1 Introduction to the Research

This research project was designed with the **aim** of finding treatments that can be used by rehabilitators to save flying-foxes such that they are releasable[2]. Once released flying-foxes need to be able to travel long distances on a daily basis (up to 500km in two days (Roberts, Catterall, Eby, et al., 2012)). The **objective** was to identify effective and efficient treatments. An effective treatment enables the injuries to heal so that full flight is achieved. Full flight includes the ability to gain height, manoeuvre, cope with strong winds and travel long distances. Efficiency is assessed in terms of: time to complete healing, stress to the flying-fox, ease of treatment, and, cost for the rehabilitator. Both efficiency and efficacy will affect the final recommendation in relation to treatment.

The **hypotheses** are that:

- all treatments will achieve healing, and,
- some treatments will enable faster healing than others.

The grey-headed flying-fox, *Pteropus poliocephalus* was the main study animal for this research. Injured individuals were sourced from rescue situations across NSW. Typically, these animals were entangled in netting thrown loosely over backyard fruit trees or were caught on barbed-wire fences. Flying-fox wings sustain injuries in these encounters, both open wounds and bruises. The grey-headed flying-fox is listed as Vulnerable on the IUCN Red List of Threatened Species (Lunney, Richards, & Dickman, 2008).

Across Australia, many native animals come into contact with artificial structures and many are injured or orphaned. These mammals, birds, reptiles and amphibians are, if rescued, treated and cared for by licensed volunteer rehabilitators until returned to the wild. Research undertaken in the interest of wildlife welfare is very limited. Wildlife rehabilitation is in its infancy and this is particularly so for any treatment knowledge relating to bats. Bats are currently grouped in the Order *Chiroptera*, which is divided into two suborders: Megachiroptera (megabats e.g. flying-

[2] Note: in rehabilitation terms ósave an animalô has a narrower meaning than in medical or veterinary use. It means that an animal is in a state where it can be returned to the wild with a reasonable chance of survival, not merely that it lives.

foxes, fruit bats, blossom bats and tube-nosed fruit bats) and Microchiroptera (microbats e.g. insectivorous bats). The common name ñflying-foxò generally refers to *Pteropus* species (Mickleburgh, Hutson, & Racey, 2002), however in Australia the term flying-fox and fruit bat are used interchangeably and generally cover all the Pteropodidae. This is possibly because a) there is only one fruit bat - *Dobsonia magna* (Strahan, 1995) - of which very few people have ever heard and b) the general media hysteria over alleged ñraidsò of ñmaraudingò bats on fruit orchards (Atkins, 2010).

All too often injured flying-foxes are either euthanised or left to heal as best they may. Typically the response to all of the following is to euthanise[3]:

- large holes in the wing membrane (greater than 60 mm radius ï used to be 30 mm radius),
- tears from the leading (and frequently trailing) edge of the wing membrane (see Figure 2),
- broken bone,
- any injury likely to take more than two weeks to heal (which is nearly all of them),
- wounds over the forelimb ï generally deteriorated and the animal died/euthanised.

The researcher had four years experience in the husbandry of flying-foxes and the handling and management of injured animals prior to the commencement of this study[4].

[3] these examples are typical of the lore at the time the researcher first became involved in flying-fox rehabilitation and were frequently stated in training courses and group meetings (pers. obs.)

[4]and is frequently called on by the rehabilitation community for advice.

2 Literature Review

2.1 Introduction

Selecting appropriate treatments for injuries and assessing the response of the injury is facilitated by an understanding of the tissue structure (see section 2.2, Skin Structure), the underlying processes of healing (see section 2.4 Normal Healing and section 2.5 Chiropteran Healing) and the environment in which the healing occurs which may modify those processes. The environment for healing flying-foxes, after rescue, involves captivity and handling by humans, who would be perceived as predators. These are stressful events which may negatively impact healing (see section 2.6 Environmental Considerations). The ôenvironmentô also includes the animal, i.e. any physiological or anatomical factors peculiar to that animal (see section 2.3, Physiology and Anatomy of Chiroptera).

Investigations of wound healing in Chiroptera are limited (see section 2.5 Chiropteran Healing) and mainly focus on observations of the healing of untreated wounds. Most injury / treatment research has been undertaken with reference to *Homo sapiens*, initially using in vitro methods and/or animal models e.g. rodents, then humans - either experimentally or via clinical trials (see section 2.7 Treatment). The general process of healing is the same for most vertebrates and therefore these models should provide adequate information for flying-fox injury treatment. Some structural components of flying-fox wings may affect healing and certainly constrain the choice of treatment. One of the unusual features of the wing is the lack of subdermal soft tissue, similar to the distal part of equine limbs. Thus, any treatments used on equine limb injuries were considered to be of particular interest.

Once the healing process is understood and mapped to observable states in a bat wing injury, the progress of individual injuries can be recorded (see section 2.8 Measurement) and the effects of different treatments compared.

The general philosophy was to find, firstly, a treatment that was efficacious and efficient, and secondly, a treatment that was practical for the average wildlife rehabilitator. This meant a treatment that was inexpensive, easily obtainable and simple to use.

2.2 Skin Structure

Vertebrate skin consists of an epidermis and a dermis. The epidermis is the superficial cellular layer while the dermis is the deeper mainly fibrous layer. In mammalian epidermis there is a germinative layer (stratum germinativum or stratum basale) and a stratum corneum and there may or may not be distinctive intermediate layers, see Figure 1. Hair forms from keratinised epidermis. Sebaceous and sweat glands may be present (Romer & Parsons, 1977). The skin covers deeper layers of tissue such as muscle, bone, organs.

The human epidermis has several layers: the stratum basale, stratum spinosum, stratum granulosum and stratum corneum. Thick skin such as on the palms of the hands and soles of the feet has a stratum lucidium deep to the stratum corneum (Creager, 1992). A basement membrane separates the dermis from the epidermis. The dermis has two layers: the papillary layer and the reticular layer. The papillary layer contains capillaries (Creager, 1992) and the reticular layer contains sebaceous and sweat glands and large blood vessels. Dermal papillae project into the epidermis, taking vascular and nervous elements close to the epidermal layers. The subdermal tissues such as muscle and bone are generally significantly thicker than the dermal layers.

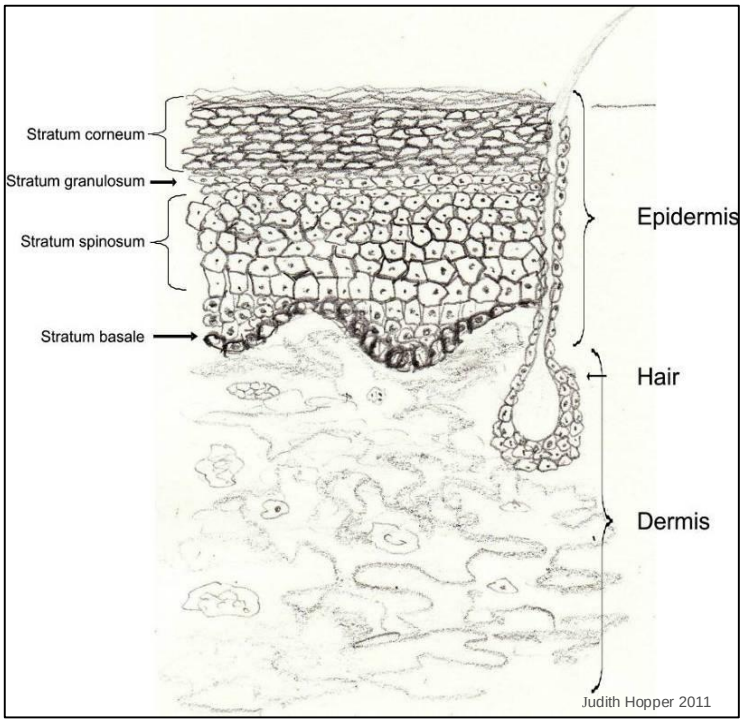


Figure 1 Diagram representing the layers of typical mammalian skin.

Flying-fox wing membrane follows the basic mammalian skin pattern, see Figure 1, but healing and treatment can be complicated by the reduction or absence of subdermal structures, see section 2.3.2 Gross Anatomy ï Chiropteran Wings, for further details.

2.3 Physiology and Anatomy of Chiroptera

2.3.1 General

Whilst the differences between Megachiroptera and Microchiroptera are documented in the literature, there remains a general lack of knowledge on bat anatomy and physiology particularly in differentiating between Megachiroptera and Microchiroptera. For instance, a paper on the comparative haematology of *Pteropus giganteus* demonstrates little knowledge of the Megachiroptera as a group, among other things that most Megachiroptera species do not hibernate (Lewis, 1977) although most Microchiroptera do to some extent. Reported information, therefore, can be difficult to interpret.

The study carried out on *P. giganteus* (Lewis, 1977) reported lower levels of some clotting factors compared with humans, in particular Hageman factor which is necessary for the complement cascade. None of the studies discussed below reported any issues that might relate to inefficient clotting processes, therefore, there is not any indication that failed clotting may cause problems after injury.

Flying-foxes are generally tropical to subtropical species (Mickleburgh, Hutson, & Racey, 1992). Grey-headed flying-foxes also occur in temperate and cool temperate regions. Flying-foxes at rest hang with their wings wrapped around their bodies (Connell, Munro, & Torpy, 2006). It has been shown that in this position the wings can maintain a layer of air around the body which is 10 degrees Celsius or more above ambient (Bartholomew, Leitner, & Nelson, 1964). This is a useful adaptation to colder temperatures.

