# USM SHORT TERM GRANT NO. 304/PPSP/6131270

# Effect of Pulsed Infrared Radiation on Fracture Healing in Rabbits

Principal Investigator:
Dr Abdul Halim Yusof

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# BORANG USM J/P
# 06

# BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
# CANSELORI
# UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: Dr Abdul Halim Bin Yusof

Nama Penyelidik-Penyelidik
Lain (Jika berkaitan) :

Prof Madya Dr Wan Ahmad kamil Wan Abdullah

Prof Madya Dr Manoharan Madhavan

Dr Abdul Rahman Mohd Ariff

2) Pusat Pengajian/Pusat/Unit : Pusat Pengajian Sains Perubatan

3) Tajuk Projek:

Effect of Pulsed Infrared radiation on Fracture Healing –an animal study
..........................................................................................................

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).


# ABSTRAK

## TAJUK: KESAN SINARAN RADIASI PULSED INFRARED KEATAS PENYEMBUHAN KEPATAHAN TULANG-SATU KAJIAN KEATAS HAIWAN

**Pengenalan:**

Stimulasi Biofizikal merupakan kaedah alternatif yang tidak memerlukan sebarang pembedahan dalam mempercepatkan lagi kadar penyembuhan kepatahan tulang. Kesan radiasi inframerah kepada kepatahan tulang belum pernah dikaji sebelum ini. Ini merupakan kajian awal yang julung kali diadakan. Kajian ini dijalankan di Pusat Pengajian Sains Perubatan, Universiti Sains Malaysia, Kubang Kerian.

**Objektif:**

Untuk melihat kesan sinaran infrared penyembuhan kepatahan tulang pada haiwan arnab berbanding dengan penyembuhan tanpa sinaran infrared

**Methodologi:**

Sebanyak 16 ekor arnab jantan yang telah dewasa di ambil sebagai subjek. Setiap satu telah di bedah dan tulang tibia dipatahkan secara pembedahan. Tulang tersebut telah disambung semula menggunakan satu dawai khas di dalam rongga sum-sumnya. Kaki tersebut dibalut dengan plaster Paris. Kumpulan kajian ini dibahagikan kepada 2 kumpulan. Satu kumpulan didedahkan kepada sinaran radiasi inframerah selama sejam sehari manakala sekumpulan lagi dijadikan kumpulan kawalanl. 4 ekor dari setiap

kumpulan akan dikorbankan pada minggu ketiga dan keenam. Tulang tibia tersebut diambil untuk pemeriksaan dan kajian dari aspek radiologi dan histologi.

**Keputusan**

Kepatahan tulang kedua-dua kumpulan didapati telah sembuh selepas tiga minggu namun kumpulan yang didedahkan kepada radiasi inframerah telah menunjukkan pertumbuhan tulang baru yang lebih banyak berbanding kumpulan control walaupun bagaimanapun pada minggu keenam tidak ada perbedaan antara kedua kumpulan ini

**Kesimpulan:**

Oleh itu boleh dirumuskan bahawa sinaran radiasi inframerah telah meningkatkan lagi proses penyembuhan dalam peringkat awal kepatahan tulang di dalam arnab.

ABSTRACT

TITLE: EFFECT OF PULSED INFRARED RADIATION ON FRACTURE HEALING-AN ANIMAL STUDY

**Introduction**

Fracture healing is a highly complex regenerative process that is essentially a replay of developmental events. Although medical technology and orthopedic management have improved greatly in the past 30 years, some fracture healed poorly, others take a long time to heal and some results in nonunion Biophysical stimulation provides an interesting non operative alternatives in tackling these problems. Infrared radiation is another biophysical stimulation using far infrared radiation (wavelength 1500nm-1mm) which is said in resonance with the process to be normalized.

**Objective**

To compare fracture healing in tibia of rabbit between a group given pulsed infrared radiation and another group allowed to undergo normal healing. Assessment are made through radiological and histological analysis.

**Methodology**

Sixteen matured male rabbits were used A transverse osteotomy were carried out over the left tibia diaphysis and they were divided equally into 2 groups of 8 rabbits each

Group A - exposed to infrared radiation.

Group B - normal healing.(control group)

They were then sacrificed at three and six weeks for assessment.

CT scan of each sample were then carried out.Each specimen is scanned in anteroposterior and lateral image (scout view or topogram). These images will show the proximal and distal extension of the hard callus. These areas is then scan with a slice interval of 0.5mm and slice thickness of 1.0mm. A three dimension image is then reconstructed. The fracture callus (hard callus) volume is then calculated The 3D images were first reconstructed. Using volumetric calculation the callus volume is then measured.For histological assessment, the specimen were first cut into 2 equal halves sagitally, along the tibial axis Representative sample were taken from each specimen. After the usual process they were then stained using Hematoxylin and Eosin stain and mounted on DPX. The sections were then examined under microscope for presence of endosteal and periosteal callus, the area of cartilage and also fracture line.

**Results:**

At 3 weeks all specimen from both the normal healing and infrared radiation group showed callus formation that bridges the fracture line that For the callus volume, at 3 weeks the infrared group does show a higher volume (almost double) but at 6 weeks the callus volume are almost equal.

At 3 weeks woven bone are seen bridging the fracture site in both group. In the normal healing group larger area of cartilage were observed as compared to the infrared group The fracture gap persisted with no intervening tissue in all the specimen except for one normal healing specimen that shows intervening fibrous tissue At 6 weeks, no area of cartilage is seen and woven bone were now seen in between the fracture gap in both the group. In the infrared group there were presence of acute inflammatory cells at both 3 and 6 weeks which were absent in the normal healing group

## Conclusion

This study had shown that in the infrared group callus volume at 3 weeks are almost double as compared with the normal healing group.At 6 weeks both the groups does not showed any significant differences in terms of callus volume. Hence it is shown that pulsed infrared radiation enhanced healing process in early stage of fracture healing

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

| Bahasa Malaysia | Bahasa Inggeris |
|---|---|
| Sinaran infrared | Infrared radiation |
| Penyembuhan kepatahan | Fracture Healing |
| Arnab | Rabbit |

5) Output Dan Faedah Projek

(a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

1-Free Papers: "Biophysical Enhancement Of Fracture Healing Using Infrared radiation.A Study in Rabbits at 55th Annual Convention of the Phhilippine Orthopaedic association, Manila Dec 4.2004

2-Mahmood Merican Award Presentation" Effect of Pulsed Infrared Radiation on Fracture Healing: A study in Rabbits" 34th Annual general Meeting and Annual Scientific Meeting of Malaysian Orthopaedic Association.2004

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan)

Kaedah ini mempunyai potensi untuk digunakan dalam rawatan klinikal sebagai kaedah alternative dan komplimentari kerana ia mempunyai kelebihan rawatan tanpa pembedahan untuk kes-kes yang biasanya memerlukan pembedahan
Namun kajian keatas subjek manusia perlu dibuat terlebih dahulu.Untuk itu beberapa kajian lanjutan akan menyusuli kajian ini

(c) Latihan Gunatenaga Manusia

Pelajar Siswazah

Dr Mohammad azril Mohammed Amin-pelajar Sarjana Otopedik

ii) Pelajar Prasiswazah: ..........................................................

