THE POTENTIAL OF SELECTED BORNEAN BUFONIDS (AMPHIBIAN: ANURAN: BUFONIDAE) AS BIOLOGICAL DRESSING IN WOUND HEALING MANAGEMENT

Suzila anak Bamic @ Jimik (28415)

Bachelor of Science with Honours
(Animal Resource Science and Management)
2013
THE POTENTIAL OF SELECTED BORNEAN BUFONIDS (AMPHIBIAN: ANURAN: BUFONIDAE) AS BIOLOGICAL DRESSING IN WOUND HEALING MANAGEMENT

SUZILA ANAK BAMIC @ JIMIK (28415)

This thesis is submitted in partial fulfilment of the requirement for the Degree of Bachelor of Science with Honours in Animal Resource Science and Management

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2013
DECLARATION

I hereby declare that this Final Year Project Report 2013 is based on my original work except for quotations and citations, which have been acknowledged as well, I declare that no portion of this dissertation has been submitted in support of an application for any other degree at UNIMAS or other institution of higher learning.

__________________________
SUZILA ANAK BAMIC @ JIMIK
Animal Resource Science and Management Programme
Department of Zoology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak (UNIMAS)
ACKNOWLEDGEMENT

Praise the Lord for the strengths and His blessing in completing this final year project. Special appreciation I offer to my supervisor, Dr. Ramlah Zainudin, who has supported me throughout my final year project research with her patience and knowledge. Not forgotten, my appreciation to my co-supervisors, Prof. Dr. Ahmad Hata Rasit and Dr. Dayangku Norlida for their support and knowledge regarding this research project.

I would like to express my appreciation to the Department of Zoology, Faculty of Resource Science and Technology and also to the Department of Pathology, Faculty of Medicine and Health Sciences for their kindness and willingness to give me the permission to do the necessary research work and to use departmental tools at their laboratory. Not forgotten, thanks to the project grant (FRGS/04(03)/840/2012(80)) in completing the research study. My acknowledgement also goes to the staffs of the laboratory of Department of Zoology and Department of Pathology for their full co-operations throughout the research work.

Sincere gratitude to Miss Elvy Quatrin, a postgraduate student who has helped me a lot in this research work. Special thanks to my research mate, Doris anak Tang and Nur Amirah bt Md Sungif for their kindness and willingness to help me during the research work. Thanks for the friendship and memories.

Last but not least, my deepest gratitude to my beloved parents, Mr. Bamic Singi and Mdm. Toning Nyumbib and also to my beloved siblings for their prayer, moral support and encouragement. Not forgotten, to those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.
# TABLE OF CONTENTS

Declaration ............................................................................................................. i  
Acknowledgement ............................................................................................... ii  
Table of Contents ................................................................................................. iii  
List of Abbreviations ............................................................................................ v  
List of Tables ......................................................................................................... vi  
List of Figures ....................................................................................................... vii  
Abstract ................................................................................................................ ix  
Abstrak .................................................................................................................. ix

## Chapter 1  Introduction & Objectives ......................................................... 1
  Introduction ........................................................................................................... 1  
  Objectives ............................................................................................................ 2

## Chapter 2  Literature Review ................................................................. 3
  2.1 Family Bufonidae ......................................................................................... 3  
  2.2 Anatomy of the Skin ................................................................................... 4  
  2.3 Histological Technique ............................................................................. 6  
  2.4 Frog Skin in Wound Healing ..................................................................... 7  
  2.5 Rat for Animal Experimentation ................................................................. 8  
  2.6 Wound Measurement Assessment ............................................................. 9  
  2.7 Wound Healing in Rats .............................................................................. 11  
  2.8 Wound Healing Process ............................................................................ 11