2.3.2 Gross Anatomy – Chiropteran Wings

Bat wings are an adaptation of the archetypal mammalian forelimb. The bones of the forelimb including the hand (with the exception of the thumb) provide a framework for the wing. The wing ñmembraneò consists of a thin flexible membrane attached along the body and hindlimbs to the talocrural region, see Figure 2. The thumb is free, equipped with a claw and used for climbing, grasping and defence.

The wing membrane of bats is unusual in comparison to most other mammals in that it is an extremely large area of skin without a subdermis (Swartz, Groves, Kim, et al., 1996).

The wing membrane consists of a pigmented, nearly hairless, highly distensible and resilient integument with distinctive and macroscopically visible bands of muscle and elastin. The membrane consists of a double epidermal layer, separated by a layer of connective tissue (Church & Warren, 1968; Church, 1970; Iversen, Bhangoo, & Hansen, 1974).

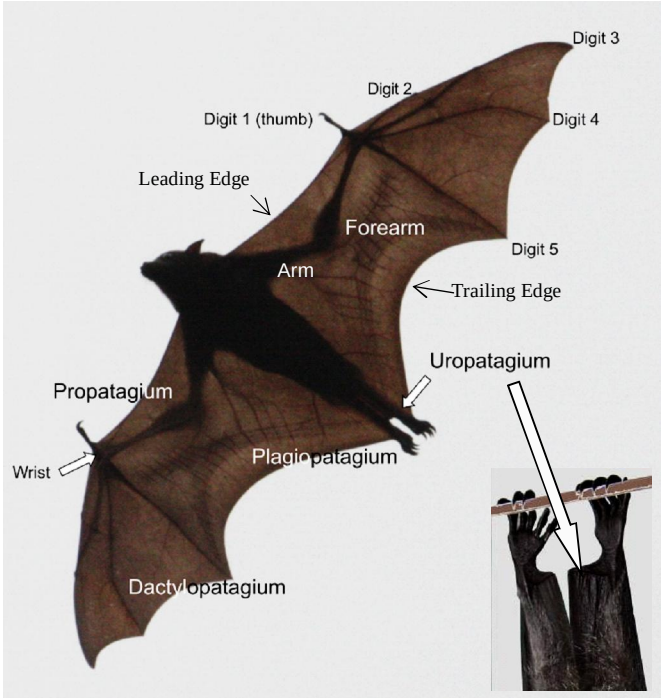


Figure 2 Flying-fox wing nomenclature.

Hair grows over the surface near the arm. In this study the term óforearmô is used to refer to the distal section of the forelimb between the elbow and wrist and the term óarmô to the proximal section of the forelimb between the shoulder and elbow joints and to the forearm. See Figure 2 for wing nomenclature.

Few studies have been conducted on the physical or mechanical properties of the patagium (wing membrane) and all of these were on microbats.

2.3.3 Micro Anatomy – Megachiropteran Wings

The most detailed description of the histology of Megachiropteran wings was by Crowley and Hall (1994) on the grey-headed flying-fox, the primary flying-fox species in this study.

The grey-headed flying-fox wing membrane (patagium) was found to consist of three layers ï a dorsal and a ventral epidermis and a dermis of normal connective tissue. Some areas of the membrane possessed hair and associated sebaceous glands but no sweat glands (Crowley & Hall, 1994).

The thickest portion of the wing was identified as the propatagium, see Figure 2, and the dactylopatagium as the thinnest. Small hair follicles and associated sebaceous glands were shown on the plagiopatagium and the uropatagium. The plagiopatagium varied in thickness (25 µm to

250 µm) and contained bands of skeletal muscle. The dactylopatagium did not contain skeletal muscle and had very few hair follicles (Crowley & Hall, 1994).

The epidermis varied between 10 µm and 40 µm in thickness with an average of 25 µm (the dorsal surface is thicker than the ventral) and the following were observed by Crowley and Hall (1994):

- basal layer (stratum basale) has low columnar to flattened squamous cells,
- stratum spinosum has scattered round, flattened cells rather than a continuous layer,
- stratum granulosum, and,
- stratum corneum has 6 - 10 layers each approximately 0.4 µm thick with interlaminar spaces giving a variable total thickness of 4 to 8 µm. Scanning electron microscopy shows a layer of closely interlocked hexagonal shaped cells. A major feature of this layer was the presence of round swellings containing a clear substance hypothesised to contain a lipid-based compound that is spread over the wing in lieu of sebum to provide waterproofing.

The dermis and epidermis were separated by a 70 nm thick basement membrane (Crowley & Hall, 1994). The dermis was found to be considerably thicker than the epidermis and contained many large papillae, up to 100 µm across. These papillae caused a distinct (even) folding of the surface. The dermis had three layers (Crowley & Hall, 1994):

- two strata papillare (one between each epidermis and the central dermis). These layers also had many micropapillae extending, from the tips of the main papillae, between the cells of the stratum basale. They consisted of ñcollagen, fibroblasts, macrophages, small blood vessels and nervesò, the main component being collagen,
- stratum reticulare consisting of large (10 µm to 200 µm) masses of elastin bound into bundles by collagen. Review of the longitudinal sections suggested that the elastin occurs in a crosshatch network. Also observed were nerves, blood and lymph vessels, macrophages and fibroblasts.

The wing had a scattering of small domes with a specialised hair arising from the centre. The hairs were short and very fine (4000 µm x 25 µm) and mostly point to the trailing edge of the wing. These hairs varied in concentration, higher over the muscle bands and along the large blood vessels (Crowley & Hall, 1994).

The combination of evenly distributed regular bundles of elastin and collagen allow the flexible membrane to stretch (Crowley & Hall, 1994). The plagiopatagium contained strips of skeletal muscle originating and inserting into the network of collagen fibres (Crowley & Hall, 1994).

The thickness of the flying-fox wing as reported by Crowley and Hall (1994) is comparable, or thicker (depending on the exact point on the wing), to the microbats examined by Studier (1972) for puncture strength and elasticity; see section 2.3.4, Micro Anatomy ï Microchiropteran Wings. Comparable flying-fox membranes should, therefore, be able to withstand comparable pressure, i.e. in the range of 5 to 9 kg/mm^2 as found by Studier (1972) for microbat wings.

2.3.4 Micro Anatomy – Microchiropteran Wings

A dorsal and a ventral epidermis, and a normal dermis were observed in microchiropteran patagium (wing membrane) (Murphy, 1960; Gupta, 1967; Quay, 1970; Holbrook & Odland, 1978; Swartz, et al., 1996). Gupta (1967) also referred to a hypodermis between the two dermal layers but this was not mentioned by other researchers.

The following cells and fibres were described in the dermis: fibroblasts, fibrocytes, histiocytes, mast cells and leucocytes, reticular fibres, elastin and collagen fibres (Gupta, 1967; Quay, 1970). Quay (1970) emphasised the amount of elastin fibres but Gupta (Gupta, 1967) only referred to ña few elastin fibresò (p. 315). Holbrook and Odland (1978) referred to an elastin and collagen fibrous mesh throughout the wing.

In microbats Carrier (1926) observed thin walled lymph vessels always running beside the larger blood vessels but this matching was not reported by Crowley and Hall (1994) in flying-foxes. Webb and Nicoll (1944) also observed the lymphatic system and described a single layer system of collecting bulbs attached to collecting vessels (alongside small blood vessels) which connect to transporting lymphatics running alongside the main veins and arteries. Carrier (1926) also commented on valves clearly visible in the blood and lymphatic vessels.

Gupta (1967) described distal wing muscles, which are species dependant, as: continuous sheet, differentiated into bands, or absent. Plagiopatagiales muscles show as parallel vertical lines (bands) (Holbrook & Odland, 1978) similar to those seen in grey-headed flying-foxes (Crowley & Hall, 1994).

Extensive testing on the strengths and elastic properties of microbat wing membranes showed very high variations depending on the orientation in the wing, the anatomical region and the taxon (Swartz, et al., 1996). Studier (1972) compared the puncture strength and elasticity of the patagium in eight microbat species with a rubber surgical glove and a plastic sandwich bag. Seven species had a comparable thickness to the sandwich bag and were 1/10 the thickness of the glove. Patagium mechanical properties are comparable to a sandwich bag in thickness and puncture strength but slightly less elastic. In contrast the patagium was only 1/10 the thickness of the rubber glove but had two to three times the puncture strength and was considerably less elastic (half to one third).

2.4 Normal Healing of Injuries

2.4.1 Introduction

The aim of treatment is to bring about or improve healing of injuries. It is, therefore, important to understand the healing processes in order to analyse treatments. Healing is a positive change to an injury and therefore ójuryô must be defined in order to understand healing.

An injury is categorised, for the purposes of this thesis, as open or closed. An open injury (also called a wound), such as a cut, is any injury which damages the epidermal layer. A closed injury such as a bruise or blister involves injury to the underlying tissues without damaging the epidermis. Blisters are pockets of interstitial fluid within the epidermis or between the epidermis and dermis. Injuries (closed and open) on the wings of flying-foxes were the focus of this research.

ñNormal healingò refers to that healing that proceeds smoothly through all stages without interruption or delay and results in the repair of tissue to restore strength and functionality. The initial healing response to open or closed injuries is the same, as demonstrated by Krawczykôs (1971) experiments on mice. This healing response addresses healing of non-fetal tissue injuries. Injury healing in fetal tissue differs from non-fetal tissue. In most post-fetal tissues healing consists of tissue repair rather than regeneration of the original tissue (McCallion & Ferguson, 1996). Fetal tissue heals without inflammation and generally without scarring. Similar healing has been demonstrated in mice incapable of generating an inflammatory response and this healing occurs at the same rate as the inflammatory healing (Martin, D'Souza, Martin, et al., 2003).

