Monitoring of Fracture Healing Process by Acousto-Ultrasonic Technique

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ABSTRACT

Presently the radiological examination is widely used for the assessment of *in vivo* bone condition. In some clinical problems such as diagnosis of the point of union of a fracture; the manual assessment is used along with radiological examination. Uncertainty regarding the significance of the radiographic and clinical findings may result in unnecessary long immobilization periods which can produce discomfort for the patient, as well as possible stiffness of the joints and sometimes permanent loss of mobility specially in older population. This paper deals with the *in vivo* analysis of bones by Acousto-Ultrasonic technique. The fracture was created through surgery on one of the limbs of rabbit and healing process was monitored through acousto-ultrasonic technique. A new index known as bone healing index was defined to calculate the end point of healing. In most of the cases, the bone healing index value was found to be 0.80, indicating that 80 % of the strength could only be restored at the completion of healing process.

Keywords: Acousto-ultrasonic, bone healing index, fracture healing, stress wave factor

INTRODUCTION

Bone fractures are the most common injury treated by the orthopaedic surgeons. Praemer et al. (1992) reported that millions of fractures occurred every year worldwide, with nearly 6.2 million fractures reported annually in the United States alone. Bone fractures can result from either trauma or pathological conditions. Fracture occurs in bones when the normal loading exceeds the load to which the bone has adapted itself during its growth and development and keeping this in mind, one may induce a trauma related fracture in bones. A pathological fracture occurs under normal loading conditions after the bone has been weakened by diseases, such as osteoporosis or bone tumors. Bone fracture healing is a complex and dynamic process of regeneration that slowly and gradually restores the structural integrity and mechanical function of the bone. The healing process involves formation of callus bone followed by change of callus bones into cortical bones and this process may take a few months. Einhorn (1995) study through statistical approach, revealed that in about 5–10% of the fractures that occurred annually, impairment of the healing process might lead to delayed union or non-union requiring further conservative or even surgical procedures.

Monitoring of fracture healing process is done by any suitable process which can evaluate the status of regaining the original strength of the healing bone. The monitoring should also be able to detect any complications in the bone healing process, and accurately assess proper healing. Blokhuis et al. (2001) reported that in routine practice, evaluation of fracture healing was performed by serial clinical and radiographic examinations, both of which depended on the orthopaedic surgeon's expertise and clinical judgment. Development of a quantitative technique would be more helpful for taking decision regarding removal of plaster cast, fixators etc. at an appropriate time.

Several non-invasive techniques have been reported in the literature for proper monitoring of fracture healing process. These include bone densitometry (Augat et al., 1997; Markel et al., 1991; Yoon & Yu, 2018), vibrational analysis (Cunningham et al., 1990; Nakatsuchi et al.,1996; Nikiforidis et al., 1990; Wang et al., 2017), acoustic emission (Aggelis et al., 2015; Claes et al., 2002; Hirasawa et al., 2002; Karaduman et al., 2018 Watanabe et al., 2001) and the attachment of strain gauges to external fixation devices as investigated by Moorcroft et al. (2001), Maffulli and Thornton (1995) and Richardson et al. (1994). All these methods aim to determine the stiffness of the healing bone, either directly or indirectly. Their ability to provide meaningful indications of healing has been validated through ex vivo and in vivo studies. However all these techniques are usually affected by extrinsic bone properties, such as bone gross geometry, and fracture type. In addition, these techniques require appropriate clinical setting, necessitating the intervention of a specialist to configure the set-up, and a number of them require removal of external fixators during examination. Ultrasonic methods have also been used for the monitoring of bone fracture

healing. Although some researchers have employed ultrasonography (Moed et al., 1998), and power Doppler ultrasonography (Risselada et al., 2006), to assess the appearance and neo-vascularisation of the callus tissue during healing, the majority of the studies have utilized quantitative ultrasound techniques. The axial transmission approach offers a unique way to examine fractures in long bones, such as tibia and radius. In axial transmission approach, a transmitter and a receiver are placed in direct contact with the skin on either side of the fracture, as is illustrated in Figure 1. The emitted ultrasonic waves propagate from the transmitter to the receiver along the long axis of the bone. Due to the complex structure of callus tissues and cortical bone, an appreciable change in the ultrasound velocity across the fracture occurs when compared to measurement of ultrasonic velocity in intact bone. Similar changes have been observed with regard to other propagation characteristics, such as ultrasonic attenuation parameter and dispersion characteristics.