## Chapter 3  Materials and Methods ....................................................... 14
  3.1 Field Work ................................................................................................. 14  
  3.2 Laboratory Work ....................................................................................... 15  
    3.2.1 Wound Healing Assessment ............................................................... 15  
    3.2.1.1 The Preparation of Toad Skin ...................................................... 15
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.1.2</td>
<td>The Excision of Skin Wounding in Rat</td>
</tr>
<tr>
<td>3.2.1.3</td>
<td>Wound Monitoring</td>
</tr>
<tr>
<td>3.2.1.4</td>
<td>Wound Healing Analysis</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Histological Techniques of the Skin Wound</td>
</tr>
<tr>
<td>3.2.2.1</td>
<td>Skin Fixing</td>
</tr>
<tr>
<td>3.2.2.2</td>
<td>Skin Grossing</td>
</tr>
<tr>
<td>3.2.2.3</td>
<td>Skin Processing</td>
</tr>
<tr>
<td>3.2.2.4</td>
<td>Skin Embedding</td>
</tr>
<tr>
<td>3.2.2.5</td>
<td>Skin Sectioning</td>
</tr>
<tr>
<td>3.2.2.6</td>
<td>Skin Staining</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Histological Slide Assessment</td>
</tr>
</tbody>
</table>

**Chapter 4**

**Results and Discussion**

4.1 Results

4.1.1 Wound Surface Area Measurement

4.1.2 Histological Analysis

4.1.2.1 Inflammation Rate

4.1.2.2 Granulation Tissue Formation

4.1.2.3 Epidermal Closure (Re-epithelialization)

4.1.2.4 Dermal Closure (Contraction)

4.2 Discussion

**Chapter 5**

**Conclusion and Recommendations**

5.1 Conclusion

5.2 Recommendations

**References**

**Appendices**
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dH₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>DPX</td>
<td>Distrene, Plasticiser, Xylene</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin</td>
</tr>
<tr>
<td>IP</td>
<td>Intra-Peritoneal Inoculation</td>
</tr>
<tr>
<td>KTX</td>
<td>ketamine/xylazine solution</td>
</tr>
<tr>
<td>mm²</td>
<td>millimeter square</td>
</tr>
<tr>
<td>R</td>
<td>Radius</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The histological skin cell parameters for assessment of wound healing (Braiman-Wiksman, 2007).</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Profile on the tissue processor</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>H &amp; E staining protocol (Brown, 2002).</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Explanation of used modified scale for the semi-quantitative evaluation of histological sections for inflammation parameter.</td>
<td>23</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Map of sampling site, point A indicates Gunung Gading National Park and point B indicates Batang Ai National Park.</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>A small incision was made at the dorsum part beneath the skin of the rats by using punch pliers</td>
<td>16</td>
</tr>
<tr>
<td>3a</td>
<td>The histological slide that was measured from <em>A. spinulifer</em>-treated wound.</td>
<td>24</td>
</tr>
<tr>
<td>3b</td>
<td>The histological slide that was measured from <em>P. juxtasper</em>-treated wound.</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>The progress of cutaneous wound healing in untreated wound, normal dressing wound (dH$_2$O) and <em>A. spinulifer</em>-treated wound.</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>The progress of cutaneous wound healing in untreated wound, normal dressing wound (dH$_2$O) and <em>P. juxtasper</em>-treated wound.</td>
<td>27</td>
</tr>
<tr>
<td>6a</td>
<td>Scanned histological slide for Day 3 experiment</td>
<td>28</td>
</tr>
<tr>
<td>6b</td>
<td>Scanned histological slide for Day 7 experiment</td>
<td>29</td>
</tr>
<tr>
<td>6c</td>
<td>Scanned histological slide for Day 14 experiment</td>
<td>29</td>
</tr>
<tr>
<td>7a</td>
<td>Wound slide of biological dressing (<em>A. spinulifer</em>), normal dressing and untreated for day 3 period of treatment.</td>
<td>31</td>
</tr>
<tr>
<td>7b</td>
<td>Wound slide of biological dressing (<em>A. spinulifer</em>), normal dressing and untreated for day 7 period of treatment.</td>
<td>32</td>
</tr>
<tr>
<td>7c</td>
<td>Wound slide of biological dressing (<em>A. spinulifer</em>), normal dressing and untreated for day 14 period of treatment.</td>
<td>33</td>
</tr>
<tr>
<td>8a</td>
<td>Wound slide of biological dressing (<em>P. juxtasper</em>), normal dressing and untreated for day 3 period of treatment.</td>
<td>35</td>
</tr>
<tr>
<td>8b</td>
<td>Wound slide of biological dressing (<em>P. juxtasper</em>), normal dressing and untreated for day 7 period of treatment.</td>
<td>36</td>
</tr>
<tr>
<td>8c</td>
<td>Wound slide of biological dressing (<em>P. juxtasper</em>), normal dressing and untreated for day 14 period of treatment.</td>
<td>37</td>
</tr>
</tbody>
</table>
9a  A graph of wound radius of *A. spinulifer* experiment in day 3.  
9b  A graph of wound radius of *A. spinulifer* experiment in day 7.  
9c  A graph of wound radius of *A. spinulifer* experiment in day 14.  
10a A graph of wound radius of *P. juxtasper* experiment in day 3.  
10b A graph of wound radius of *P. juxtasper* experiment in day 7.  
10c A graph of wound radius of *P. juxtasper* experiment in day 14.  
11a Comparison between the scales of the inflammation for the wound treated with biological skin of *A. spinulifer*, normal dressing wound and untreated wound.  
11b Comparison between the scales of the inflammation for the wound treated with biological skin of *P. juxtasper*, normal dressing wound and untreated wound.  
12a Comparison between the percentages of the granulation tissue for the wound treated biological skin of *A. spinulifer*, normal dressing wound and untreated wound.  
12b Comparison between the percentages of the granulation tissue for the wound treated with biological skin of *P. juxtasper*, normal dressing wound and untreated wound.  
13a Comparison between the percentages of the epidermal closure for the wound treated with biological skin of *A. spinulifer*, normal dressing wound and untreated wound.  
13b Comparison between the percentages of the epidermal closure for the wound treated with biological skin of *P. juxtasper*, normal dressing wound and untreated wound.  
14a Comparison between the percentages of the dermal closure for the wound treated with biological skin of *A. spinulifer*, normal dressing wound and untreated wound.  
14b Comparison between the percentages of the dermal closure for the wound treated with biological skin of *P. juxtasper*, normal dressing wound and untreated wound.
THE POTENTIAL OF SELECTED BORNEAN BUFONIDS (AMPHIBIAN: ANURAN: BUFONIDAE) AS BIOLOGICAL DRESSING IN WOUND HEALING MANAGEMENT

