubbed using chlorhexidene antiseptic and Povidone solution. The pigeons were placed on sternal recumbenc, 2-cm long incision was given on the skin, muscles and fascia were incised (Doneley, 2011). At the point, a transverse fracture was created at the mid-shaft of the left ulna. Mid shaft transverse fracture was created using ESF pins and fixed with external skeleton fixation (ESF) Type-1, size 0.045″ (Imex, Veterinary Inc., TX, USA). The Safil-violet 5/0 was used to close the fascia, muscles and skin. After that latex Penrose tubing size (3/8″ wide) was used on the top of the all ESF pins parallel to and above the ulna bone. Penrose tubing was filled with a mixture of acrylic material at 10 ml acrylic liquid and 5 ml acrylic powder (Redig, 2001; Sanaei *et al.*, 2011). When the acrylic material had dried completely, the ESF pins over the column were cut using a pin cutter. Torbugesic 2mg (Fort Dodge Animal Health, USA) was injected subcutaneously once only for post-operative analgesia. Terramycin ointment was applied on the incision and the ESF pin site. The wound was covered with woven gauze sponge to prevent any infection and wing was wrapped with a figure-of-eight bandage.

Specimen collection

After 12 weeks post-fracture healing, all birds were sacrificed by intravenous administration of Pentobarbital (182.0 mg, Lure Cedex, France) for biomechanical testing. After post-mortem examination, all healed ulna bones showed complete clinical union on palpation and specimens were tested for biomechanical examination. To compare with healed bone normal intact ulna bones of right side ulna were collected for negative control. The specimens were labeled and kept in sterile sealed plastic bags, separately. All bones were frozen at -19 °C (Kraus *et al*., 1998; Jalila, 2002; Savaridas *et al*., 2012).

Three-point bending test procedure

Specimens were un-freezed overnight in an ice cooler at room temperature and moistened in saline immersed gauze (Wander *et al.,* 2000; Jalila, 2002; Nam *et al*., 2004). Mechanical stable specimens were tested with three point bending (Van-Wettere *et al*., 2009) and this test was initially conducted on non-experimental normal ulna bone of pigeons, when this method had satisfied all criteria for this test. This test was performed at the strength of material laboratory, Faculty of Engineering, Universiti Putra Malaysia. The specimens were tested using Instron machine (Model 3365) 5kN capacity (Instron®, Corp, USA) associated with three-point bending flexure fixator and fixed in frames series 3300 (Figure 1). Three-point bending test of 4 pigeons left treated with ESF and four ulna not treated considered as a negative control were used to find out the maximum flexure load (N), flexure stress (strength) at maximum flexure load (MPa), flexure stress at break (Standard) MPa, and flexure strain at break (Standard) mm/mm, as per study protocol (Shuid *et al.,* 2010). This test was adopted according to the technique used by Van Wettere *et al.* (2009) in birds, Savaridas *et al.* (2012) in rats, and Koivukangas, (2002) in mice. The tubular ulna bone of 5.5 cm length and 0.2 cm wide, was mounted horizontally anteroposterior position on fixture with two supports. The three point bending (flexural force) test was applied vertically at mid -point of diaphysis of the operated left ulna at site of healed fracture with the span length of 4.5 mm and each specimen was subjected to three-point bending test at a loading speed of 0.15500 mm/sec until the specimen was broken (Kokubo *et al*., 1982; Savaridas *et al*., 2012). Bone was unloaded and examined for fracture location. Subsequently, untreated right ulna was tested for negative control (Koivukangas, 2002) to compare with left treated ulna. During performing the bending test bone failure data were generated in the computer system connected with Bluehill®, material testing software package for universal testing systems v: 2.8 (Instron®). Following three-point bending test properties of fracture healing were calculated such as: (1)

Maximum flexure load (N), (2) Flexure stress(strength) at maximum flexure load (MPa), (3) Flexure stress at break (Standard) MPa, and (4) Flexure strain at break (Standard) mm/mm, parameters were compared with treated left ulna with intact right ulna in pigeon model.