The application of quantitative ultrasound to monitor the healing process has been investigated through animal experimentations and also in clinical studies over a long period of time. Simple experiments on bone phantoms and bone specimens have also been performed, aiming to examine the effect of the fracture characteristics (gap width and depth) on the measured quantities. The recent introduction of computational methods into bone research has extended our understanding of the underlying propagation phenomena and has helped researchers to propose new measurement techniques, such as the use of guided waves.

By considering the fact that two of the main functions of long bones are to support the body weight and locomotion, it is essential that the healing of a fracture should be assessed mainly in terms of increasing mechanical strength.

In this work an attempt has been made to use a combination of acoustic emission and ultrasonic technique for monitoring the fracture healing process. This is the first study wherein acousto-ultrasonic technique has been used for monitoring fracture healing process. Animal experimentation with rabbits were conducted using acousto-ultrasonic technique for monitoring the fracture healing process.
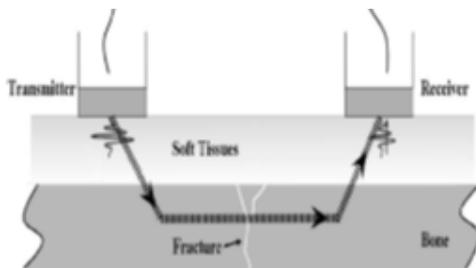

Figure 1. The axial transmission technique for ultrasonic evaluation of fracture healing process in long bones.

EXPERIMENTAL STUDY

An experimental work with rabbits was conducted in the Animal House of Birla Institute of Technology and Science (BITS) Pilani. Twenty (20) healthy rabbits of both sexes were selected from the groups available. The average weights of the rabbits were ranging from 1.5 kg to 2.0 kg and the approximate age of the

rabbits were 24 weeks. They were fed with standard diet during the whole experimentation period. The whole experimental work was approved by Animal Ethics Committee (Ref No.: IAEC/RES/12/03).

Creating Closed Fracture

Many investigators used several animal fracture models for the study of fracture healing, even with inherent advantages and disadvantages. The adjustments have been made between the reproducibility of osteotomy and the actual fracture. Generally, the production of real fractures increases the chances of variation in fracture site and location, which in turn results in making retesting more difficult. As it is difficult to grip the whole bone in vivo; the most common fracture process adopted by the researchers is bending (Ashhurst et al., 1982) and (Claes & Cunningham, 2009), where the bone can be supported against two points and a load can be applied from the opposite side. When a single point loading is adopted, the location of the fracture is determined by the loading point, and the mode is termed as "three point bending". A more uniform stress distribution over the test section is obtained when using two parallel loading points within the test span, termed as "four point bending", but the exact location and direction of the fracture is not so well controlled. Burstein and Frankel (1971) reported that the bone fractures at the weakest section as it would be expected in normal service conditions. They proposed a device to create a closed fracture in rats. However, all the methods mentioned above required very sophisticated equipment, and expert post-treatment care for proper healing. The experimental fracture created sometimes separated the rabbit's tibia in two parts, which needs intramedullary nailing to keep them aligned. This again required skill of a surgeon. Keeping in view all of the limitations; we went for creating a surgical fracture giving an oblique cut of 2 to 2.5mm depth in rabbits' left tibia. As this is only the first attempt of its kind, the main goal is to investigate the application of acousto-ultrasonic technique for monitoring the process of fracture healing. The type of fracture produced is not important at this point of study, but definitely it will be needed in the future study once the proof of the acousto-ultrasonic technique has been obtained.