Despite early work using microbats to study inflammation (Paget, 1850) very little information is available for either megabat or microbat injuries and healing, see section 2.5 Chiropteran Healing. Consequently, skin injuries in other mammals have been used as alternative models. The major focus of studies for normal healing in the literature is adult *Homo sapiens* skin. Healing studies for humans use humans as the research animal or an animal model - most often pigs, rabbits or rodents. As these other animals are proxies for humans and no more apparently related / relevant to flying foxes than humans most of the literature review reports on human trials unless an element of the study was considered particularly relevant e.g. distal limb healing of equines and canines (due to the lack of subdermal tissues between the skin and the bone). Field trials were considered particularly relevant as the conditions could be considered closer to those pertaining to flying-fox injury conditions than injuries caused and healed in controlled laboratory situations[5].

Normal wound healing is by:

- primary intention (where the edges of the wound, i.e. the cut epidermis, are in, or are brought into, contact),
- secondary intention (where the wound edges are apart and the wound fills in to heal), see Figure 3, or,
- tertiary intention (where the wound edges are deliberately kept apart, e.g. for debridement, for some time before being drawn together).

Normal healing begins within seconds of injury and may continue for months depending on the size of the wound, degree of contamination and the resultant scarring. Generally a wound is expected to be covered (healed) within days or weeks and then proceed through remodelling of scar tissue (Clark, 1985). During repair, the developing tissue may produce some form of scar, depending on the severity of the injury, and this may lead to the loss of function of the organ, in the case of flying-fox wing injuries - the loss of flight.

For convenience, healing is usually divided into a series of sequential phases but this is an extreme simplification. The process is complex, with cells and biochemicals playing multiple roles in different phases overlapping in time. Not only do most of the activities begin within the first day, even within the first hour (Kirsner & Bogensberger, 2002), different portions of the

[5] It is against the principles of the researcher to accept the practice of inflicting harm on an animal for any reason other than the benefit of that animal or its species.

wound can be at different phases at any time (Clark, 1985). Possibly, because of this complexity and overlap of processes, different authors / researchers do not group the various processes into precisely the same phases.

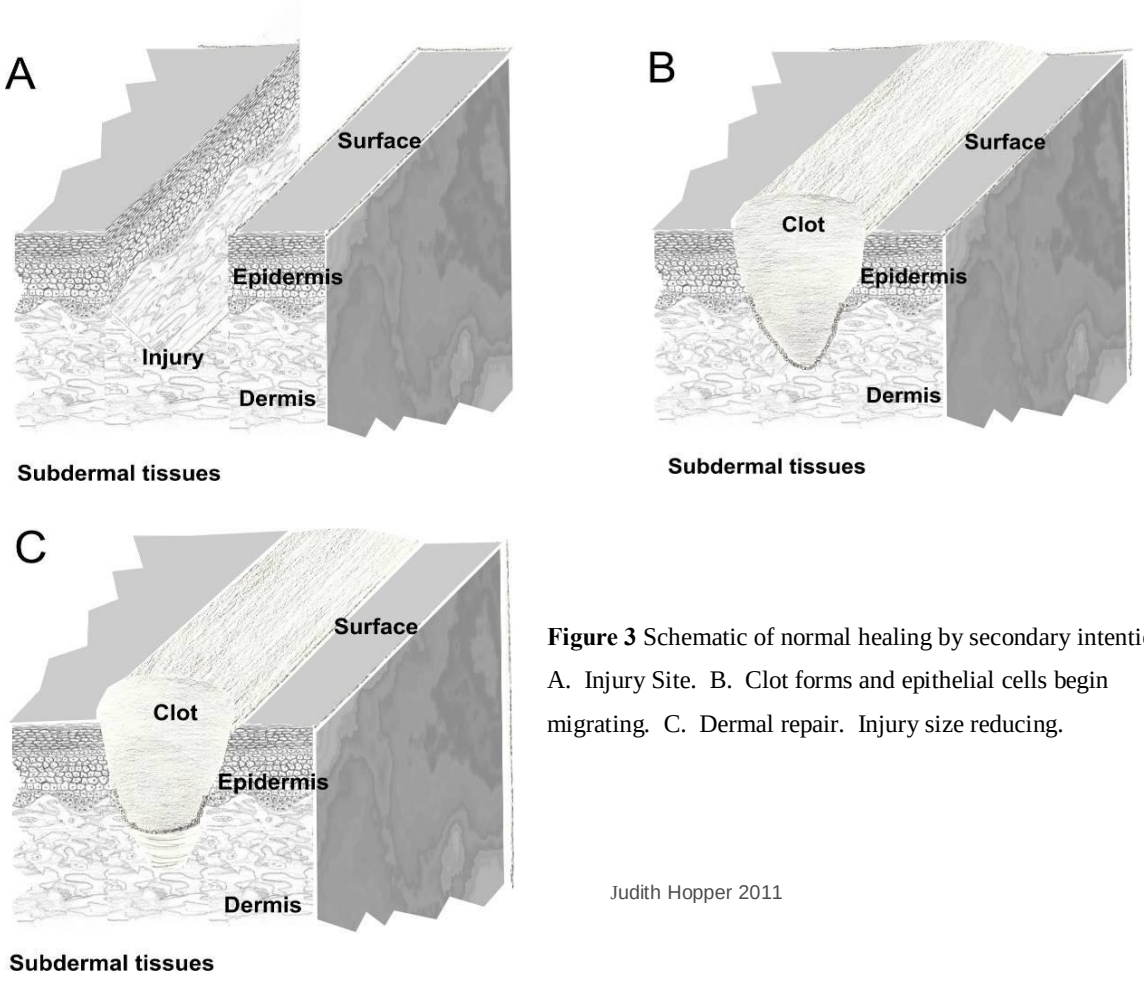


Figure 3 Schematic of normal healing by secondary intention. A. Injury Site. B. Clot forms and epithelial cells begin migrating. C. Dermal repair. Injury size reducing.

Four phases are defined here for use in this project:

1. haemostasis (forming the ñprovisional matrixò and generating the initial cytokines to attract leucocytes),
2. inflammation (the influx of first neutrophils then monocytes leading to asepsis of the wound, debridement of damaged tissue and cytokine production to develop and control granulation),
3. granulation (fibroplasia, angiogenesis and re-epithelialisation), and,
4. remodelling (the remodelling of the extra cellular matrix and final scar or tissue repair).

Each phase is dominated by various cell types and is initiated and controlled by chemicals, e.g. cytokines and growth factors, which signal the proper phase of the wound cascade and mediate the distinct processes (such as clotting, cell recruitment and collagen replacement) which usually produce further chemotaxins to initiate the next phase. Each phase, therefore, can be described in terms of inputs, processes and outputs. The initiation and control of healing by chemotaxins is a growing field of complexity. The focus of this thesis is to develop treatments and therefore an in-depth description of healing is not considered appropriate. Only a broad outline is given here, for more detailed reviews see (DiPietro, 2010), Grazul-Bilska et al. (2003), Martin et al. (1992) and Steenfos (1994).

2.4.2 Haemostasis

Haemostasis is the first stage of healing. During haemostasis the primary element of interest, for healing, is the platelet and the primary activities are:

- vasoconstriction (the vascular phase) then the production of a platelet plug (platelet activation) (Kirsner & Bogensberger, 2002),
- the coagulation cascade producing a fibrinous clot (Martin, et al., 1992) to form the provisional matrix (Clark, 1996b),
- the triggering and controlling of inflammation through the release of cytokines from platelet granules that attract leukocytes and the initiation of the complement cascade and prostaglandin cycles(Hunt, 1990; Riches, 1996; Sylvia, 2003) (Kirsner & Bogensberger, 2002), and,
- to a lesser extent, the release of cytokines to start some of the processes of granulation.

Haemorrhage and tissue damage initiate haemostasis by activating platelets and triggering the release of thromboplastin (Martini, 1998) which triggers the coagulation cascade (Clark, 1985).

It should be noted that the failure of this phase might result in the death of the flying-fox before rescue and therefore may not be observable within the bounds of this study. There are not, however, reports of numerous dead flying-foxes from unknown causes or from obvious bleeding at rescue sites. Further, experience with ósurgicalô procedures, e.g. wing punches for DNA studies and surgery to open constricted membranes, has shown a rapid sealing of the wound in less than five minutes and in many cases less than one minute (pers. obs.). Many of the initiators of inflammation, therefore, should be present.

2.4.3 Inflammation

Inflammation is a normal response to injury and part of the process of repair (Haslett & Henson, 1996). It begins soon after injury and is evidenced by swelling, redness, pain and heat (Kirsner & Bogensberger, 2002).

Inflammation involves vascular and cellular changes at the injury site, primarily:

- vasodilatation and increased endothelial permeability to allow more cells and fluids into the site (Majno & Palade, 1961; Church & Levi-Schaffer, 1997; Sherratt & Dallon, 2002),
- injury clean-up and defence (Clark, 1985), and,
- the production of growth factors for the granulation phase (fibroplasia, angiogenesis , and epithelialisation) through leukocyte attraction and activation (Simpson & Ross, 1972; Fernandez, Henson, Otani, et al., 1978; Newman, Henson, & Henson, 1982; Wahl, Hunt, Wakefield, et al., 1987).

These are followed by the final process - resolution: the normal conclusion of the inflammation phase with the reduction or inactivation of mediators, the removal of leucocytes and the restoration of normal permeability (Hall, Savill, Henson, et al., 1994; Haslett & Henson, 1996; Bannenberg, Chiang, Ariel, et al., 2005; Serhan, Brain, Buckley, et al., 2007). Other immune responses occur simultaneously but are less important in the context of this research than the processes listed.