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iii) Lain-Lain :

JuruXray Jabatan radiology
Juruteknik Rumah Haiwan

6. Peralatan Yang Telah Dibeli:

2 set infrared emitters

## UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

Penyelidikan yg baik.
Perlu diterbitkan di jurnal

T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

PROFESSOR ABDUL AZIZ BABA
Chairman of Research & Ethics Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan

# BOOK OF ABSTRACTS

55th Annual Convention of the
Philippine Orthopaedic Association
Combined with
2nd Asian Congress of the
International Federation of
Foot and Ankle Societies
and the
4th Annual Convention of the
Philippine Orthopaedic
Foot and Ankle Society

November 30 - December 4, 2004
Edsa Shangri-la Hotel
Manila, Philippines

## BIOPHYSICAL ENHANCEMENT OF FRACTURE HEALING USING INFRARED RADIATION - A STUDY IN RABBITS

*Mohd Amin Azril, MD, Y HALIM, MA ISKANDAR*
*(Orthopaedic Dept Kulliyah Of Medicine, International Islamic University Malaysia, Orthopaedic Department School of Medical Science University Sains, Malaysia)*

Fracture healing is an evolutional biological process that allows the bone to regain their pre-injury function. Biophysical stimulation such as infrared light provides a non invasive alternative to enhance fracture healing. It was observed that skin wound healed faster when it is exposed to infrared light . However the effect of infrared radiation on bone healing had never been evaluated. We evaluated the role of non invasive biophysical stimulation of infrared radiation in fracture healing. This is a pilot descriptive experiment, based on New Zealand white rabbits, whereby the healing of bone are being assessed by CT scan and histologically confirmed.

The source of infrared radiation is from the heating of a non-luminous lamp coated with ceramic oxide material. The population samples consist of 16 adult male rabbits. Each was subjected to a surgically induced transverse osteotomy on the right tibia. They are then divided into 2 groups. One group is exposed to infrared radiation for 1 hour a day while the other group which serves as a control was not exposed to infrared radiation. Four were sacrificed from each group at 3rd and 6th weeks. The operated tibia is harvested. Radiological and histological assessments were then carried out. Both groups showed the fracture unites at 3rd week but a much larger volume of hard callus was noted in the infrared group.

At 6 weeks the volume of hard callus are almost equal in both groups. In conclusion, infrared radiation modifies bone union by forming larger amount of callus in the early stage of healing.

# MAHMOOD MERICAN AWARD PRESENTATIONS

## 5 JUNE 2004, SATURDAY

0900 hrs **The Effect of Rofecoxib and Alendronate on Fracture Healing : A Laboratory Study on the Rabbit Ulna Osteotomy Model** 35

Sureshan Sivananthan
*Department of Orthopaedic Surgery, University Malaya Medical Centre, Kuala Lumpur, Malaysia*

0910 hrs **Post Mortem Study on Bone Allograft Bacterial Colonization** 36

Haris Ali, Robert Penafort
*Department of Orthopaedic Surgery, University Malaya Medical Centre, Kuala Lumpur, Malaysia*

0920 hrs **Vancomycin Release from Vancomycin Impregnated Calcium Sulfate and PMA Discs** 37

C P Lau, M K Kwan, Tunku Sara Ahmad
*Department of Orthopaedic Surgery, University Malaya Medical Centre, Kuala Lumpur, Malaysia*

0930 hrs **The Significance of Using Pooled Human Serum in Human Articular Cartilage Tissue Engineering** 38

Azmi B*, S Ibrahim*, Samsudin O C*, Aminuddin B S ***/****, Munirah S***, Chua K H***, Ruszymah B H I**
**Department of Orthopaedics and Traumatology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*
***Department of Physiology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*
****Tissue Engineering Laboratory, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*
****ENT Specialist Clinic, Ampang Puteri Hospital, Kuala Lumpur, Malaysia*

0940 hrs **Study of Clinical and Radiological Factors Influencing the Rate of New Bone Formation in Distraction Osteogenesis** 39

S Manimaran, Saw Aik
*Department of Orthopaedic Surgery, University Malaya Medical Centre, Kuala Lumpur, Malaysia*

0950 hrs **Effect of Pulsed Infrared Radiation on Fracture Healing : A Study in Rabbits** 40

M A Azril, A Y Halim, M A Iskandar, W Zulmi
*Department of Orthopaedic Surgery, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia*

# MANUSKRIP

ABSTRACT

**TITLE: EFFECT OF PULSED INFRARED RADIATION ON FRACTURE HEALING -AN ANIMAL STUDY**

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**Introduction**

Fracture healing is a highly complex regenerative process that is essentially a replay of developmental events. Although medical technology and orthopedic management have improved greatly in the past 30 years, some fracture healed poorly, others take a long time to heal and some results in nonunion Biophysical stimulation provides an interesting non operative alternatives in tackling these problems. Infrared radiation is another biophysical stimulation using far infrared radiation (wavelength 1500nm-1mm) which is said in resonance with the process to be normalized.

**Objective**

To compare fracture healing in tibia of rabbit between a group given pulsed infrared radiation and another group allowed to undergo normal healing. Assessment are made through radiological and histological analysis.

**Methodology**

Sixteen matured male rabbits were used A transverse osteotomy were carried out over the left tibia diaphysis and they were divided equally into 2 groups of 8 rabbits each

Group A - exposed to infrared radiation.

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They were then sacrificed at three and six weeks for assessment.

CT scan of each sample were then carried out.Each specimen is scanned in anteroposterior and lateral image (scout view or topogram). These images will show the proximal and distal extension of the hard callus. These areas is then scan with a slice interval of 0.5mm and slice thickness of 1.0mm. A three dimension image is then reconstructed. The fracture callus (hard callus) volume is then calculated The 3D images were first reconstructed. Using volumetric calculation the callus volume is then measured.For histological assessment, the specimen were first cut into 2 equal halves sagitally, along the tibial axis Representative sample were taken from each specimen. After the usual process they were then stained using Hematoxylin and Eosin stain and mounted on DPX. The sections were then examined under microscope for presence of endosteal and periosteal callus, the area of cartilage and also fracture line.

**Results:**

At 3 weeks all specimen from both the normal healing and infrared radiation group showed callus formation that bridges the fracture line that For the callus volume, at 3 weeks the infrared group does show a higher volume (almost double) but at 6 weeks the callus volume are almost equal.