Procedure for Creating Surgical Fracture

Pre-Requirements. The rabbits were kept fasting for nearly 6 hours to avoid any complication due to anaesthesia. The rabbits were anesthetized using a combination of xylazin (5-6 mg/kg body weight) and ketamine hydrochloride (9-13 mg/kg body weight)). Before starting the surgery, all surgical equipments, cottons, distilled water, surgery gown, green cotton cloth were autoclaved at 121°C, for 20 minutes.

Procedure. The Fibula is thin and there is an elongated interosseous space between the two bones. Comparatively less musculature is present on the medial aspect of bone. This was the reason for choosing rabbits' tibia for this study. The following procedure is adapted for creating surgical fracture in rabbit's tibia:

1) Legs of rabbit were washed with disinfectant soap (savlon) water and with clean cloth.

2) The hairs on the right hind legs were removed with the help of scissors and commercial hair remover.

3) The skin was then cleaned with 70% ethyl alcohol and povidon was applied as an anti-bacterial reagent.

4) Local anesthesia lignocaine hydrochloride (approx. 2ml) was in filtered at different places on the medial aspect of tibia region.

5) The skin was removed by giving a vertical incision of 40-50 mm with the help of surgical blade.

6) Facia were removed with the help of scalpel.

7) The muscles were removed by giving irregular cut with the help of point to blunt scissor.

8) At the distal end of posterior side, deep digital flexor muscle was separated from tibia by blunt incisions, at proximal third, bone was cut to the depth of 2.5 - 3mm by electric saw.

9) The muscles were sutured by catgut no.2 in lock and stitch method.

10) The facia and skin was sutured by silk no.2 by horizontal mattress method.

Figure 2 shows the surgical fracture created for the study.

Post Surgical Treatment. The post-surgical treatment provided to the rabbits is as follows:

1) The rabbits hind leg was immobilized by applying Plaster of Paris cast from the stifle joint to hock joint, immobilizing the ankle joint in zero position (neutral) and the knee joint in 900 of flexion.

Figure 2. Photograph of surgical fracture in right limb

2) The size of the plaster cast was reduced 3 weeks post operatively, as it was cut just distal to the knee and proximal to the ankles permitting full mobility of these joints.

3) The very next day after the surgery 3-4 holes were drilled on the plaster of Paris near the surgical site, to provide air passage.

4) The antibiotic/painkiller injection fortivir (0.5 ml I/M) and

Diclab (0.30 ml I/M) were given to all the rabbits for 3-4 days.

5)The injection T.T (0.25ml I/M) was also given to all the rabbits for pain relaxation. The antibiotic injection was given continuously for three days.

6) Few rabbits, which were given injection Belamyl (0.5 ml I/M) and injection cobacal D (0.75 ml I/M), showed symptoms of anorexia.

All the rabbits were kept on healthy diet during the study.

ACOUSTO-ULTRASONIC MEASUREMENT

Acousto-ultrasonic measurements were made from the healing limb and also from the contra lateral limb at every two week during the healing period. The contra lateral limb was used as a control for the acousto-ultrasonic measurements. The plaster cast was carefully removed during the acousto-ultrasonic measurements and if required applied again afterwards. In most of the cases the plaster cast was totally removed after 8 weeks. As the fracture was just a cut of 2-2.5 mm only, by examining the X-ray after 8 weeks, the decision was taken that whether the plaster cast should be applied again or not. Figure 3 shows the arrangement for acousto-ultrasonic measurement.