The primary cell of interest is the macrophage (Peacock, 1984) which provides the initiators and mediators for the inflammatory processes. Several processes lead into the inflammation phase:

- coagulation cascade, part of haemostasis, (Bar-Shavit, Kahn, Fenton, et al., 1983; Hunt, 1990),
- prostaglandin cycle, triggered by damaged cell membranes (Smith, 1983),
- complement cascade, part of the immune response triggered by the injury (Fernandez, et al., 1978; Sylvia, 2003),

Acute inflammation lasts 24 to 48 hours and is followed by a sub-acute phase lasting up to two weeks (Kirsner & Bogensberger, 2002). The change in leukocyte composition from primarily polymorphonuclear leucocytes (mainly neutrophils) to primarily macrophages marks the change

from early to late stage inflammation (Clark, 1996b). Failure to change may result in chronic wounds including over-granulation (Wilmink & van Weeren, 2005).

2.4.4 Granulation

Granulation is the formation of new tissue (stroma) in the injury. Granulation tissue begins to appear within four days (Tonnesen, Feng, & Clark, 2000) and is a complex of macrophages, fibroblasts, extra cellular matrix (ECM) molecules (such as collagen, fibronectin) and new blood vessels (Clark, 1990; Davidson, 2002). Macrophages provide cytokines, fibroblasts provide the ECM and the new blood vessels provide nutrients and oxygen (Tonnesen, et al., 2000; Theoret, 2005).

Fibroplasia, matrix deposition, angiogenesis and re-epithelialisation occur concurrently at different sites throughout the wound bed (Kurkinen, Vaheri, Roberts, et al., 1980). They are described in the following sections.

2.4.4.1 Matrix Deposition and Fibroplasia

Most of the wound extra cellular matrix is from the provisional ECM provided by the blood clot (Theoret, 2005). This is replaced during granulation through fibroplasia and associated matrix deposition (Theoret, 2005). Fibroplasia is the formation of new tissue by fibroblasts which are one of the key cells in tissue repair and wound closure (Gabbiani, 2003) producing the ECM (Mori, Bellini, Stacey, et al., 2005).

The primary activities are:

- fibroblast migration and proliferation: within two to three days fibroblasts enter the wound site, proliferate and, by approximately day five , begin to produce the new ECM. Proliferation and ECM production require and stimulate changes in the phenotype of the fibroblast to form myofibroblasts (Gabbiani, Ryan, & Majno, 1971; Falanga, Zitelli, & Eaglestein, 1988; Welch, Odland, & Clark, 1990; Stephens & Thomas, 2002),
- ECM Production: fibroblasts synthesise various macromolecules, such as collagen (initially appearing around day five to seven), glycoproteins (primarily fibronectin matrix), proteoglycans and elastin and remodel the ECM (Saarialho-Kere, Chang, Welgus, et al., 1992; Falanga, 1998; Parks, 1999; Stephens & Thomas, 2002),

- contraction: fibroblasts containing microfibrils (myofibroblasts) link to other myofibroblasts and to the collagen bundles and contracts across the wound . Initial collagen production in response to a wound (open injury) is of type III rather than the type I collagen in normal skin. Further, in normal tissue, the collagen forms fibres arranged in bundles, but in scar tissue it is not well formed into fibres. Elastin is not involved in early wound healing but is important in the remodelling phase (Gabbiani, et al., 1971; Bhangoo & Church, 1976; Gabbiani, Le Lous, Bailey, et al., 1976; Desmouliere, Chaponnier, & Gabbiani, 2005; Zheng, Choi, Rouleau, et al., 2006).

Resolution is the final activity: myofibroblasts likely undergo apoptosis when epithelialisation is completed. While this seems to be well accepted very little is known about it (Desmouliere, et al., 2005).

2.4.4.2 Angiogenesis

Injury angiogenesis is the formation of new blood vessels during granulation and follows fibroblast ECM deposition in the wound (Tonnesen, et al., 2000). The main cells involved are the microvascular endothelial cells (Madri, Sankar, & Romanic, 1996). Angiogenesis is necessary for successful granulation, providing new blood vessels that bring nutrients and oxygen into the developing granulation tissue.

Angiogenesis is initiated, stimulated and controlled by: the ECM composition; macrophages; balance of fibrin / fibronectin; certain cytokines e.g. bFGF, TGF_{β} IL-1, VEGF, FGF, and kinin; interaction between the cells, and, fibrin and collagen (Schweigerer, Neufeld, Friedman, et al., 1987; Madri, Pratt, & Tucker, 1988; Clark, 1990; Madri, Marx, Merwin, et al., 1991; Madri & Marx, 1992; Qu, Liebler, Powers, et al., 1995; Clark, Tonnesen, Gailit, et al., 1996; Feng, Clark, Galanakis, et al., 1999; Plendl, Snyman, Naidoo, et al., 2000). The angiogenic activities are:

- activation and migration of endothelial cells into the site (Clark, 1996b),
- proliferation: production of cells for buds and loops of the new vascular system . Proliferation occurs only at the tip of the angiogenic sprout (Madri, et al., 1996; Stephens & Thomas, 2002; Crivellato & Ribatti, 2005),
- remodelling: regression of the vascular bed and maturation of the remaining vessels (Madri, et al., 1996) to develop a normal vascular system.

2.4.4.3 Re-epithelialisation

Re-epithelialisation is the resurfacing of the wound through keratinocyte migration and proliferation (Santoro & Gaudino, 2005) and commences within 24 to 48 hours (Odland & Ross, 1968). Keratinocytes detach from the basement membrane and migrate from the wound edges until they make contact in the centre. Migration ceases and proliferation commences (Larjava, Salo, Haapasalmi, et al., 1993). Replacement of the epithelial layer occurs concurrently with granulation, growing from the wound edge over the new ECM (Davidson, 2002).

The process is initiated and controlled by cytokines from the cells and from the ECM from previous phases (Rubin, Osada, Finch, et al., 1989; Werner, Peters, Longaker, et al., 1992) and the activities are:

- activation / transformation and migration of keratinocytes are stimulated by keratinocyte growth factor (KGF) (Martin, et al., 1992; Larjava, et al., 1993). Migration begins within 24 hours (Krawczyk, 1971; Theoret, 2005) for simple clean cuts,
- barrier completion: proliferation is inhibited in migrating cells (Krawczyk, 1971; Jacinto, Martinez-Arias, & Martin, 2001) but continues to occur near the periphery of the wound (Krawczyk, 1971). Once a monolayer of keratinocytes is established migration ceases and a new stratified epidermis is established from the wound edge (Odland & Ross, 1968; Martin, 1997).

Epithelialisation occurs more quickly, where dermal appendages are present. Time until complete closure varies between species (Theoret, 2005).

2.4.5 Summary

Normal healing proceeds through a series of sequential processes that overlap in time, see Figure 4. Initial processes may be defined as:

- haemostasis to produce a platelet plug and fibrin clot, regarded as the initial ECM,
- inflammation to remove debris from the wound and provide a major source of cytokines,
- granulation to remodel and develop the ECM and produce new blood vessels, and, re-epithelialise the wound (reduced or not required for closed wounds).

A fourth process, remodelling of scar tissue, may continue for up to two years (Clark, 1985).

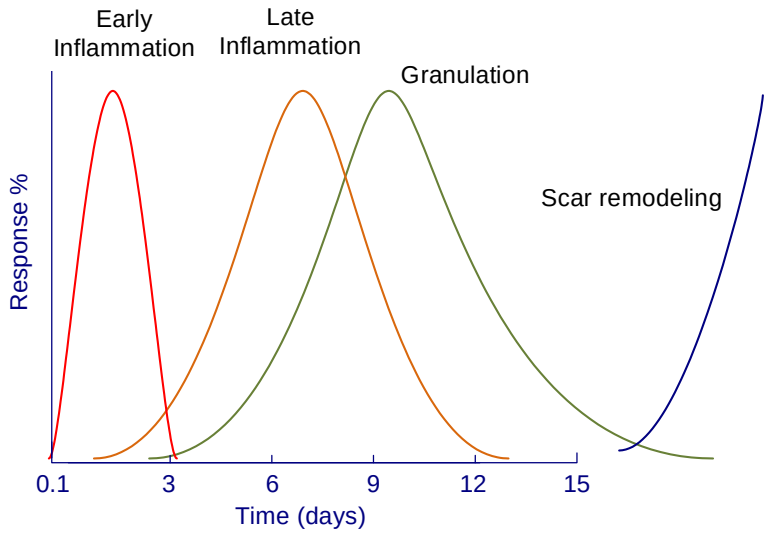


Figure 4 Typical healing times for human injuries.

2.5 Chiropteran Healing

Despite the elasticity and strength of a bat wing membrane, the patagium can be torn or otherwise damaged, particularly when entangled in barbed-wire or fine stranded netting. These entanglements are common and produce both bruises and wounds. There have been few studies of injury healing in bats. Possibly the first was a study of inflammation presented in a series of lectures by Professor Paget to the Royal College of Surgeons of England in 1850 (Paget, 1850).

Healing of full thickness wounds in *Eidolon helvum* wings was studied by Church and Warren (1968). Full thickness wounds, 20 mm x 20 mm, made in the membrane, immediately opened a further 40% due to wing elasticity. During the first three days, wounds increased slightly in size, and ventral and dorsal epithelial layers fused across the woundsô edges. Note: the wounds were not examined until day three and therefore ñfusionò between the edges may have occurred prior to this. This was also seen in other wound healing experiments in this species (Bhangoo, 1974; Bhangoo & Church, 1976). After contraction began, the wound edges thickened up to eight times that of the thickness of normal epidermis with a rolled pale edge of thickened epidermis over the granulation tissue. The dermis also thickened with increased amounts of collagen by day 10. By day 14 the granulation tissue was primarily fibroblasts and a small amount of collagen and capillary network. The dermis became a thin layer of loose connective tissue between the

overlying thickened epidermal layers, contracting fastest between day 5 and day 12 (of this 20 mm x 20 mm hole) as increasing amounts of tissue formed at the edge. The new skin was 10% of the wound area by day 8, 40% by day 12 and complete by day 28. It was noted that the hole became square and the new tissue became star shaped. The central hole was reduced by the developing new tissue during wound healing (Church & Warren, 1968).