Suzila anak Bamic @ Jimik

Animal Resource Science and Management Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

This study was conducted to assess the wound healing potential of selected species in family Bufonidae. The assessment of the healing process was compared with the normal dressing wound by using dH_2O and the untreated wound. The study was conducted on day 3, day 7 and day 14. Three laboratory rats were utilised for each day of the experiment, for the purpose of the study. Ruler measurement data were collected and histological study were done. Histological sections of the skin specimens were stained by hematoxylin and eosin. Ruler measurement data suggested that the wound treated with skin *Ansonia spinulifer* healed faster than *Phrynoidis juxtasper*. However, histological evaluation suggested that both species *A. spinulifer* and *P. juxtasper* could not be justified their healing potential due to some limitations, such as no replication of the experiment, difficulty in handling the rats and error during analysing the histological slides.

**Key words:** wound healing, potential, Bufonidae, histological, limitations

ABSTRAK


*Kata kunci:* penyembuhan luka, potensi, Bufonidae, histology, batasan
CHAPTER 1

INTRODUCTION

1.1 Background of Study

Frog skin is said to be useful in wound healing management. Frog skin can accelerate wound healing faster than the commercial wound dressing available at the market due to its microbicidal peptides content (Raghavan et al., 2010). According to Mashreghi et al. (2013), from the latest century, traditional medicine which used the frog skin as a biological dressing in Vietnam and South America had showed good effects in wound healing. Frog skin secretions also have wound healing properties and can reduce inflammation (Mashreghi et al., 2013). But most of the research study of the anuran skin in wound healing is on the frog species while there is still no research study on the toad skin in wound healing management. Therefore, the selected species in family Bufonidae were chosen to screen their skin potential in wound healing management via histological method.