At 3 weeks woven bone are seen bridging the fracture site in both group. In the normal healing group larger area of cartilage were observed as compared to the infrared group The fracture gap persisted with no intervening tissue in all the specimen except for one normal healing specimen that shows intervening fibrous tissue At 6 weeks, no area of cartilage is seen and woven bone were now seen in between the fracture gap in both the group. In the infrared group there were presence of acute inflammatory cells at both 3 and 6 weeks which were absent in the normal healing group

**Conclusion**

This study had shown that in the infrared group callus volume at 3 weeks are almost double as compared with the normal healing group.At 6 weeks both the groups does not showed any significant differences in terms of callus volume. Hence it is shown that pulsed infrared radiation enhanced healing process in early stage of fracture healing

Keywords: fracture healing, infrared radiation, rabbits

## INTRODUCTION

Fracture healing is a highly complex regenerative process that is essentially a replay of developmental events. These events include the action of many different cell types, a myriad of proteins and an active gene expression that in majority of cases it ultimately restore the bone natural integrity (Hadjiargyrou et al 1998a). Each year approximately 33 million people sustain musculoskeletal injury in United States. Of these injuries, nearly 6.2 million are fractures. Although medical technology and orthopaedic management have improved greatly in the past 30 years, some fracture healed poorly, others take a long time to heal and some results in nonunion (Eiahorn TA 1998b). Of these around 5-10% ended up with nonunion.

Biophysical stimulation provides an interesting non operative alternatives in tackling these problems. Studies on the effect of Pulsed electromagnetic field and low intensity ultrasound on enhancement of fracture healing has already been done on animals with only few clinical trials (Inoue et al 2002, Hadjiargyrou et al 1998a, Mayr et al 2001). Infrared radiation is another biophysical stimulation proposed by Rustam Rakhimov, an

Uzbekistan biophysicist. His method of treatment is based on using far infrared radiation (wavelength 1500nm-1mm) which he said is in resonance with the process to be normalized. These narrow spectrum far infrared radiation are generated by heating ceramic oxide material. This method of treatment is considered safe, effective with no adverse consequences and is widely used in Uzbekistan (Rakhimov R 2000a, Tikhonova N 2000). Currently there is no english literature on this method of treatment. Furthermore the therapeutic uses of infrared radiation were previously confine to the near infrared spectrum(wavelength 760-1500nm) which were widely used by physiotherapist. The far infrared spectrum were thought to be of no therapeutic use.

The purpose of this study is to evaluate the response of bone healing exposed to infrared radiation as compared to normal healing in a fractured rabbit tibia. The healing effect if truly present should then be clinically evaluated as it provide a non invasive solution in problems of bone union, therefore might be more acceptable to general population.

**METHODOLOGY**

This is a pilot study, descriptive in nature. Ethical approval were obtained from the USM Health Campus Research and Animal Ethics Committee. The population sample were decided by the animal ethical committee. Sixteen matured male New Zealand White rabbits (Oryctolagus cuniculus) aged 4-5 months and weighing between 2-3kg were used in this experiment. These rabbits are supplied by USM Health Campus Animal House. They were maintained in good health status, each cage housed a pair of rabbits and the cages are cleaned daily. A transverse osteotomy were carried out over the left tibia diaphysis and they were divided equally into 2 groups of 8 rabbits each (figure 5):

Group A - exposed to infrared radiation.

Group B - normal healing.(control group)

They were then sacrificed at three and six weeks for assessment.

CT scan of each sample were then carried out using General Electric(GE) computed tomography light speed plus system. Each specimen is scanned in anteroposterior and lateral image (scout view or topogram). These images will show the proximal and distal extension of the hard callus. These areas is then scan with a slice interval of 0.5mm and slice thickness of 1.0mm. A three dimension image is then reconstructed. The fracture callus (hard callus) volume is then calculated using volume rendering software at the GE Advantage Workstation. The 3D images were first reconstructed. The Hounsfield unit for bone is 1000 and for soft tissue is less than 200 (Stimac GK and Kelsey CA 1992). By setting the Hounsfield window between 250-1000 the tibial bone and soft tissue images were then filtered out by digital image substraction leaving behind only the hard callus. Using volumetric calculation the callus volume is then measured. Bone union were defined radiologically as the presence of callus, bridging the fracture gap in 3 out of 4 cortex. This assessment were from the anterioposterior (2 cortex) and lateral (2 cortex) radiology image (Heckman et al 1994, Kristiansen et al 1997). In this study the bridging callus is assessed by the 3D reconstructed images. The axial cut of these images at the fracture site were analysed and if the callus covers more than 240 degrees circumferentially, union is then considered as had occurred.

For histological assessment, the specimen were first cut into 2 equal halves sagitally, along the tibial axis by using a water cooled diamond edge cutting saw. Representative sample to accommodate into the universal cassette measuring 1.5cm proximal and distal to the fracture site were taken from each specimen. It is then decalcified by immersing in 5% Nitric acid solution. After that the tissue were routinely processed using histokinete machine (Tissue-take VIP) overnight. The processed sample were then cut into 5um thick section using a microtome. They were then stained using Hematoxylin and Eosin stain and mounted on DPX. The sections were then examined under microscope for presence of endosteal and periosteal callus, the area of cartilage and also fracture line. This is assessed by a single pathologist who were blinded on which specimen belong to which group.

## RESULTS

Out of 16 rabbits, only 13 specimen were obtained. One rabbit died during surgery, one specimen was accidently fractured while harvesting the bone and another specimen was excluded due to a technical error.

Radiological assessment:

From the CT scan 3D reconstructed images, the union is assessed through the presence of bridging callus and the callus volume is then calculated. At 3 weeks all specimen from both the normal healing and infrared radiation group showed callus formation that bridges the fracture line that denotes that union had occurred. The calculated callus volume are shown below (Table 1). The same are seen in the 6 weeks group and the callus volume is stated below (Table 2).

Table 1: Callus volume at 3 weeks.

| Infrared radiation<br><br>Callus volume ($cm^3$) | Normal Healing<br><br>Callus volume ($cm^3$) |
|---|---|
| 0.207 | 0.125 |
| 0.276 | 0.146 |
| 0.762 | Died on table |
| 0.447 | 0.248 |

Table 2: Callus volume at 6 weeks

| Infrared radiation<br><br>Callus volume ($cm^3$) | Normal Healing<br><br>Callus volume ($cm^3$) |
|---|---|
| 0.122 | Fractured |
| 0.718 * | 0.141 |
| 0.373 | 0.314 |
| 0.116 | 0.245 |

* technical error ( K-wire outside of distal fragment )

For the callus volume, at 3 weeks the infrared group does show a higher volume (almost double) but at 6 weeks the callus volume are almost equal.

Histological assessment are made using Leica DMPE microscope and Leica Qwin colour image analyzer software. At 3 weeks woven bone are seen bridging the fracture site in both group. This is seen at both the periosteal and endosteal side. In the normal healing group larger area of cartilage were observed as compared to the infrared group

The fracture gap persisted with no intervening tissue in all the specimen except for one normal healing specimen that shows intervening fibrous tissue .These cortical gaps were around 50-250um.