A study, on the same species (*E. helvum*), investigating epidermal proliferation after wounding was conducted by Iversen et al. (1974). This was an epidermal layer wound only, produced by tape stripping on the ventral surface. In this case, the wound area remained constant for two to three days before decreasing. All wounds completely re-epithelialised between 7 and 11 days from an initial size of 12 mm x 50 mm (Iversen, et al., 1974). Inflammatory responses were visually observable from approximately 24 hours after epithelial layer wounding (Iversen, et al., 1974). This is comparable to other animal studies (Odland & Ross, 1968; Theoret, 2005). The paper also gave details on mitotic rates and epidermal cell numbers in the wound area and in unrelated areas.

The role of elastin was studied using full thickness 5 mm^2 wounds in the wing membrane (Bhangoo & Church, 1976). As with Church and Warren (1968) the wounds became oval due to the effects of elastin, the cut ends of which were clearly visible. By the end of the week fibroblasts and inflammatory cells surrounded the elastin bundles. Epidermal hyperplasia was also evident. By two weeks, many spindle shaped cells, lying with their long axis parallel to the elastin bundles, were seen, apparently arising near the elastin bundles. By four weeks these cells had migrated further into the new tissue which at this stage was primarily collagen. By six weeks fine elastin fibres were seen between the original cut ends of the elastin bundles and the advancing spindle cells. These fibres were well oriented in the elastin bundles but disorderly at the scar centre. By 10 weeks the elastin producing cells were taking the shortest distance between the original cut ends of the parent bundles, establishing continuity between them. By 12 weeks newly laid elastin occurred throughout the scar and fibres had increased in diameter. At this time the scar appeared wrinkled, more like normal wing membrane. During the following weeks (up to six months) of remodelling the "elastoblasts" reduced and the elastin fibres became more compact. It is unclear whether these ñelastoblastsò are a special cell or a type of fibroblast (Bhangoo & Church, 1976).

Church and Griffin (1968), in a study of *Mycobacterium buruli* lesion development, observed that megabat wings (*E. helvum*) damaged by biopsy showed marked or complete healing by two

weeks and complete contraction, of the initial 7 mm by 11 mm hole, in less than 6 weeks (Church & Griffin, 1968).

Full thickness holes in the membrane recover due to regeneration of the entire tissue not just contraction (Goss, 1980). This ability to regenerate an entire structure is unusual in mammals (Goss, 1980).

Closed injuries have also been studied. Crushing bruises were induced in the wing membrane of *E helvum*, 8 mm x 1 mm in size (Church & Noronha, 1965; Church, Noronha, & Allbrook, 1966). These bruises are reported as healed within 20 days (Church & Noronha, 1965). After the initial event, the muscle fibres were observed to be divided and crushed but the ECM and epithelial layers were intact. There was little bleeding (Church & Noronha, 1965; Church, et al., 1966). Neutrophils were present during the first few hours only (Church, et al., 1966). Tissue macrophages (histiocytes) appeared within three hours and within 24 hours were observed within damaged fibres and cleanup proceeded rapidly over the first four days (Church & Noronha, 1965; Church, et al., 1966). Satellite cells (myogenic cells which proliferate after injury to regenerate muscle tissue (Morgan & Partridge, 2003)) were found during the first two days but were gone by three to four days in the damaged section, replaced by myoblasts. They reappeared after eight days (Church, et al., 1966). Presumably the satellite cells become myoblasts in order to regenerate the muscle (Church, 1970). The new actomyosin (of the muscle fibres) appeared within 10 days. Myoblasts underwent mitosis to produce new muscle fibres (Church, et al., 1966). By four weeks many muscle fibres were again continuous throughout the lesion and regeneration was complete (Church, 1970). Interestingly collagen was not laid down in the scar tissue (Church, et al., 1966) of these closed (bruise) injuries.

The flying-fox patagium is subjected to great tensile forces during flight and must have appropriate compliance and strength to withstand the forces to which it is exposed. Healed membranes and scars therefore must be pliable to accommodate the necessary stretching. The exact stretch required is not known but microbat patagium has a compliance of 0.97 - 1.58 mm N^{-1} (reported in terms of an elasticity of 9.5 mm/kg to 15.5 mm/kg - millimetres of stretch per kilogram of weight producing the stretch) (Studier, 1972) which may provide a guide to the requirements of a healthy flying-fox patagium. Some areas of flying-fox wings, however, are thicker than that of the microbats studied. If the structure is the same then the increased thickness would mean a reduction in normal stretch length however if the structure of elastin and collagen is different then the microbat results may not provide a useful guide.

2.6 Environmental Considerations

Physiological Stress

Chronic and acute stress can impair injury healing. University students displayed a 40% slower healing rate of mucosal wounds during examination times than during vacation time. Patients recovering from surgery also displayed a marked degradation of healing and an increase in pain (Broadbent, Petrie, Alley, et al., 2003) when under the additional stress from the patientôs perception of the coming operation. The study also indicates that some individuals experience more stress than do others in the same circumstances. In people with chronic stress (such as care givers for Alzheimerôs sufferers) wounds also are slower to heal (e.g. 24% slower, (Kiecolt-Glaser, Marucha, Mercado, et al., 1995)).

Healing also slowed significantly in mice stressed by repeated restraint and the wound size initially increased more in stressed mice. The study indicated that the early inflammatory response was impaired (Eijkelkamp, Engeland, Gajendrareddy, et al., 2007). Restraint induced stress is a significant concern in relation to healing flying-fox injuries. Most injuries were sustained during restraint (e.g. net entanglement) and it is necessary to further restrain the flying-fox during treatment.

Diet

Healing is a complex biological process which requires energy and nutrients (Arnold & Barbul, 2006). Deficiencies of key nutrients can impair healing (Lim, Levy, & Bray, 2004) leading to increased risk of wound infection and poor wound strength or delayed healing (Arnold & Barbul, 2006). Protein synthesis requires 0.9 kilocalories per gram and a section of granulation tissue 30 mm x 30 mm x 1 mm contains 10mg collagen (Arnold & Barbul, 2006). A wound with an area of 50 mm x 10 mm x 0.5 mm (a fairly normal moderate wound for an injured flying-fox) would therefore require 2.7 mg of collagen and 0.0025 kcal. Healing requires positive energy and nutritional balances to meet the increased needs of developing new tissue.

Weather

Wound healing is affected by temperature with colder temperatures reducing healing rate (Campbell & Cuthbertson, 1967). Flying-foxes at rest hang with their wings wrapped around their bodies and in this position the wings can maintain a layer of air around the body 10 degrees

Celsius or more above ambient (Bartholomew, et al., 1964). This is a useful adaptation to colder temperatures and may also keep the inner wing warmer and assist healing in cold weather.

2.7 Treatment

2.7.1 Introduction

ñMany smaller wounds heal despite the misguided efforts of disinterested cliniciansò (Pavletic, 1999). While this may be the case for domestic animal injuries, once damaged, bat wing membranes are extremely delicate and disinterest is more likely to result in death or euthanasia (pers. obs.) than healing. Effective and efficient treatments are needed to prevent minor wounds causing a non-functional wing and a euthanised animal.

Rehabilitators are busy people who look after wildlife in their spare time and with their own money. Developing effective treatments is not sufficient. Treatments must be easy and quick. As previously discussed it is important to minimise stress to flying-foxes in rehabilitation, for their welfare and to minimise the negative effects of stress on healing. This means minimising the handling and constraint of wild caught adults. Even human treatment protocols emphasise the importance of reducing nursing time in selecting a treatment (Ovington, 1999).

The successful treatment of wounds in animals requires ñpractice, attention to small details and adherence to the basic principles of wound managementò (Pavletic, 1999). Continued habitat degradation and human activities incompatible with wildlife survival will provide rehabilitators with more ópracticeô than they require. The protocols from this research will provide the information and techniques to give the necessary guidance on óattention to detailsô and óbasic principlesô.

2.7.2 Treatment Aim

The aim of treatment is to achieve and maintain stable wound healing processes with minimum stress to the flying-fox. Stable healing results in healthy granulation tissue and, in the case of open injuries epithelialisation, with minimal scarring.

2.7.3 Types of Treatment

Treatments may be systemic or local. Systemic treatments can be delivered orally or by injection or by suppository and can reach all areas of the body; however, they cannot be easily directed and

do not protect the site from ongoing injury. Local treatments can be applied to a specific areas, provide treatment even when the local vascular system is compromised (as is often the case with entanglement injuries) and can protect the injury site physically. For these reasons, this research focussed on topical treatments.

Topical treatments can be:

- dressings (a ócloth ô covering the wound site),
- topical medicines (ointments, creams, infusions, tinctures,), or
- washes (medicinal liquids used to flush the wound).

Note however, that in modern wound treatment the boundaries between categories are becoming less clear. This is particularly so in the area of bioactive or combination dressings that are both dressing and medicinal application.

Topical treatments may be used for:

- debridement,
- antimicrobial action,
- boosting part of the healing process, and / or
- protection / pain management.

Sources of topical treatments included:

- mainstream human and veterinary medicine,
- alternative human and veterinary medicine, and,
- research.