Wound radius reduction rate is determined by estimating the wound area (multiplying the two longest diameters) and then calculating the radius of the wound and plotting changes over time (Zimny et al., 2003). According to Zimny et al. (2003), the wounded area (A, in mm$^2$) was measured its maximum length and width and calculated its wound radius (R) by the equation $R = \sqrt{A/\pi}$.
During the research study, the incision of the wound area was done by using punch pliers. This gives an accurate and similar surface area measurement (25mm$^2$) of each of the wound area. The finding suggested that the wound treated with skin of *A. spinulifer* healed faster as compared to the normal dressing and the untreated wound. However, the wound treated with skin of *P. juxtasper* healed slower than the normal dressing wound and the untreated wound. The untreated wound healed the fastest. Differ from the histological analysis which based on the four parameters, the overall finding suggested that both species of Bufonidae was not effective for the wound healing assessment.

1.2 Objectives

The objectives of the research are:

(i) to screen the potential of Bornean Bufonids skin of selected species among family Bufonidae in wound healing; and

(ii) to assess the healing process by determining the healing time and comparing structure between untreated, normal and biological wound dressing via histological study.
CHAPTER 2

LITERATURE REVIEW

2.1 Family Bufonidae

Bufonidae is known as true toads, from the order Anura (frogs and toads). This family has a world-wide distribution, but Borneo and Southeast Asia have a distinctive set of genera and species (Inger & Stuebing, 2005). There are a few toads that have adapted to drier environments such as deserts by burrowing (Skeel, 2011).

Toads of the family Bufonidae have a thick, warty and a glandular skin (Frost, 2009). They possess large parotoid glands on the side of their heads and have rather short hind limbs than the frogs. According to Frost (2009), the parotoid gland releases an alkaloid poison when the toads are in stress condition. The poison in the glands contains a number of toxins which cause different effects to the particular predator (Frost, 2009). They also have pectoral or shoulder girdles that are not fused together and have no maxillary teeth (Skeel, 2011). They usually feed on insects and other invertebrates. According to Skeel (2011), some species in family Bufonidae are not harmful, but important members of their ecosystems and food chain which serves as both predators and preys.

Genus *Phrynoidis* was recently removed from the synonymy of *Bufo* by Frost *et al.* (2006). There are two species in this genus which are *Phrynoidis aspera* and *Phrynoidis juxtasper* (Frost *et al*., 2006). *P. aspera* is one of the two large river toad
species and also known as River Toad (Haas & Das, 2012). The other species in Genus *Phrynoidis* is *P. juxtasper*. It is a very large toad and also known as Giant River Toad. The males can reach add up to 120 mm, while the females 215 mm snout-vent length and it can get as big as a small rabbit (Haas & Das, 2012). Haas and Das (2012) stated that the toads secrete large amounts of highly toxic, milky poison from their warts when molested. The skin, eggs and the tadpoles of *P. juxtasper* are poisonous (Haas & Das, 2012). *P. juxtasper* is a good swimmer and jumper as well, as stated in Haas and Das (2012). *P. juxtasper* is an adaptable species and tolerance to a degree of habitat modification (Inger *et al.*, 2004a).

Genus *Ansonia* is relatively small, slender toads with dry, rough skin but lack of parotoid gland (Inger & Stuebing, 2005). Most species have slender, elongated bodies and relatively long legs. According to Inger and Stuebing (2005), the species in Genus *Ansonia* have snout which usually projects beyond the mouth. The adults are occasionally seen on the leaves of herbaceous plants and usually will move to clear, rocky streams to breed (Inger & Stuebing, 2005). Species *Ansonia spinulifer* is common lowland stream toad. The adult males are usually 30-40 mm snout-vent length, while the females are up to 45 mm (Haas & Das, 2012). Genus *Ansonia* is much smaller than genus *Phrynoidis*. *A. spinulifer* is unable to adapt to modified habitats (Inger *et al.*, 2004b).

### 2.2 Anatomy of the Skin

Bufonidae have a rough, drier skin with warts, live on land and use water for breeding purposes. They have large parotoid gland behind their eyes. Amphibian skin is morphologically, biochemically and physiologically complex organ which
fulfils a wide range of functions necessary for the organism’s survival (Govender et al., 2012). The skin is highly vascular which facilitates dermal respiration.