At 6 weeks, no area of cartilage is seen and woven bone were now seen in between the fracture gap in both the group It is also noted that the infrared group showed presence of acute inflammatory cells at both 3 and 6 weeks which were absent in the normal healing group.

## DISCUSSION

The primary goal in fracture treatment is to restore mechanical stability and function as rapidly as possible. The most common biological problem that hinder healing is the inability or failure to form callus that provides early fracture stabilization (Frost HM 1989). Treatment that target the stimulation or enhancement of callus formation have the potential significantly to impact the quality and rate of fracture healing.

The rationale of biological stimulation eg: Transforming Growth Factor Beta (TGFß) and Fibroblast Growth Factor (FGF) at the fracture site is to increase the number or rate of differentiation of reparative cells at the damage site. Studies by Radomsky et al 1998 and Rosier et al 1998 had shown that TGFß and FGF results in stimulation of callus formation, increased in callus size and earlier restoration of mechanical strength at fracture site. However these agents had to be delivered to the fractured site either by injection or surgical implantation.

Biophysical stimulation provides an alternative non operative technique to enhance fracture healing (Otter et al 1998). While Pulsed Electromagnetic Field (PEMF) and Ultrasound stimulation had been studied to show an effect on fracture healing enhancement (Inoue et al 2002, Hadjiargyrou et al 1998a, Mayr et al 2001), no published study has ever been done on the effect of far infrared radiation on fracture healing as suggested by Rustam Rakhimov.

The purpose of this study is therefore to find out if infrared radiation has an effect on fracture healing. Once its effect has been confirmed then the optimum dose, time exposure, wavelength and mechanism of action need to be investigated.

The interval time for assessment were chosen at 3 and 6 weeks as the normal bone healing in rabbits mentioned in literature and other studies varies from 3-6 weeks (Murray et al 1996, Richardson VCG 2000c). In a study by Mathias PG Bostrom and Nancy P Camacho 1998 on effects of Bone Morphogenetic Proteins on bone healing, the overall hard callus area tended to increase in size in between 2-3 weeks in the rabbits fractured ulna that was given BMP as compared to the control group. Therefore 3-6 weeks were decided as it is believed to be able to demonstrate any differences in the healing process both radiology and histologically. The specimen were also sacrificed at only 2 specific interval so as to have a much larger subject in each group.

In an experimental model, mechanical testing and quantitative roentgengraphic densitometry is widely used as it is more superior than normal radiographic evaluation and scoring in assessing fracture healing (Lane and Sandhu 1997). However both are not available in the institution where this study is done. Bone union were defined radiologically as the presence of callus, bridging the fracture gap in 3 out of 4 cortex. This assessment were from the anterioposterior (2 cortex) and lateral(2 cortex) radiograph (Heckman et al 1994, Kristiansen et al 1997). In this study both the group showed bony union at 3 weeks and this is more clearly seen in the reconstructed 3D images that showed callus all around the fracture. Although both had achieved bony union at around the same time, the infrared exposed group seams to have a lot more callus formation which is almost double compared to the control group. This is also evident in the histological assessment that shows more woven bone in the infrared group as compared to the control group that had larger area of cartilage still present. This relation of increasing callus volume when stimulated by biological or biophysical agents were also noted by several other authors. Mathias Bostrom and Nancy Camacho 1998 in their review of potential role of BMP in fractured healing reported that Den Boer et al had showed increase callus volume in fractured goat tibia after injecting BMP7 as compared to the control group. He also showed increase torsional stiffness in those specimen. The callus volume were measured by 3D CT scan. Increased in total callus and bone area were also demonstrated by Radomsky et al 1998 in fractured rabbit fibula which had been given Fibroblast Growth Factor. Shimazaki et al 2000 and Mayr et al 2001 also showed increased callus in rabbit and sheep model treated with low intensity ultrasound. Black et al 1984, showed that while radiograph were useful to show callus progression, it proved unreliable for quatitative evaluation of healing. He compared mechanical behaviour of a single transverse osteotomy in a rabbit fibula and showed the linear correlation between peak sustainable load in Newton with increasing bone formation. Therefore he had suggested the possibility of using computer assisted

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# LAPORAN AKHIR

# The Effect Of Pulsed Infrared Radiation On Fracture Healing -a study in rabbits

## 1.0 INTRODUCTION.

Fracture healing is a highly complex regenerative process that is essentially a replay of developmental events. These events include the action of many different cell types, a myriad of proteins and an active gene expression that in majority of cases it ultimately restore the bone natural integrity (Hadjiargyrou et al 1998a). Each year approximately 33 million people sustain musculoskeletal injury in United States. Of these injuries, nearly 6.2 million are fractures. Although medical technology and orthopaedic management have improved greatly in the past 30 years, some fracture healed poorly, others take a long time to heal and some results in nonunion (Eiahorn TA 1998b). Of these around 5-10% ended up with nonunion.

Biophysical stimulation provides an interesting non operative alternatives in tackling these problems. Studies on the effect of Pulsed electromagnetic field and low intensity ultrasound on enhancement of fracture healing has already been done on animals with only few clinical trials (Inoue et al 2002, Hadjiargyrou et al 1998a, Mayr et al 2001). Infrared radiation is another biophysical stimulation proposed by Rustam Rakhimov, an Uzbekistan biophysicist. His method of treatment is based on using far infrared radiation (wavelength 1500nm-1mm) which he said is in resonance with the process to be normalized. These narrow spectrum far infrared radiation are generated by heating ceramic oxide material. This method of treatment is considered safe, effective with no adverse consequences and is widely used in Uzbekistan (Rakhimov R 2000a, Tikhonova N 2000). Currently there is no english literature on this method of treatment. Furthermore the therapeutic uses of infrared radiation were previously confine to the near infrared spectrum(wavelength 760-1500nm) which were widely used by physiotherapist. The far infrared spectrum were thought to be of no therapeutic use.

The purpose of this study is to evaluate the response of bone healing exposed to infrared radiation as compared to normal healing in a fractured rabbit tibia. The healing effect if truly present should then be clinically evaluated as it provide a non invasive solution in problems of bone union, therefore might be more acceptable to general population.