2.7.4 Topical Treatments

2.7.4.1 General

Topical applications have been used to treat injuries for thousands of years. In some form or another, from a simple leaf poultice to an autograft, they are commonplace (Caldwell, 1990). The first purpose of the application is to protect the injury from physical or microbiological contamination and from further damage. A second but increasingly important purpose is to

provide an environment that enhances healing. These needs may be met by a topical application, a dressing or by both (either separately or combined in one product) (Myer, 2002). By definition, a wash is usually a substance used to clean or debride the injury prior to a treatment application.

An enhanced environment may be as simple as keeping a wound moist. Moist wound healing in a pig model was shown to be faster than dry in 1962 (Winter, 1962; Hinman & Maibach, 1963) and remains the current best practice for humans (Jones & Harding, 2001).

The early work by Winter (1962) with pigs demonstrated that in moist wounds keratinocytes move freely across the wound exudate but in dry wounds they must push through the scab. Parallel work on human wounds showed that in dry scab healing the cells grew at right angles to the surface whereas they spread freely across the wound in moist wound healing (Hinman & Maibach, 1963). Increased rate of re-epithelialisation on pigs was also demonstrated, grossly and histologically, under Opsite® occlusive dressings compared with dry gauze (Dyson, Young, Pendle, et al., 1988).

Dressings and other applications can provide far more healing factors than simply a moist environment. They can provide antimicrobial activity and / or chemical mediators for various aspects of healing. This can be through chemicals embedded in the dressing or by holding a topical treatment, e.g. ointment or cream, in place.

Many of the characteristics of an ideal dressing for wounds, as listed below (Myer, 2002; Vloemans, Soesman, Suijker, et al., 2003), also apply to any topical application:

- flexible and durable,
- provide mechanical and bacterial protection,
- maintain a moist environment over the wound,
- allow gaseous and fluid exchange (absorb wound exudate),
- not stick to the wound,
- safe to use,
- sterile,
- not require frequent changing (changing dressings can easily damage new skin (Wu, Bao, Yoshii, et al., 2001)).

The dressing must also be:

- easy to use (for a single rehabilitator working alone) and apply to the peculiarities of a wing shape without folding the membrane[6],
- acceptable to the flying-fox (i.e. not irritate or be easy to lick off or pull off),
- inexpensive and packaged in small numbers[7],
- available ñover the counterò in a range of sizes to accommodate injury size.

Dressings / topical applications fall into the following categories (for the details of dressing types see Table 1):

- moisture keeping,
- bioactive,
- moisture keeping and bioactive,
- specialist,
- dry.

Topical applications are also used for closed injuries. While a closed injury may not immediately appear to require a moist environment, it is important to maintain the epidermal layer. Dehydration of the epidermis can cause it to break down and result in an open injury. The principles remain the same in closed injuries but assessing the treatment may differ, e.g. a dressing normally considered ñdryò may maintain a sufficiently ñmoistò environment for a closed injury. For practical purposes most of the other characteristics of an ideal wound dressing, as listed above, apply to closed injuries.

[6] the membrane must remain flat to avoid damage, such as bruising from the folding or from holding exudate against healthy membrane

[7] many rehabilitators may only treat an injured animal every few months or more and so do not want to waste opened and unused dressings or buy large (and hence expensive) packets

Table 1 Dressings Descriptions
Description taken from Myer (2002) unless otherwise indicated

Product Category	Dressing Type	Description	Use / Comment
Silicone meshes	Moisture keeping	Occlusive or semi-occlusive inert transparent sheet or gel	May reduce hypertrophic and keloid scarring
Semi-permeable adhesive films	Moisture keeping	An adhesive layer on a polyurethane membrane	Very thin and flexible for use on wing curves, clear view of wound Well tolerated by bats (pers. obs.) Debridement of necrotic tissue, barrier against bacteria, mechanical protection Not indicated for infected wounds, wounds with delicate edges (due to adhesive) and can cause maceration if excessive exudate present, peri wound skin must be dry and clean for adherence
Hydrogels	Moisture keeping	High amounts of water with glycerin or similar material, either as an amorphous gel or as part of a dressing Cross-linked macromolecules with an insoluble network structure which swells in contact with water (Wu, et al., 2001)	Space filling, hydrating, debridement, protection, insulation, pain reduction Frequent (greater than daily) changes are required for gels, dressings can be left several days May cause maceration to the wound edge Suitable for infected wounds and protects granulation tissue

Product Category	**Dressing Type**	**Description**	**Use / Comment**
Hydrocolloids	Moisture keeping, Interactive	Compounds of gelatin, pectin, carboxymethylcellulose, elastomers and adhesives on a polyurethane membrane	May be left on up to seven days Maintain a moist environment Assist autolytic debridement of necrotic tissue Protect granulation tissue Manage low to moderate exudative wounds Adhesive on the dressing may damage epithelial growth Insulate, reduce pain, control bacteria Indicated for partial to full thickness granulating wounds with up to moderate exudate, traumatic injuries, necrotic tissue
Combination dressings	Moisture keeping, interactive	Depends on the dressing type	Depends on the properties of the individual components
Antimicrobial dressings	Bioactive	Silver sulphadiazine cream Silver dressings Iodine	Active agents silver and sulphadiazine - antibacterial and antifungal, possible side effects (pain, rashes, necrosis) and systemic absorption with prolonged use Silver dressings: effective in preventing and controlling bacterial infection, noncytotoxic, slow release of silver over several days Iodine impregnated gels & dressings ï antimicrobial, may cause thyroid problems

Product Category	**Dressing Type**	**Description**	**Use / Comment**
Collagen containing dressings (engineered skin substitute)	Interactive	Various sources of collagen in various presentations	Change weekly but if changed daily may be used on infected wounds, assist autolytic debridement, exudate absorption and haemostasis, stimulates formation of granulation tissue, not for use on dry wounds
Single component biological dressings and engineered skin substitutes	Interactive	Biological and semi-biological dressings from a skin source, such as autografts, allografts and xenografts, biologic systems such as amniotic fluid or acellular human dermis	Expensive and difficult to acquire and use, require suitable donors and / or laboratory processing, require hospital admission, frequently short-lived and time consuming to develop
Synthetic foam dressings	Dry	Polyurethane base	Space filling, exudate absorptive, debridement, protection, insulation, pain reduction, suitable for infected wounds Thick (several millimetres to centimetres ï pers. obs.) Can be left on for up to seven days

Product Category	**Dressing Type**	**Description**	**Use / Comment**
Gauze	Dry	Woven cotton or non-woven synthetic with or without additives impregnated	Thick (several millimetres to centimetres ï pers. obs.) Tends to dry onto the wound Space filling, mechanical debridement (but may damage newly forming tissue), exudate absorptive (but may therefore allow desiccation of the wound), insulation Suitable for infected wounds
Alginates	Dry or moist	Calcium salt of alginic acid from seaweed	Not suitable on dry wounds Suitable for infected or bleeding wounds, promotes granulation, space filling, exudate absorptive, debridement, haemostasis, pain reduction Changed every 12 hours to four days
Impregnated gauze	Dry or moist	Gauze with various compounds added: saline, paraffin, iodine, hydrogels, etc	Thick (several millimetres to centimetres ï pers. obs.) May cause maceration of surrounding tissue, cheap and readily available

2.7.4.2 Dressings

There are hundreds of dressings from which to choose (e.g. 359 in 17 categories in the USA reported in emedicine (Ruszczak, Schwartz, Joss-Wichman, et al., 2009)). There are also many papers comparing one or more dressings. Most of these are human clinical reports and many report single case studies. Guidelines from the Agency for Healthcare Policy and Research - Pressure Ulcer Treatment made recommendations based on evidence from medical literature from between 1966 to mid-1993 (Ovington, 1999). Ovington (1999) reviewed these guidelines and the relevant literature from between 1993 and 1998 and concluded that the overall strength of evidence does not support any one treatment. Chaby et al. (2007) identified 2330 studies by reviewing three major databases (MEDLINE, Embase, and the Cochrane Controlled Clinical Trials Register) between 1990 and 2006 but reduced these to 99 ñreasonableò trials. Of the 99, none satisfied their level A category of large randomised double-blind controlled studies (Chaby, et al., 2007). None of the above provides much guidance in the initial selection of a dressing.

The obvious first choice is between wet and dry dressings. Either dressing type can be used as a simple dressing or in combination with another topical application.

Dry dressings range from a simple gauze bandage to complex foam sponges. They provide mechanical protection and can also provide a delivery mechanism for wound healing chemicals. Wet dressings also range from the simple, e.g. a film, to more complex.

Winterôs work clearly demonstrated an improvement in the rate of re-epithelialisation in a moist environment (Winter, 1962) at least twice that of a dry environment (Winter & Scales, 1963). He also indicated faster regeneration of the underlying connective tissue. Hinman and Maibach (1963) also demonstrated this increase in re-epithelialisation rate using a polyethylene film occlusive dressing on human subjects. Pig wounds treated with an occlusive dressing also contracted faster and had smaller scars (Winter, 1963).

Dyson et al. (1988; 1992) studied the effects of moist versus dry dressings using Opsite® (an occlusive polyurethane dressing) and dry gauze on experimental wounds in pigs. Histological examination of healing pig wounds showed a more regular and advanced development of granulation tissue earlier in a moist compared with a dry healing environment (Dyson, et al., 1988). The moist environment wounds showed a more rapid resolution of the inflammatory phase and therefore an earlier granulation stage (faster reduction of numbers of neutrophils and

macrophages and a more rapid increase in fibrocytes and epithelial cells). The study suggested that fibroblasts activated to myofibroblasts much earlier in moist than in dry healing environments, increasing contracture and decreasing scarring (Dyson, et al., 1988). Further, vascularisation was more rapid and more orderly in a moist than in a dry wound environment (Dyson, et al., 1992). In summary, their results were in favour of moist healing (with Opsite®). Moist wound healing is generally regarded as ideal (Jones & Harding, 2001) and is recommended by the USA Agency for Healthcare Research and Quality[8] as the prime treatment for ulcers (Ovington, 1999).