Amphibians (frogs and toads) rely upon their skin to breathe for the exchange of oxygen and carbon dioxide with their environment (Chiasson & Underhill, 1951). Toads spend most of their time on land and therefore are in danger of desiccation (Chiasson & Underhill, 1951). The skin provides a defence against bacteria, fungi and other invaders. The glands in the skin of amphibian produce substances that are toxic to other animals. The chemical compounds secreted by the glands play various roles, either in the regulation of physiological functions of the skin or in the defence against predators and/or pathogens (Govender et al., 2012). Bettin and Greven (1986) also stated that the granular glands are the site of synthesis of a wide range of chemical compounds which provide protection against bacterial and fungal infection as well as predators. In *Bufo*, the granular gland cells are covered over the skin surface or arranged in enlarged clusters which forming discrete, compact glands such as the parotoid glands (Clarke, 1997). The skin glands produce a range of biochemical active compounds that may cause death to mammalian. Daly et al. (1987) stated that the main categories of secretions include biogenic amines, bufogenines and bufotoxins (steroids), alkaloids and peptides. Thus, the extraordinary range of biochemical found in amphibian granular gland secretions and the high probability of their containing compounds with a novel molecular structure as well as their clinically useful function, makes the amphibians an additional target group for medicinal purpose (Clarke, 1997).

Cei et al. (1972) found that the biogenic amines in the skin of *Bufo* are varied between species and subspecies. Meanwhile, bufogenines and bufotoxins have
cardioacceloratory properties which could increase the strength of heart beat and
decrease the heart rate (Clarke, 1997). Clarke (1997) stated that dried toad skin
secretions have been used in Oriental medicine for the past 3000 years and were
introduced into Europe in the 1600s, while Habermehl (1981) stated that many of the
bufogenines and bufotoxins were used as a marked effect for local anaesthetic.
According to Daly et al. (1987), alkaloids are found mainly in poison dart frogs
(family Dendrobatidae), however they also can be found in toads such as short-
headed toad (Brachycephalus ephippium) and cane toad (Bufo marinus). Clarke
(1997) stated that the literature suggested that peptides may be either absent (Cei et
al., 1968) or present in small amounts (Daly & Witkop, 1971) in Bufo species,
because the peptides found mainly in frog species.

In fact, toad and frog skin extracts have been used in medicinal purpose.
Gomes et al. (2007) stated that frog and toad skin has probably been the most
exploited for their antimicrobial components. It is said that the granular glands
produce secretions that might be effective against microbial and fungal infections.

2.3 Histological Technique

Histology is the scientific study of biological tissues via histological
techniques. Histological technique is a branch of biology which concerned with the
demonstration of minute tissue structures in living organism. It is carried out by
examining a thin slice of tissue under a light microscope or an electron microscope
(Kiernan, 2008).
Histological stains are often used in order to examine structural details (Kiernan, 2008). During the preparation for histology slides, hematoxylin and eosin (H&E) staining protocol will be used. According to Kiernan (2008), hematoxylin solution is used for nuclear staining while eosin solution for cytoplasmic staining. Hematoxylin usually gives staining blue or blue-purple in colour while eosin gives staining pink or pink-red in colour.

2.4 Frog Skin in Wound Healing

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers (Mercandetti, 2011). Wound healing involves a complex series of interactions between different cell types (Mackay & Miller, 2003). Each phase of wound is different even though the process is continuous. The main purpose in wound management is to heal the wound in the shortest time possible without making or causing the patient suffering from the pain.