## 2.0 LITERATURE REVIEW.

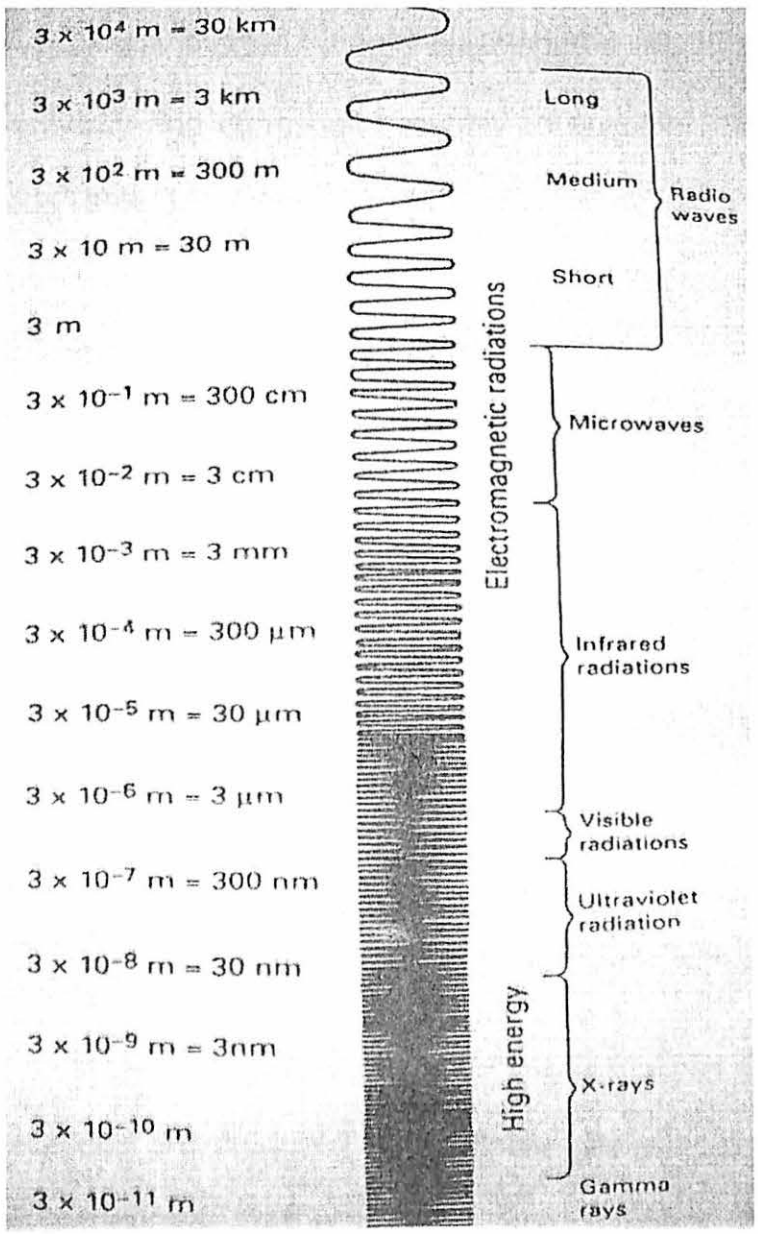
### 2.1 Infrared radiation.

#### 2.1.1 Introduction.

Historically, there has always been a powerful association between the rays of the sun and healing. The latin word for god 'Deus' is said to derive from the sun-worshipping Aryans' name for the sun. The healing effects of heat and light were widely known to the ancient. In 1800, a British Astronomer Sir William Herschel discovered the infrared region (Jackson et al 1997). Of all radiations, perhaps the most 'natural' from a human perspective are those infrared radiations constantly emitted and absorbed by all humankind. Infrared radiations lie within the part of the electromagnetic spectrum which gives rise to heating when absorbed by matter. The radiations are characterized by wavelengths which are longer than that of visible red light, extending to the microwave region which is from 760nm to 1mm. These wave length's can be detected by using a spectrometer (Figure 1).

Figure 1: Electromagnetic radiation wavelength.

Source : John Low and Ann Reed :Infrared and visible radiation, Electrotherapy Explained principle and practice 3rd Edt, Butterworth & Heinemann.



2.1.2 Types of infrared radiation.

These infrared radiations can be subdivided into three bands A, B and C (Sheila Kitchen 2000a) approximately distinguished by their absorption characteristics. Therapeutically only the shorter infrared wavelengths are utilized. A and B are utilized therapeutically and correspond roughly to an older classification of 'near' and 'far' infrared (Table1).

Table 1: Classification of infrared (IR) radiation.

| Type | Wavelength |
|---|---|
| IRA | 760-1400nm (0.76-1.4um) |
| IRB | 1401-3000nm (1.4-3um) |
| IRC | 3001nm-1mm (3um-1mm) |
| Near/Short IR | 760-1500nm |
| Far/Long IR | 1500nm-1mm |

Infrared radiations are produced in all matter, by various kinds of molecular vibration. When atoms move further apart or closer together without breaking free from one another, the molecules formed by them alter shape and infrared radiations are emitted. Any given molecule is already in a state of vibration and rotation and this can be altered by absorbing heat which leads to the emission of many different wavelengths of infrared. The result is that any heated body emits infrared radiations, indeed any material that is at a temperature above absolute zero emits infrared. Although the radiations will be of a whole range of different frequencies, the frequencies at which the maximum intensity of radiation is emitted are proportional to temperature. Thus the higher the temperature, the higher the frequency and hence the shorter the wavelength. To understand this better, it is known that light is emitted in photons. When an atom changes its energy, the differences in the energy ($\Delta E$) is released as a photon with frequency ($\lambda$).

Quantum energy of a photon thus depends on the wavelength of light to an equation; $\Delta E = hv = hc/\lambda$ where h is the Planck constant ( $6.626 \times 10^{-33}$ joule/sec ), v is the radiated frequency, c is the speed of light in vacuum and $\lambda$ is the light wavelength (Rakhimov RK 2000).

The human body also emits a whole range of infrared radiations (multiple wavelength), mainly type C, with a peak around 10 000 nm(John Low and Ann Reed 2000a). The shorter, visible radiations not only cause molecular and atomic motion but can also break chemical bonds when they are absorbed. It is this that provokes chemical changes in the retinal pigments which are detected via the optic nerve as sight.

### 2.1.3 Source of infrared.

Any heated material will produce infrared radiations, the wavelength being determined by the temperature. If short infrared is to be produced efficiently the material must not be oxidized (burnt) by the higher temperatures used. The most convenient method is to heat a resistance wire by passing an electric current through it. An ordinary household electric fire can be made of a coil of suitable resistance wire, such as nickel-chrome alloy, wound on a ceramic insulator.