Dry dressings can be combined with materials to enhance their wound healing potential by giving them occlusive properties or healing components. Examples include:

- soaking gauze in paraffin (occlusive),
- polyurethane foam dressings (occlusive) (Banks, Bale, Harding, et al., 1997),
- impregnated sponges (occlusive and / or deliver enhanced healing components) (Choi, Lee, Hong, et al., 2001).

Although some of these improved dry dressings show positive results for both human and non-human (rat) animal models (Banks, et al., 1997; Choi, et al., 2001) they do not offer an advantage over moist dressings and require restraint of the flying-fox, therefore, this research focussed primarily on moist dressings.

Within this category the options are:

- occlusive or semi-occlusive adhesive film,
- saline,
- hydrogel / hydrocolloidal,
- silicon meshes,
- combination dressings,
- collagen dressings.

[8] formerly the USA Agency for Health Care Policy and Research

Moist Dressings

The simple moist dressings are saline / gauze, adhesive films and hydrocolloidals / hydrogels. For a description see Table 1.

Many clinical trials over the last 20 to 30 years comparing the different moist dressings gave varying results as to the efficacy of particular dressings (Leicht, Siim, & Sorensen, 1989; Feldman, Rogers, & Karpinski, 1991; Tan, Roberts, & Sinclair, 1993; Banks & Harding, 1994a, 1994b; Thomas, Banks, Bale, et al., 1997; Viciano, Castera, Medrano, et al., 2000). Thomas (2008) presented a review of these studies regarding several aspects such as healing time, pain reduction and cost. A Cochrane Review[9] of dressings on burns indicated some slightly positive results for hydrocolloids, polyurethane films, silver dressings (but not silver sulphadiazine) and biosynthetic dressings (Wasiak, Cleland, & Campbell, 2008).

One particular prospective clinical trial examined saline moistened gauze, hydrogel sheet, and a hydrocolloidal sheet (Mulder, Altman, Seeley, et al., 1993). No significant difference was found in efficacy of these three moist healing dressing styles but the trial highlighted significant differences in other areas which would be important in the treatment of flying-foxes, such as the number of dressing changes. Similar differences were indicated in a review of the AHCPR guidelines (Ovington, 1999) and a study comparing gauze and hydrocolloid dressings (Viciano, et al., 2000). The most important conclusion, with respect to treating flying-foxes, was that saline dressings need to be changed up to 10 times more often than occlusive dressings (Ovington, 1999). This is a significantly negative attribute for a dressing used for treating wild animals.

It is neither practical nor desirable to handle wild animals frequently and therefore dressings requiring fewer changes are preferred. It was also noted that clinicians in the Mulder et al. (1993) trial preferred the hydrogel sheet because they could monitor the wound and therefore its progress without removing the dressing. This was a common comment in many of the trials. The non-adhesive surface of the particular hydrogel sheet also reduced pain when the dressing was removed (Mulder, et al., 1993). Minimising pain is very important for animal welfare to reduce stress. Most wounds are not a clean cut as in a deliberate experiment but are more in the nature of

[9] ñCochrane Reviews are systematic reviews of primary research in human health care and health policy, and are internationally recognised as the highest standard in evidence-based health careò ("Cochrane reviews," 2011)

a graze, i.e. patches of intact epidermis and patches of torn epidermis or dermis and therefore are painful and easily damaged.

A dressing is only useful if it remains in place. When treating wildlife the removal of the treatment by the patient is a significant factor.

Barrier Films

Barrier films are a polymer dissolved in a quick drying carrier and applied as a spray. A meta-analysis of one of these (3M$^{\hat{E}}$ Cavilon$^{\hat{E}}$ no-sting barrier film) found that it effectively protected peri-wound skin although not more so than other dressings (Schuren, Becker, & Sibbald, 2005). Other sprays have been reported as neither better nor worse than standard treatments (Dover, Otto, Nanchahal, et al., 1995; Quinn, Lowe, & Mertz, 2000).

Special Dressings

The ósSpecial Dressingsôcategory covers engineered skin substitutes, either organic or inorganic, and dressings that contain wound healing biochemicals. Most of these require specialised facilities or resources to obtain or to use.

Amniotic membrane: this is available fresh or preserved and is considered extremely effective with burns in humans (Gajiwala & Gajiwala, 2004). It is clear, enabling the wound to be observed. Disadvantages are that it needs to be changed daily, covered with a thick gauze dressing and is not readily available.

Graft materials: acellular matrices, xenogenic, allo and auto-grafts. These are widely used in humans, are generally successful and are used in a variety of situations from leg ulcers (Siedler & Schuller-Petrovic, 2002) to burns (Herndon & Tompkins, 2004). Availability is limited, the materials are expensive and application requires hospital admission. Graft rejection is also an issue.

Alginates: these dressings are made from seaweed, are highly absorbent and can promote granulation (Myer, 2002). Alginates are not recommended for dry wounds because they do not form a gel, unless in contact with moisture, and therefore can dehydrate the wound (Myer, 2002).

A Note on Injury Infection

Bacteria in a wound may lead to infection. Some dressings, e.g. alginates and carboxymethylcellulose dressings can trap bacteria (Tachi, Hirabayashi, Yonehara, et al., 2004) and hold them away from the wound surface. Tachi et al. (2004) used a carboxymethylcellulose dressing and two alginate dressings with *Staphylococcus aureus* and *Pseudomonas aeruginosa* inoculated wounds. All dressings trapped bacteria but the carboxymethylcellulose dressing was better at retaining both bacteria. While this favours a carboxymethylcellulose dressing as the moist environment dressing, it is not an active treatment against bacteria.

2.7.4.3 Topical Applications

General

Topical applications other than dressings were reviewed. These were sourced from conventional medical and veterinary sources, from alternative medical sources and from research.

Silver

Silver as a topical application is available in the form of silver impregnated dressings and as a topical cream. It has long been used as an antiseptic (Lansdown, Sampson, Laupattarakasem, et al., 1997) and experimentally proved effective against infection (Konrad, Tsunoda, Weber, et al., 2002). Silver (as silver nitrate solution or as silver sulphadiazine) has also been indicated to improve wound healing (Lansdown, et al., 1997). Silver (incorporated in a foam) has been indicated as beneficial in the reduction of ulcer size in chronic ulcers (Jørgensen, Price, Andersen, et al., 2005). The relevant Cochrane report, however, concluded that there was insufficient evidence to recommend the use of silver over other treatments for infected or chronic wounds (Vermeulen, van Hattem, Storm-Versloot, et al., 2007).

The primary use for silver sulphadiazine (SSD) seems to be specifically for burns and infections but it is not rated as clearly the best treatment in the literature. Further, the use of SSD to treat burns has been questioned compared with other standard dressings, with silver impregnated dressings (Wyatt, McGowan, & Najarian, 1990; Wasiak, et al., 2008) and with honey (Subrahmanyam, 1991, 1993).

Veterinary Keratinolytic Solutions

There are a range of veterinary wound cleansing solutions which contain keratinolytic agents for debridement and microbial inhibition - such as salicylic acid (Bashir, Dreher, Chew, et al., 2005) and which are promoted as enhancing granulation (A. Gallard, e-mail, April 8, 2009). There is a lack of literature supporting one solution over another although a particular product may be in fashion in wildlife rehabilitation at any one time point.

Growth Factors

Theoretically, if the specific growth factor was deficient and introducing it did not interfere with the injury, directly applying one or more of the chemical factors involved in the healing cascade should improve healing.

Basic fibroblast growth factor (FGF) applied to the wounds of healing impaired mice improved the healing rate to that of normal mice (Tsuboi & Rifkin, 1990) and improved the healing rate of wounds in pig skin by 20% (Hebda, Klingbeil, Abraham, et al., 1990). A small human clinical trial of wound healing using EGF (epithelial growth factor) showed a slight increase in the healing rate (Brown, Nanney, Griffen, et al., 1989). Positive results have been reported for transforming growth factor type Ŭ(TGF$_{Ŭ}$) and for vaccinia growth factor (VGF) (Schultz, White, Mitchell, et al., 1987) but they were only tested on two animals and the results varied between wounds on the same animal. A single growth factor may be insufficient and the method of application can have a major impact on success (Lynch, Nixon, Colvin, et al., 1987).

Honey

Honey has been used in healing, including wounds, for many centuries (Molan, 1992a) but is still regarded as a complementary medicine. In the last 20+ years honey has attracted increased interest in its properties in both the popular press and the scientific field.

Oryan and Zaker (1998) tested its use on cutaneous wounds in rabbits. By day two the treated wounds demonstrated clear signs of healing (in contrast with the untreated) such as reduction of inflammation and by 14 days treated wounds had visibly healed ï well ahead of untreated wounds. Oryan and Zaker (1998) concluded that, due to the lack of polymorphonucleic cells in the wound, honey has clear antimicrobial properties. They also concluded that honey speeds healing by reducing oedema, cleaning the wound and increasing epithelialisation and wound contraction. Bergman et al. (1983) demonstrated (histologically) faster healing in honey treated

wounds in mice over saline treated wounds. They attributed this to the honeyôs ñenergy producing properties", hygroscopic effect and antimicrobial properties (Bergman, et al., 1983).