Traditional healers in India use the dorsal skin of frogs from the genus *Hoplobatrachus* to cover the wounds of their patients (Govender et al., 2012). According to Govender et al. (2012), the lack of adequate medical supplies to treat napalm burns during the Vietnam War in the 1960s lead surgeons to investigate the traditional Vietnamese remedies for burns. They found that the use of amphibian skins from the genus *Hoplobatrachus* as temporary grafts for patients with severe skin loss was a successful means of treatment. The testing of the grafts to the rats showed that experimental wounds dressed with frog skin healed much faster than the wounds dressed with cotton gauze (Govender et al., 2012).
Amphibian skin is a hidden treasure with exciting biomedical potential (Sai & Babu, 1998). Using frog skin as wound healer had been practically used as traditional medicine in India (Sai et al., 1995). Another proof had been shown when a faster healing was observed in the experimental study which was conducted on skin wounds from female albino rats that was dressed with the dorsal skin of freshly sacrificed frogs as compared to cotton gauze. Raghavan et al. (2010) also found out that the frog skin possess lipid components with pharmaceutical and therapeutic potential. The current study had expected the role of frog skin lipids in the inflammatory phase of wound healing.

2.5 Rat for Animal Experimentation

Rodents are the most common type of mammal used in experimental studies. Many researchers have been using rats, mice, guinea pigs and hamsters as their study organisms. Rat is the most used as an animal experimentation due to its similarity to human in terms of genetics, anatomy and physiology (Simmons, 2008). Simmons (2008) stated that rats are being used abundantly in research studies due to their genomes which are similar to that of humans, easy to handle, highly reproductive rates and relatively low cost of use.

Rats give an excellent model for skin wound healing because it provides a standard size, type, shape and the depth of the wound injury and thus make it possible to compare the data between studies healing in all mammalian species (Dorsett-Martin & Wysocki, 2008). Dorsett-Martin & Wysocki (2008) also stated that the rat is often selected for skin wound healing models due to its availability,
low cost and small size, which result in a more economical and efficient use of limited laboratory space and housing facilities.

2.6 Wound Measurement Assessment

Wound assessment is very important in wound healing management. It is a complex process which included wound appearance, wound aetiology, prediction and monitoring of healing rates, identifications of factors delaying healing and wound documentation (Shaw & Bell, 2011). Wound measurement is needed in order to investigate the healing time of that particular wounded area. According to Fette (2006), the assessment of wound measurement is important in order to monitor the progress of healing process through the changes occurring in the length, width, area or volume of a wound. Wound measurement is an important component to provide a baseline measurement and accurately determine the percentage of reduction or increase in wound area over time (Shaw & Bell, 2011).

Wound measurement should be done routinely as the size of a wound is considered as one of the main indicators in order to look for the progress in healing time. According to Shaw and Bell (2011), wound measurement techniques can be categorised as contact or non-contact in their application. Tracing of the wound, the used of depth gauges and volume measurement by using casts or saline are the examples of contact techniques (Shaw & Bell, 2011). Non-contact techniques involving the use of the structured light and lasers, photography, video image analysis, magnetic resonance imaging and stereophotogrammetry (Williams, 1997).
Simple wound measurement method is one of the popular methods. It is the simplest and cheapest method to calculate the wound surface area which include the technique that measure the length and width measurement by using a ruler (Fette, 2006; Shaw & Bell, 2011). This method assumes that the wound has a geometric surface shape, as for example, surface area of a circle is measured by (diameter x diameter), while for an oval, (maximum diameter x maximum diameter) perpendicular to the first measurement (Fette, 2006). According to Shaw and Bell (2011), in order to determine the wound surface area, two important issues are taken: the identification of the wound margin (typically using a wound tracing or alternatively a digital image) and the calculation of wound area. Another popular method in determining the wound area is by tracing method. Wound area also can be determined by tracing the outline of the wound (wound circumference) onto a grid or graph paper with 1cm squares area and the number of squares of a known area are counted (Shaw & Bell, 2011). However, the accuracy of area measurement by using this type of method depends on correct and consistent identification of the wound margins (Chang et al., 2011).

Zimny et al. (2003) state that in order to determine the wound radius reduction rate, the wound area (multiplying the two longest diameters) is estimated and then plotting the mean wound radius against time to investigate the time course of wound healing, thus the slope of the regression curve is interpreted as the daily reduction of the mean wound radius. The mean wound radius (R) is calculated from the mean wound area (A, in mm²) by the equation \( R = \sqrt{A/\pi} \) (Zimny et al., 2003). Thus, by taking the measurement of wound radius, the wound healing time can be measured.
2.7 Wound Healing in Rats

Rats have been widely used in the study of skin wound healing and the efficacy of different treatment model (Dorsett-Martin, 2004). Dorsett-Martin (2004) said that rat is chosen as the model due to its availability, low cost and small size. According to Vidinsky et al. (2006), the rat skin structure is similar to human skin. The specific structural characteristics may vary depending on the body region (Marcelo et al., 2003).