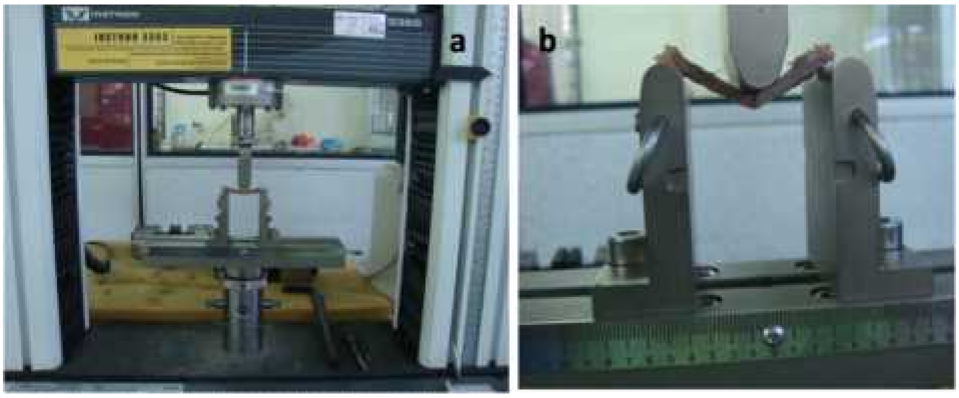

Figure 1.(a) Photograph showing Instron machine used for flexural testing (b) Three-point bending test and specimen bended at mid-point of ulna.

Statistical analysis

Data obtained from flexure force applied for testing three-point bending test on treated left side and untreated intact-right ulna was compared. Statistical analyses were performed using SPSS v20 (IBM. Inc., USA). The $P< 0.05$ values were considered significant.

RESULTS AND DISCUSSION

There was no any complication observed in birds used in this study and no any birds died post-operatively. However, all birds increased their body weight during the period of three months study. All pigeon used in this experiment had tolerated the load of external skeletal fixation pins well and half-pins provided excellent mechanical support for fracture healing. All birds were sacrificed at 12 weeks post-surgery for biomechanical evaluation. The ulna bones were palpated after postmortem and all bones found with complete clinical union at 12 weeks and all birds were moving operated wing nicely. In results, all experimental birds showed (100%) complete healing and healed ulna bones were not movable. This fracture healing showed that ESF pins along with acrylic column fixation had mechanically supported in bird fracture healing. There were notable clinical union and good bone alignment and no pin site infection also seen. The results of the various parameters showed that mean maximum flexure load (N), and mean flexure stress at maximum flexure load (MPa) were significantly higher in right intact ulna as compared to the left-treated ulna in both parameters. Furthermore, the mean values of flexure stress at break (MPa) of right intact and left-treated ulna when compared statistically were not significant at $p> 0.064$. In addition, there were no significant differences at *p*> 0.312 seen in the means of flexure strain at break (mm/mm) with left- treated and right intact ulna in pigeon (Table 1). When three point bending force applied the specimen broken at mid-point at bone union upon flexure force. When tested bending force on normal intact ulna breakdown of bone occurred at higher load as compared to left operated ulna stabilized with ESF pins. The force was significantly higher in normal intact ulna than treated left ulna. The aim of this fracture study was to evaluate biomechanical properties healing of ulna fracture and to restrict the movement of broken bone during healing time, while ulna fractures were stabilized with ESF (Imex, Inc, USA) fixator type-1. In birds, fracture usually occurs due to trauma during flying and hitting to windows where radius and ulna are frequently broken (Hatt, 2008) thus, open and comminuted fractures happened (Bennett and Kuzma, 1992) and such fracture can be diagnosed by manual palpation of the wings (Hatt, 2008). The important aspect of fracture healing is return bone integrity with mechanical properties (Chao and Aro, 1991). Therefore, successful rigid stabilization of bird fracture is important to restore in this study skeletal function (Redig, 2000). However, in this study three point bending test was conducted according to Marralli *et al*. (2013). As it is the standard procedure and this test was carried out using a universal testing instron machine. However, bending test has been proved a sensitive test for mechanical properties of fracture healing in many animal models (Molster *et al*., 1983; Hiltunen *et al*., 1993). Three point bending test was used in this study because this test provides helpful information for properties of fracture healing (Walsh *et al*., 1997). However, three point bending test was conducted as it is commonly used to confirm the bone healing. Therefore, following biomechanical parameter such as flexural strength is considered useful in determining the bending strength of fractured bones (Comelekoglu *et al*., 2007). Mechanical integrity of the skeletal system is important because it provides structural support for fracture healing (Reddy *et al*., 2001; Liebschner, 2004).