Two kinds of infrared lamps are used generally :

a) Non-luminous generators - One type is made in a similar way to an electric fire. In these heaters, the wire glows red thus giving some radiations in the visible region but peak emission in the short infrared. The ceramic material, being heated to a lower temperature than the wire, gives only infrared and no visible radiations. Some infrared lamps for therapy have the wire embedded in the insulating ceramic (or porcelain or fireclay) so that no visible radiations are given out. The heater wire can also be mounted behind a metal plate or inside a metal tube which does not become red-hot, but emit infrared in the same way. As such a lamp becomes hotter all the parts - the emitter, the metal plate on the end of the emitter, the protective wire mesh and the reflector become heated, giving of a range of wavelengths from near to far infrared. The infrared emitter is placed at the focus of a hemispherical or parabolic reflector to reflect the radiations into an approximately uniform beam. However, the beam does diverge somewhat due to the relatively large size of the emitter compared to the reflector, and this serves to reduce the risk of 'hot spots'. The reflector and emitter are mounted on a strong, firmly supported metal stand which can be adjusted to alter the height and angle of the reflector. When such lamps are switched on they require some time to warm up because of the thermal inertia of the considerable mass of metal and insulating material that has to be heated, thus small lamps may take about 5 min but larger one may take up to 15 min to reach maximum emission(Forster and Palastanga, 1985). In spite of the fact that lamps with an exposed coil will give of a red glow, they are collectively designated as non-luminous source to distinguish them from those that emit visible as well as infrared radiations, which were called 'luminous' lamps. The non luminious lamps produce radiation mainly between 3000-4000nm with about 10% between 1500nm to visible light (300nm-700nm).(John Low and Ann Reed 2000b).

b) Luminous generators - Luminous generators (incandescent lamps) consist of a tungsten filament in a large glass envelope which contains inert gas at low pressure. Part of the inside of the glass bulb is often silvered to provide a reflector. These lamps work on the same principle as a simple electric light bulb, the filament is heated to a high temperature (around 3000°C) by the current passed through it and so gives off a continuous spectrum in the infrared and visible regions. Oxidation of the filament does not occur because there is no oxygen present, only a trace of some inert gas. The peak emission occurs at near 1000 nm but radiation, extends from the long infrared throughout the visible to the ultraviolet wavelength. These latter radiations are absorbed by the glass and are not therefore transmitted by the lamp. Luminous generators are sometimes called 'radiant heat' generators, indicating that heating is by both infrared and visible radiations.

### 2.1.4 Therapeutic uses of infrared radiation

The therapeutic use of infrared radiation had long been based on the ability of its wavelength to be absorbed by tissue and produce heat. It is generally assumed that the infrared photons does not give rise to photochemical effects, thus the physiological effect of infrared are therefore, the result of local tissue heating (Sheila Kitchen 2000b). The penetration depth is the depth at which approximately 63% of the radiation energy has been absorbed and it differs with different wave length. Very long infrared wavelength (around 40000nm) behaves like microwave and penetrates several centimeters (John Low and Ann Reed 2000c). The radiation used therapeutically by the physiotherapist are mainly IRA and IRB in which at around 3000nm, the penetrating depth is about 0.1mm as compared to the maximum penetrating depth of 3mm at around 1000nm (Harlen 1980). The amount of energy received by the patient will then be governed by the intensity of the lamp output (measured in watts), the distance between lamp and patient (between 50-75cm) and the duration of treatment.

It's biological effect includes an increase in metabolic activities of superficial tissue due to direct effect of heat on chemical process, vasodilatation of skin cutaneous vessel, extensibility of collagen fibre after being heated and also pain relief. Therapeutically, the neurological effect may be due vasodilatation that brings cell and chemicals to assist healing and remove the breakdown product of injury. An increase in sensory nerve conduction, influence the sensory response via an increase in endorphins that affect the pain gate mechanism (Kitchen S and Partridge 1991). Infrared radiation has also been used in the treatment of psoriasis, on the grounds that moderate hyperthermia can affect cell replication and therefore could benefit a hyperproliferative disease like psoriasis. However, psoriasis is more commonly and effectively treated with ultraviolet.

More recently, there is a new intrest in light therapy. It has been shown that Light Emitting Diode Irradiation (LED) can stimulate wound healing. The wavelength responsible for this are those of visible red light and near infrared radiation (680-880nm). In a study by Whelan et al 2001, it is shown that exposure to these radiation results in, invitro increases of cell growth of 140-200% in mouse derived fibroblast, rat derived osteoblast and an increased growth of 155-170% of normal human epithelial cells. When these radiation were exposed to US submarine crew whose living condition are those of low oxygen, high carbon dioxide and low exposure to sunlight, laceration wound healed 50% faster than the control group. (7 days compared to 14 days in control). It provide low energy stimulation of tissue that results in increased cellular activity during wound healing. Process like fibroblast proliferation, synthesis of collagen and procollagen, macrophage and lymphocyte stimulation, Growth factor production ( TGFß, PDGF) and greater rate of extracellular matrix production have been reported with LED treatment. It was also demonstrated that mitochondria are receptive to monochromatic infrared radiation and that LED increases respiratory metabolism of the cells. (Whelan et al 2001, Tiina Karu 1999). Another surprise finding from Whelan et al study is that most photon at wavelengths of 630-800nm travel approximately 23cm through the skin surface and muscle.

### 2.1.5 Pulsed far infrared radiation.

The healing effect of far infrared were suggested by Rustam Rakhimov. Human emits infrared photons secondary to chemical/photochemical processes in our cell. Any pathological processes will lead to a change in the energy state of an organ. Being aware of these processes, it can be corrected using definite wavelengths. The wavelength of infrared radiation for medical applications should have quantum energy not exceeding that produced by man himself, as otherwise we may come across undesirable damaging processes. At the same time, it must be such that it could be used for equalization of photochemical processes of an organ, normalization of immune processes, dissolution of pathological formations (eg: collagen) and also destruction of bacteria.

His approach implies the creation of composite systems of energy conversion, on the basis of ceramic materials with desired properties which are capable of absorbing electromagnetic radiation of a broad spectrum and to release infrared in a narrow long-wave range. The ceramics are made by special multistage and very complicated process at temperature up to 3000°C in a solar furnace. It allows him to obtain ultra-pure materials. The process takes between 1-12 months. The ceramics is then applied to the surface of any source of light energy (eg; electric lamp) or any other heating source. When the primary source radiation is on, its energy is converted into long infrared wavelength of a desired narrow spectrum range. This process is called autocatalytic and occurs whenever the primary source of radiation is energised. The process of absorption and emission continues constantly throughout the ceramic layer, as a result of which the ceramics generates radiation of a definite frequency due to the absorption of 'disorganized' energy. The process of conversion is based on the fact that, as a result of miscellaneous wavelength energy absorption, certain photochemical reactions occurring in the ceramic material, increases the overall energy of the system. After reaching a saturation point (energy of activation or power barrier) the system reverts to the initial energy state, releasing the absorbed energy as a desired wavelength. The duration of every single process is about 1/100 microsecond.

The wavelength that is produced, is between 8-50 um, which is almost similar to the one produced by our body and it is determined by the type of ceramics used. The main objective of this method is to normalize physiological processes and eliminate pathologic ones by exposure to infrared radiation that is in resonance with the processes to be corrected. In other words, the radiation normalizes metabolic processes and eliminates the root cause of the disease and not only its manifestations ( Rakhimov R 2000).

## 2.2 Bone Morphology.

### 2.2.1 Introduction.

Bone can be studied in three ways, as a material, as a tissue, and as an organ. Bone as a *material* is a compound composite that is very resistant to compression, similar to reinforced concrete with the collagen fibrils representing the steel rods and the mineral matrix as the cement. Bone has remarkable properties. It is strong yet light, equal in bending strength to oak and in tensile strength to cast iron but three times lighter and ten times more flexible. Three factors contribute significantly to the material properties of bone (1) laminar construction of the cortex and trabeculae, (2) tubular design of long bones with radial distribution of the mass, and (3) internal reinforcement by a trabecular mesh.