Most of the studies are using rat’s dorsum part as the wound location. According to Vidinsky et al. (2006), the skin of the dorsum of a normal rat is formed by epidermis, dermis and subcutaneous striated muscle. Thus, the healing of three different tissue layers can be studied due to the ability of proliferation. Only the epidermis has the capability to regenerate (Vidinsky et al., 2006).

According to Kumar et al. (2003), the complete regeneration of the rat epidermis is finished on the fifth day after the surgery, which is comparable to human being. The process of inflammation proliferative and remodelling phases in rat are faster than in human but comparable (Kumar et al., 2003). Therefore, it is also important to know the histological study of wound healing in rat skin.

2.8 Wound Healing Process

A wound is defined as damage or disruption to the normal anatomical structure and function (Velnar et al., 2009). Normal wound healing is a dynamic and complex process which involves the restoring of cellular structures and tissue layers
(Mercandetti, 2011; Velnar et al., 2009). Velnar et al. (2009) stated that a completely healed wound is one that has been returned to a normal anatomical structure, function and appearance of the tissue within a reasonable period of time. Mercandetti (2011) stated that the human adult wound healing process can be divided into three different phases; the inflammatory phase, the proliferative phase and the remodelling phase. The inflammatory phase is marked by platelet accumulation, coagulation and leukocyte migration, while proliferative phase is characterized by re-epithelialization, angiogenesis, fibroplasias and wound contraction, whereas the remodelling phase takes place in which the dermis responds to injury with the production of collagen and matrix proteins (Kirsner & Eaqlstein, 1993).

Braiman-Wiksman et al. (2007) stated that the initiation of healing is primarily dependent on the epidermal migration which derives re-epithelialization. This parameter is also known as epidermal closure. Re-epithelialization is widely accepted to be one of the major processes in wound healing that ensures a successful repair (Martin, 1997). According to Braiman-Wiksman et al. (2007), re-epithelialization can be observed through the hyperplastic epidermis at the wound edges, constitutes an essential component of the migrating cell pool which migrates to seal the wound gap. The migration of the keratinocytes towards the gap of the wound had provided a basis for another stage, such as granulation tissue formation (Braiman-Wiksman et al., 2007).

Granulation tissue formation was considered fully formed which is 100% when a continuous layer of granulation tissue formed across the entire wound gap and the layer of granulation tissue filled the entire wound depth (Braiman-Wiksman et al., 2007).
Another major process involved in the early stages of healing is the inflammation which is related to the inflammatory response (Jones et al., 2004). According to Kirsner and Eaqlstein (1993), the initial inflammatory response involves the recruitment of cells that fight potential bacterial contamination of the wound and activate cytokine secretion in order to activate dermal and epidermal processes. However, the inflammation should not go beyond a certain level; otherwise it will cause healing impairment.

In the final stages of healing, remodelling occurred in which the wounds are completely re-epithelialized and the final process is the dermal reorganization or dermal closure (Braiman-Wiksman et al., 2007). At this stage, the wounded skin regains its strength and elasticity and thus proceeds to the reorganization of the collagen and elastic fibers for the final reconstruction of the dermis (Braiman-Wiksman et al., 2007). Table 1 shows the histological skin cell parameters for assessment of wound healing according to (Braiman-Wiksman et al., 2007).

<table>
<thead>
<tr>
<th>Healing Parameter</th>
<th>Assessment Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal closure</td>
<td>Basal layer of the epidermis. To assess the newly formed epidermis.</td>
</tr>
<tr>
<td>Granulation tissue formation</td>
<td>Fibroblast, new blood vessels</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Inflammatory cells</td>
</tr>
<tr>
<td>Dermal closure</td>
<td>Abscess matrix remodelling</td>
</tr>
</tbody>
</table>