Similarly, maximum flexure load is commonly used parameter to determine compressive strength until a fracture happens on bone (Comelekoglu *et al*., 2007). However, mechanically bone fragility could be replaced by developing bone strength (flexure force and stress) and by reducing brittleness (flexure strain) at break of bone (Turner, 2002). In present study, there was significant increase in maximum flexure load (N) and flexure stress at maximum flexure load (MPa) in right intact ulna as compared to fractured healed left ulna, which was stabilized with ESF pins in same bird. This was also in agreement with Estai *et al*. (2012). They reported that control group had significant improvement for bone strength than treated with calcium group in rat study. It was found that mechanical stabilization of fractured bone is important in clinical cases (Fu *et al*., 2009). This shows that bone strength is the important determinant for restoration of bone integrity. Thus, stabilization of fracture with ESF had improved the bone strength and provided biomechanical effectiveness. However, in present study, there was no significant difference observed in the flexure stress at break (MPa) and flexure strain at break (mm/mm) in intact right and treated left ulna. This study finding is in agreement with results of Estai *et al*. (2012) where they described that strain is reciprocal to brittleness remained dependable in rat experiment. The results of this experiment revealed that right non-operated ulna have good bending characteristics than the left operated ulna healed fracture after 12 weeks of surgery. Milton, (1985) showed that transverse fracture presents bending and rotation force and comminuted fractures are prone to rotation, bending and compression force are the problems. Furthermore, another problem is that birds have thin and brittle cortex (Bennet and Kuzma, 1992), which has less holding power to hold the load of fixation devices (Olsen *et al.*, 2000). Whereas, special concern must be taken while device fixation (Bennet and Kuzma, 1992). Therefore, in present study an ESF half pin (Imex, Inc, USA) played an important role in providing superior bone holding strength and was well accepted by all birds (Altman *et al.,* 1997). This technique is useful and no adverse effects were observed on birds (Bennett and Kuzma, 1992). ESF supports faster fracture healing with minimal callus formation and devices were almost tolerated by many birds. In birds, cortical bone is thin and breakable because of high calcium contents which decrease the tensile strength (Farner and King, 1972). James *et al*. (1978) pointed out that medullary type bones heal faster than air-filled bones in birds. Similarly, in this study results also proved good bone healing in medullary bone like ulna in pigeons. Meanwhile, in the present study, ESF pins used in birds had no problem and advanced the fracture healing. Its main advantage is that ESF pins are small size 0.045″, adjustable and connected with external acrylic bar for mechanical support (Redig, 2001). Lewallen *et al.* (1984) compared the bone plates and ESF in dog model and reported that ESF provided less bending stiffness after 120 days. However, in our study 12 weeks post-surgery found that ESF had provided good bending stiffness in bird ulna. Redig (1983) considered that the disadvantage of ESF is that it is expensive, but safe for fracture treatment in birds. In this study, ESF half-pins were connected with light acrylic column and similarly, Jalila (2002) and Sanaei *et al*. (2011) used an acrylic mixture for ESF pin holding in birds. Based on the results, ESF used for bone fracture treatment provided complete clinical union and best mechanical stability.

Table 1. Three-point bending test of fracture healed ulna compared with normal intact ulna in pigeon.

Parameters	Treated Left Ulna	Right Ulna	p-value
Maximum flexure load (N)	$46.96^{a} \pm 11.30$	$57.69^{b} \pm 6.67$	0.014
Flexure stress at maximum load (Standard) MPa	$27.09^{a} \pm 6.52$	$33.28^{b} \pm 3.85$	0.014
Flexure stress at break (Standard)-MPa	$29.55^{NS} \pm 1.55$	$35.68^{NS} \pm 3.99$	0.064
Flexure strain at break (Standard) mm/mm	$0.44^{NS} \pm 0.05$	$0.68^{NS} \pm 0.60$	0.312

[a, b.] Values within row with different superscripts are significant at $p<0.05$. [ns]. Non-significant

CONCLUSION

The results of this experiment indicate that ESF pins had provided good bending strength after 12 weeks fracture fixation in pigeons. It is concluded that ESF technique would be suitable for fracture stabilization in pigeon and further studies on more birds are suggested to determine biomechanical properties of the fracture healing process.

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