Bone as a *tissue* consists of living cells embedded in a highly vascular, mineralized, osteoid matrix. Osteoblasts lay down osteoid seams with an average width of 12 micrometers. Bone is 92% solid and 8% water. *60%* of bone dry weight is mineral, and 40*%* is matrix (Table 2). Collagen is the major organic matrix component at *95%.* Osteocytes, osteoblasts, and osteoclasts make up about 3% of bone volume.

Table 2: General composition of bone.

| | |
|---|---|
| Solid | 92% |
| Water | 8% |
| Solid composition<br>Inorganic (Mineral)<br>Organic | <br>60%<br>40% |
| Mineral phase composition<br>Calcium<br>Phosphorus, Mg, Na | <br>60%<br>40% |
| Organic phase composition<br>Collagen<br>Cells<br>Non collagen proteins | <br>95%<br>3%<br>2% |

Bone as an *organ,* contains many different tissues such as cartilage, nerve, fibrous tissue, marrow, adipose tissue, and vascular tissue. As an organ, bone changes with age. Young bones are porous, flexible, and contain red hematopoietic marrow. Old bones are more rigid and have a fibrofatty marrow. Marrow can also serve as a source of bone cells. The blood vessels in marrow is critical to the circulatory system in bone thus, disorders or mechanical disruption of the marrow can affect the activities of bone and periosteal cells.

Bones are living, changing organs of support, locomotion, protection, and metabolism, constantly responding and remodeling to internal and external stimuli. They serves a variety of different functions : -

1. Act as internal support for the trunk and extremities and as a scaffold for the attachment of muscles and ligaments.
2. Bones protectively cover the brain, spinal cord, and also the thoracic organs.
3. They provide a home for hematopoletic tissue.
4. Bones function in mineral ion homeostasis and as an ion reservoir containing 99% of body calcium, *85%* of phosphorus, 66% of magnesium, and 60% of sodium.

2.2.2 Classification of bone tissue.

Bone may be classified on the basis of shape, macroscopic appearance, developmental biology, or microscopic organization. Each classification has certain advantages and disadvantages, but no one scheme completely satisfies all scientific and clinical needs.

Table 3: Classification of bone.

| | |
|---|---|
| Shape | Long, short, regular, irregular, flat |
| Macroscopic | Cortical(compact), cancellous(trabecular). |
| Developemental | Membranous, enchondral. |
| Microscopic | Woven, lamellar. |

*External geometry* is the basis for the shape classification. Bones are grouped as long or short, flat or tubular, regular or irregular.

*Visual Inspection* is the basis for a macroscopic classification of bones into three types ie. cortical, cancellous, and fine cancellous. Cortical (or compact) bone comprises the diaphysis of long bones and the cortex of all others. Most of the skeletal mass (80% by weight) is cortical bone, but most of the skeletal volume is cancellous bone. Cancellous (or trabecular) bone occurs in the metaphysis of long bones and in the interior of flat bones such as the scapula or the innominate. Fine cancellous bone, found only in the embryo, makes up both the diaphysis and metaphysis of newly ossified embryonic bone.

*Origin* is the basis for a developmental classification of bones, which are intramembranous and endochondral. Intramembranous bones (skull and clavicle) are formed by osteoblasts that differentiate directly from a membranous periosteum. Endochondral bones, which are the majority, initially appear as cartilage models that are converted to bone.

*Matix* and cellular arrangement form the basis for a microscopic classification of bone into two types ie. woven bone and lamellar bone (figure 2). Woven bone is the first bone made at the growth plate, in fracture callus, and in certain pathological conditions such as Paget's disease. Cells and collagen fibrils assume an irregular and random arrangement in woven bone. It contains about 4 times as many osteocytes per unit volume. The matrix is cellular and stains unevenly and patchily with basophilic dyes. Lamellar bone replaces woven bone in the process of remodeling.

Lamellar bone makes up the cortex and trabecula of mature bone and is subdivided into Haversian lamellar bone and non-Haversian lamellar bone. All lamellar bone has an orderly cellular distribution and regularly arranged collagen fibrils laid down within osteoid seams. In each successive osteoid layer, the collagen fibrils differ in the angle of fibril orientation, similar to plywood in appearance and function. Lamellae of Haversian bone are circumferentially arranged around a central vascular canal, and individual osteocytes communicate with the vessels in the central canal by cellular processes running through an elaborate system of canaliculi.

Cancellous or trabecular bone has few if any Haversian systems. The construction consists of three or four lamellae sandwiched together to form a three-dimensional osseous lattice arranged along lines of force. Since the trabecular width is small, canaliculi communicate directly with capillaries in the medullary canal, so blood vessels do not course through trabeculae , but they run along the side.

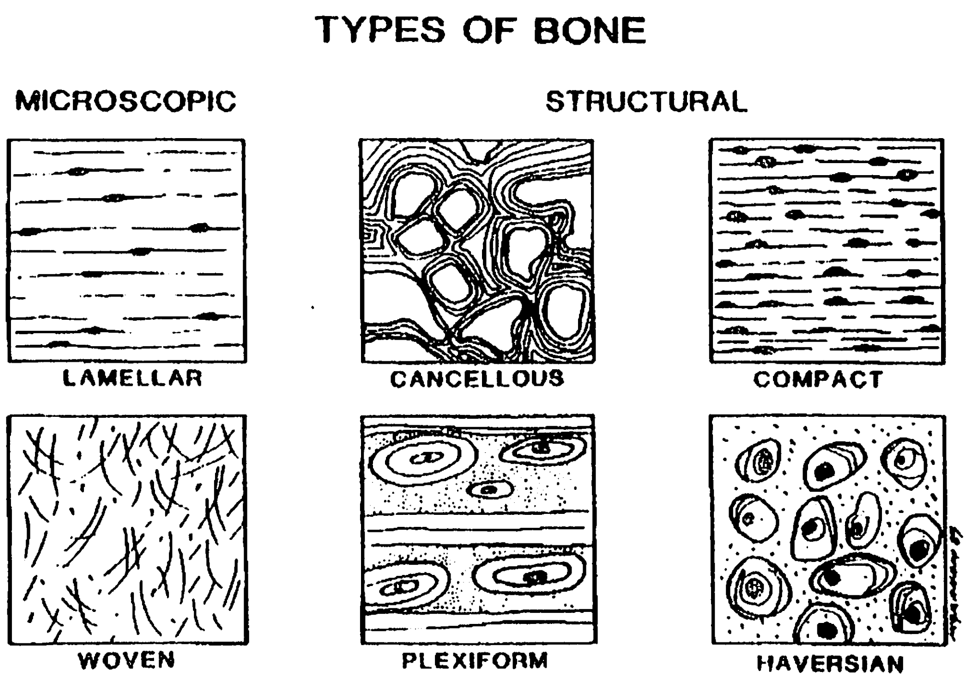



Figure 2 – Types of bone
(Source : Miller Review of orthopaedics 2nd Edt, Chapter 1)