The pocket hand held acousto-ultrasonic unit from NDT Automation, a member of MISTRAS group has two spring-loaded, wheeled, rolling transducers attached with it. One sensor is the ultrasonic pulser and the other sensor is the acoustic emission receiver. The distance between the two sensors is one inch fixed. Here using the ultrasonic pulser, the low amplitude ultrasonic pulses of fixed frequency 250 kHz were injected in to the rabbit's tibia for the duration of 100µs. The pulser has the capability of generating the burst frequencies in the range of 50 kHz-1MHz, and the output voltage of 20 Volts peak to peak. The 25% of the noise signal were removed through the inbuilt system within the unit. The calibration of the amplitude height, threshold and other equipment settings were done with respect to contralateral limb before testing the healing bone. Once the calibration was done, the entire equipment setting was kept constant during the whole monitoring process for all the rabbits. The threshold setting was done with the help of setting detection gate facility with the instruments. The stress waves after interacting within the tibia bone were picked up by the 10kHz – 1MHz frequency response acoustic emission sensor. The care was taken while placing the sensors over the bone while taking the measurement. The instrument was somewhat modified, so that adequate amount of pressure is maintained so that proper sitting of the sensors could be assured. The sensor placement sites were so chosen such that the soft tissue thickness is the minimum at those places. The site chosen for the surgical fracture had the minimum soft tissue thickness. The received signal was them amplified by the built-in preamplifier of 40dB gain and bandwidth of 10kHz-1MHz. The equipment had the facility of activating the high pass filter and low pass filter within

it. The high pass filter frequency of 100 kHz and the frequency of 400 kHz for low pass filter were chosen for the in vivo study.

The Pocket AU system has its own built-in AU software program that uses A-Scan and C-scan analysis to perform Acousto-ultrasonic inspection. Only the A-Scan analysis feature was used for this study, as the scanning of the rabbit's tibia was not possible. The received signal was digitised through the dedicated feature extraction processor within the unit. The waveform parameters were then exported and converted to ASCII files and transferred to the personal computer for further signal processing.

The stress wave factor for the study is taken as the energy content of AU signals. The relative energy is given by formula (1) [12]

$$\text{Energy integral} = SWF = \int_{t1}^{t2}[v(t)]^2 dt \qquad 1$$

Where, v(t) is the amplitude distribution with respect to time.

The Stress Wave factor (energy content) corresponding to both the control and healing limb were measured after every two weeks during the healing period up to 12 weeks from the date the fracture was made. A Healing Index which is a ratio of SWF of healing limb and SWF of control limb was calculated.

Radiographic examination was also made every month for qualitative assessment. The software was developed in MATLAB 6.05 for calculating the different parameters of the acoustic emission signal and for calculating the healing index, the term used for monitoring the healing process,

RESULTS AND DISCUSSIONS

The software specially developed for the present study took the AU waveforms recorded during the AU measurements as input and process the signals to find different parameters such as energy content of the signal, peak amplitude, rise time, and time of flight. The signals were also plotted directly on the screen and the calculated Healing index was also displayed.

Figure 3 gives the details of the acousto-ultrasonic measurements made in rabbit No. 4 just after the surgery. It clearly mentions the various parameters calculated for both the control and healing limb just after the fracture was created. In Figure 3 output for "good" stands for control limb and "bad" for Healing limb.