### 2.2.3 Bone structure.

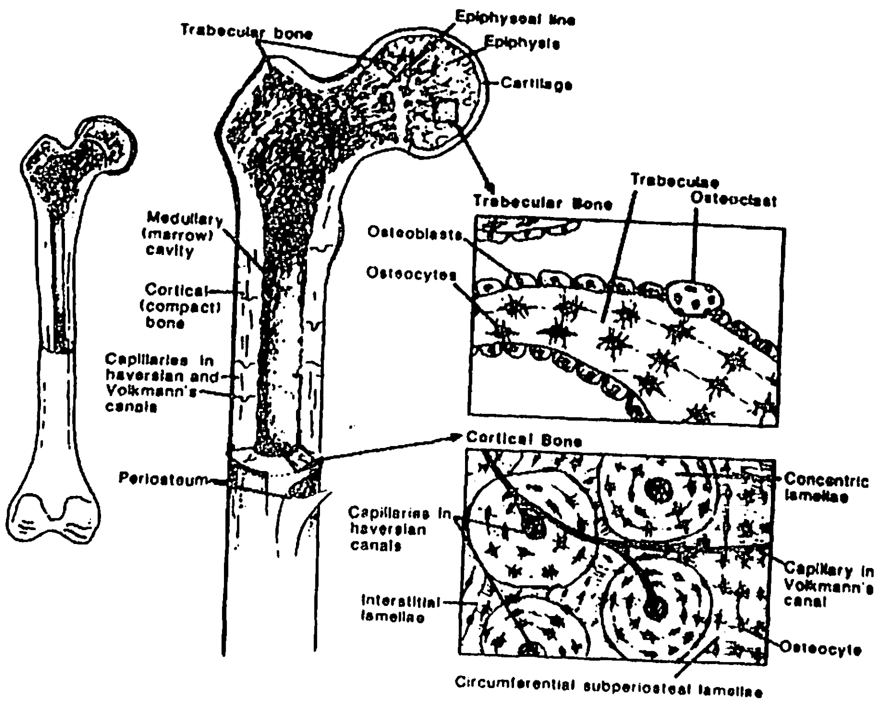
Bones are a mixture of cortical and cancellous tissue. As stated before, cortical and cancellous tissue have identical composition but different morphology. In a long bone, cortical tissue of the diaphysis has the texture of ivory and is arranged as a hollow cylinder to best resist bending forces. The metaphysis flares to increase volume and surface area, thus decreasing stresses at contact points (joints). Cancellous tissue within the metaphysis is a meshwork of interlocking longitudinal and perpendicular plates arranged along internal lines of *force.* Cancellous plates support a thin layer of subchondral cortical bone and distribute weight-bearing and joint reaction forces into the bulk of bone tissue.

The structure of both cortical and cancellous bones changes in response to applied loads, immobilization, hormonal influences and other factors. Cancellous bone has approximately 20 times more surface area per unit of volume than does cortical bone. Its cells lie primarily between lamellae or on the surface of trabeculae, where they can be directly influenced by adjacent bone marrow cells.

Table 4 – Differences between Cortical and Cancellous bone.

| | Cortical | Cancellous |
|---|---|---|
| Skeletal mass | 80% | 20% |
| Mechanics | Rigid | Flexible |
| Cells | Osteocyte in lacunae | Osteocyte in lacunae |
| Osteocyte arrangement | Radial into osteones | Linear along trabaculae |
| Matrix | Mineralised osteoid | Mineralised osteoid |
| Blood supply | Penetrating vessel | Surface vessel |
| Surface area-volume ratio | Low | High |
| Mineral metabolism | Minor role | Major role |

Both cortical and cancellous tissues are lamellar bone, but cortical tissue is Haversian lamellar bone, whereas cancellous tissue is non-Haversian lamellar bone. Haversian lamellae make up the functional units of cortical bone, the Haversian system, or the *osteone.* Each osteone is a series of concentric lamellae constructed around a central Haversian canal containing small arterioles, venules, and nerves. The long axis of the osteone is parallel to the long axis of the bone. Haversian canals communicate with the medullary cavity by transverse or oblique Volkmann's canals. ( Figure 3)




<u>Figure 3 – Cortical and trabecular bone demonstrating the various microstructures.</u>
(Source : Miller Review of orthopaedics 2nd Edt, Chapter 1)

Within the circular lamellae, osteocytes live in the seclusion of their own separate lacunae, sending multiple cytoplasmic extensions running through canaliculi. The canaliculi are the lifelines of osteocytes, providing communication channels to other osteocytes and nutrition conduits to the central Haversian canal for metabolic exchange, ion metabolism, and hormone delivery.

Osteones vary in size and shape, but on the average, each cylindrical osteone contains six concentric lamellae (range 3-15), and each lamella is *5-7* micrometers thick. The theoretical maximum diameter of an osteone is about 150 micrometers, a distance set by the limits of nutrient diffusion from the central canal. Osteones also vary in degree of mineralization. Older osteones contain more mineral salts and show greater microradiographic density than young osteones, and the lamellae closest to the central canal contain more mineral than the peripheral lamellae. A basophilic cement line delineates the peripheral limits of an osteone and bonds it to its neighbors. The cement line is 1 micrometer thick and has a different composition from the surrounding matrix, being high in glycosaminoglycan and noncollagen proteins. Both primary and secondary osteones have cement lines.

A primary osteone (primary Haversian) is the first Haversian system to be formed on calcified cartilage bars or in woven bone. Secondary osteones replace primary osteones during the remodeling process. Secondary osteones (secondary Haversian systems) develop from resorption tunnels made by cutting cones during the process of remodeling. Non-Haversian lamellae make up trabeculae of the metaphysis and medullary canal plus the area between osteones, including the outer and inner surfaces of bone. Lamellae encircling the outside and inside of a bone are called the outer and inner circumferential lamellae. Osteones between the circumferential lamellae are interstitial lamellae.

2.2.4 Bone surfaces and membrane

A surface that divides space into inside and outside has been referred to as an envelope. Each long bone has three surfaces enclosed within an envelope of two membranes. The three surfaces are the periosteal surface, the endosteal surface, and the Haversian surface. The two membranes are the periosteum on the outside and the endosteum on the inside.

The periosteum is a two-layered membrane. it covers the external surfaces of bones except in the regions immediately around or within synovial joints. The outer fibrous layer consists of dense white fibrous tissue and elongated, highly differentiated fibroblasts, the inner cambium layer is the germinal layer and contains reticulin and elastic fibers, fibroblasts, plus undifferentiated