Figure 4 displays the energy content (used as SWF for the present study) for both the control and healing limbs of rabbit no. 4 as well as healing index just after the surgery. The healing index was found to be 0.003, as the maximum signal experienced attenuation due to the presence of fracture. The X-ray picture of the limb post-surgery is shown in Figure 5.
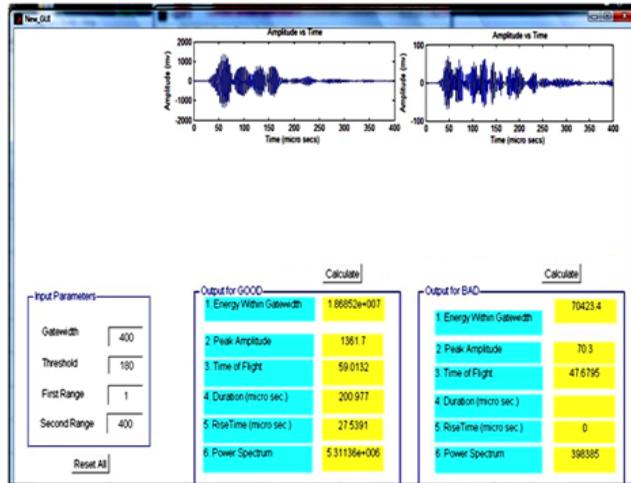
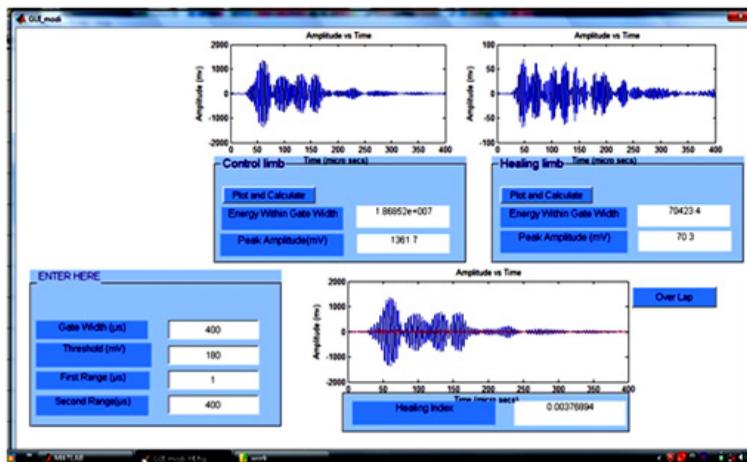*Figure 3.* Acousto-ultrasonic parameters calculated for rabbit number 4 just after surgery

Figure 4. Acousto-ultrasonic measurements for rabbit No. 4 just after the surgery
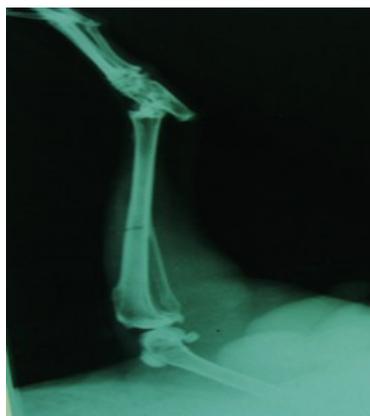

Figure 5. X-radiograph of rabbit number 4 just post-surgery

The value of bone healing index in the fourth week during the healing period indicates that the callus formation around the fracture has taken place at this time. The value of the healing index clearly shows an increasing trend during the healing period. The value of the healing index after week 12 was found to nearly constant when measured in the fourteenth week. Hence the healing index value of 0.86 obtained after week twelfth was considered to be the end point of healing process.

Figure 6 and 7 show the value of the healing index and the X-ray photograph of rabbit 4 at the end of week twelfth. The value of the healing index clearly indicates that it increased considerably till week eighth but after that its value increased very slowly. This was due to slow remodeling process in the final stage of fracture healing process, which continued after week 12 also.
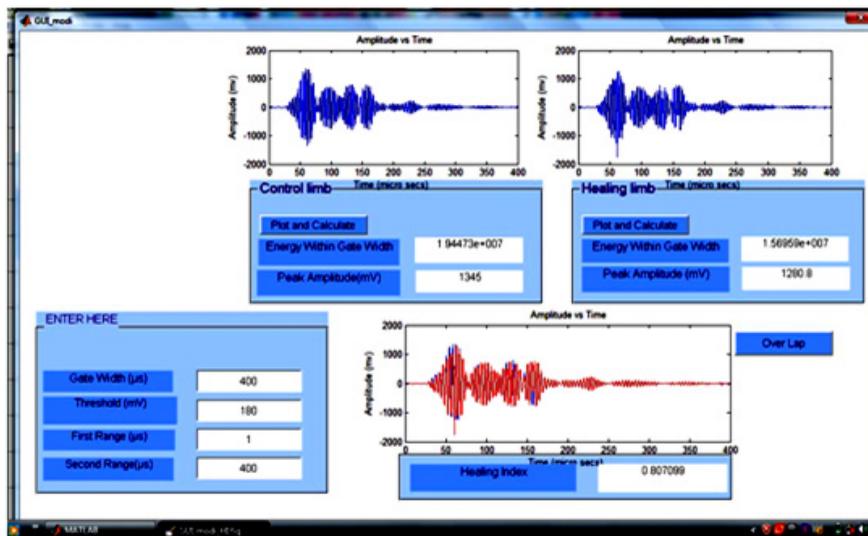

Figure 6. Acousto-ultrasonic measurements for rabbit number 4 at twelfth week

Figure 7. X-ray of rabbit number 4 at twelfth week

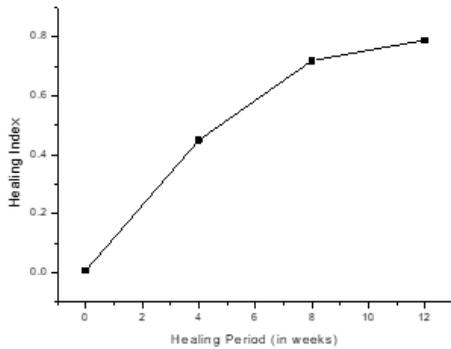Subsequent graphs Figure 8 to 10, shows the Bone healing index values for rabbits number 10, 11 and 13 respectively.

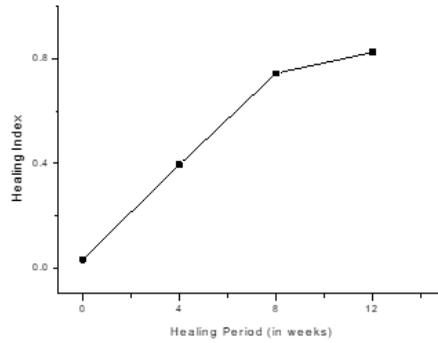*Figure 8.* Healing Index values for rabbit number 10 during the healing period

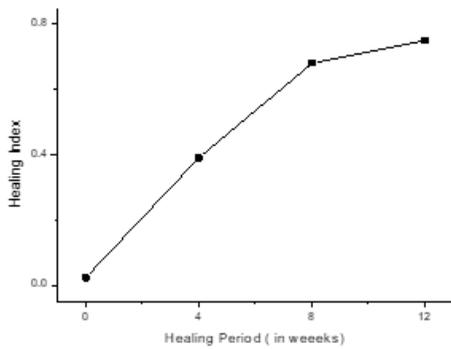*Figure 9.* Healing Index values for rabbit number 11 during the healing period

Figure10. Healing Index values for rabbit number 13 during the healing period

CONCLUSION

The results of the present experimental study indicate that Acousto-ultrasonic technique can be used for monitoring the fracture healing process.

The calculated Healing Index from the acousto-ultrasonic measurements was found to be increasing uniformly during the healing period. The end point of the healing process was indicated by the more or less constant value of Healing Index value at 12-14 weeks of the healing period.

The X-ray examination clearly show the healing has almost completed after the eighth week, but the Healing Index value at the same time does not confirm it. Hence it can be concluded that the healing index value which was calculated with respect to energy content of the signal, clearly assembled the strength of the bone also. Thus, it is concluded that the acousto-ultrasonic technique, apart from monitoring the fracture healing process also gives the information of the strength of the bone achieved. The correlation between the energy content (SWF) and the strength of the bone has already been validated in the *in vitro* study with this technique. Due to the limitations we could not perform the same correlation for the *in vivo* studies.

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