Subrahmanyan (1991, 1993, 1997, 2001) evaluated honey in the healing of burns in several human clinical trials. Three studies compared honey to SSD (Subrahmanyam, 1991, 1997, 2001) involving a total of 254 patients evenly distributed between the two treatments. In all three trials the healing was significantly faster with honey than with SSD. Interestingly, the times for honey healing were reasonably consistent, with approximately 100% healed by day 21, however the times for SSD healing were considerably more variable, with 42% to 100% fully healed by day 21. In the earlier study (Subrahmanyam, 1991) Subrahmanyan noted a faster onset of granulation in, and more effective sterilisation of, the wounds treated by honey over the SSD group. In 1997, studying clinical evidence, faster healing of sterile wounds was also noted. In addition, histological evidence demonstrated earlier shifts from inflammation to granulation and faster epithelialisation in the honey treated group. In a latter study (Subrahmanyam, 2001) the focus was on the biochemical aspects of burn injury and again honey proved more effective in correcting these than SSD.

Wounds treated with honey also healed faster with a lower infection rate than those treated with Opsite® (a polyurethane film) (Subrahmanyam, 1993). While interesting, Opsite® is merely a film without biochemical properties. Its primary purposes are to retain moisture and provide a mechanical barrier. A prospective, randomised, double-blind controlled trial on abrasions and shallow wounds sustained by mineworkers did not find any significant difference between honey under Opsite® or a hydrogel under Opsite® (Ingle, Levin, & Polinder, 2006).

Honey has also been used in the healing of skin graft donor sites (Misirlioglu, Eroglu, Karacaoglan, et al., 2003). In this study honey performed significantly better in healing rate than the paraffin gauze or the saline soaked gauze but there was not a significant difference in the healing time between honey and hydrocolloid dressings. Histological examination confirmed the clinical findings.

The Cochrane review - Honey as a Topical Treatment for Wounds (Jull, Rodgers, & Walker, 2008) - reviewed 19 human trials. The review concluded that honey may improve healing times in mild-to-moderate burns but there is insufficient evidence in other areas (Jull, et al., 2008).

There is much discussion on the reason for honey's reported success in treatment (Molan, 1992a, 1992b). Two reasons for success are considered to be its antibacterial and antifungal properties.

These properties have been attributed to osmolarity, acidity, hydrogen peroxide or to an unidentified substance frequently called inhibine (when this is not equated with hydrogen peroxide) which is usually attributed to the properties of the supplier plant (Molan, 1992b; Willix, Molan, & Harfoot, 1992; McCarthy, 1995; Zaghloul, El-Shattawy, Kassem, et al., 2001). Honey is likely to have different constituents depending on the source of its raw ingredients: nectar and pollen. Zaghloul (2001) reports no significant difference in honeyôs antimicrobial properties from different regions across Egypt. Others also report no significant difference, but attribute the results to different components of the honey - hydrogen peroxide in non-manuka honey and to ñnon-peroxide factorsò or unknown ñphytochemical componentsò in the manuka honey (Willix, et al., 1992; Cooper, Molan, & Harding, 1999).

In summary, honey has the potential to:

- provide a moist wound environment,
- clean wounds,
- reduce inflammation,
- improve re-epithelialisation, and,
- reduce infection.

Aloe vera

There are many commercial claims for the use of *Aloe vera* (Syn *Aloe barbadensis*) in moisturising, wound healing and many other applications (Joshi, 1998). It is used in complementary medicine. Several studies have examined the impact of various components of *Aloe vera* such as acemannan (Bradley, 1998), mannose-6-phosphate (Davis, Donato, Hartman, et al., 1994) and the effect on glycosaminoglycans (Chithra, Sajithlal, & Gowri, 1998). These studies have reported improved healing over untreated controls. This does not mean an improvement over other treatments nor that the results can be extrapolated to a whole plant extract. Chithra et al. (1998) reported increased collagen production in rat wounds with systemic and topical *Aloe vera* extract. The sample size was not given. Thomas et al. (1998) reported ñno significant differenceò between acemannan and saline. Heggers et al. (1995) reported some benefits for the use of *Aloe vera* over SSD and antibacterial topical applications. Schmidt and Greenspoon (1991) reported delayed healing with the use of *Aloe vera*, however, the standard deviation was up to 50% of the mean and a large number of data points were lost from the non-

aloe group. Published results appear confusing and / or contradictory and several reviews comment on the lack of clear direction (Maenthaisong, Chaiyakunapruk, Niruntraporn, et al., 2007; Feily & Namazi, 2009) and the requirement for proper trials.

Arnica

Systemic or topical arnica (a plant extract from *Arnica montana*, *A. chamissonis*, *A. fulgens, A. codifolia* or *A. sororia*) has long been accepted by the general public, as a suitable treatment for bruises (Alonso, Lazarus, & Baumann, 2002). It is a complementary medicine used topically to treat bruises in Europe and the United States (Kouzi & Nuzum, 2007).

Alonso et al. (2002) did not find any benefit in using arnica on bruises caused by laser treatment. Stevinson et al. (2003) carried out a placebo, double blind trial with oral homeopathic arnica and concluded that there was not any significant difference in the pain or bruising which resulted from hand-surgery. This result sparked an interesting debate by the homeopathic practitioners (Fisher, Mathie, van Haselen, et al., 2003) but unfortunately it failed to reach consensus or provide proof as to technique or efficacy. Jeffrey et al. (2002) carried out a trial on hand surgery patients using both topical and oral arnica in a blinded clinical trial. There was some evidence of a decrease in pain (at two weeks) based on a subjective assessment by the patient, but not in swelling. There appears to be even fewer scientific investigations into arnica than *Aloe vera* and most are reviewing arnica as a homoeopathic treatment rather than as a topical application.

Macadamia Oil

Macadamia oil[10] was recommended by a massage therapist for use on a flying-fox unrelated to this research and was successful (pers. obs.). It was used in lieu of vitamin E oil, which was difficult to obtain at the time.

There appears to be very little scientific data on the action of macadamia oil on skin despite enthusiasm from manufacturers and the cosmetic industry. Macadamia oil is high in palmitoleic acid (Ricks, 1991) and cosmetic retailers are very positive about the value of palmitoleic acid for the skin. This seems to be primarily due to the claim that palmitoleic acid is very close to the fatty acids of sebum which decreases as people age (Ricks, 1991). Macadamia oil is promoted as

[10] Macadamia, *Macadamia integrifolia,* is an Australian tree native to Queensland and northern New South Wales (Ryan, November 2006). The oil is a fine non-greasy oil and is produced by mechanical pressing of the nut kernel, information supplied by Macadamia Oils of Australia, Wollongbar NSW

an anti-ageing and moisturising treatment, because of its high levels of palmitoleic acid (Macadamia Oils of Australia, 2006). ñMacadamia oil helps replenish the palmitoleic acid in the skin's oil (sebum), which diminishes as we get olderò (Graig Farm Organics, 2009). Macadamia Oils of Australia reports the GC profile for macadamia oil as 15-21 % palmitoleic acid C16.1, 14.5% palmitic acid C16:0, 7% linoleic acid C18:2 and 47% oleic acid (Macadamia Oils of Australia, 2006).

Akhtar et al. (2006) referred to palmitoleic acid in children's skin being responsible for their "dewy skin" and that it is not present in adult sebum. Their paper focused on the physical properties of the oil to prove that it would be non-toxic and useful in the preparation of cosmetics rather than its biological worth.

Essential fatty acids applied topically can restore barrier integrity to mammalian skin (Houtsmuller & van der Beek, 1981) and decrease water loss (Hartop & Prottey, 1976). Palmitoleic acid has been shown to increase keratinocyte proliferation to excess in normal skin (Katsuta, Iida, Inomata, et al., 2005). This effect may be an advantage when applied to wounded skin. Palmitoleic acid also inhibits gram positive bacteria (Wille & Kydonieus, 2003). Fatty acid composition varies in different mammalian skins (Gray & Yardley, 1975; Ge, Gordon, Hsuan, et al., 2003) and the fatty acid composition of flying-fox skin is unknown.

2.7.4.4 Other Solutions

A variety of other solutions from alternative and complementary medicines were reviewed: e.g.:

- catfish gel (Wang, Thomson, Ali, et al., 2001),
- chitosan (Kweon, Song, & Park, 2003; You, Park, Koh, et al., 2004),
- curcumin (Sidhu, Singh, Thaloor, et al., 1998),
- mitogenic whey (Rayner, Cowin, Robertson, et al., 2000),
- oriental hornet venom (Dayan, Barr-Nea, Sandbank, et al., 1983), and,
- pine resin (Simbirtsev, Konusova, McHedlidze, et al., 2002).

Refer to section 3.4 Treatment Selection for details on the selection process.

2.8 Measurement

To determine the effectiveness of treatment, the healing progress must be assessed and measured. While the assessment of injuries is not regarded as an exact science, and lacks general agreement as to the best method (van Rijswijk, 2001), it needs to be reliable and valid.

To assess wounds clinically the area, depth and appearance are recorded. Injury area is measured using three methods (van Rijswijk, 2001; Lampe, 2002):

- rule,
- tracing, and,
- photography.

Tracing involves placing a clear plastic film over the injury and tracing the injury edges with a pen. The method is very accurate (Lampe, 2002); W. Corbridge, unpublished research, 2005). Alternatively, the sheet can be scanned and the area calculated using a software tracing package.

Photography requires a grid marked lens or a rule in the photograph for scale and the area can then be calculated manually or with appropriate software. Photography and tracing correlate well (van Rijswijk, 2001; Lampe, 2002).

Measurement reliability will be affected by the stretch on the injury and the position with reference to the plane of the lens (for photography). Variation can cause inaccuracy in calculations of size change.

Wound depth is commonly measured by inserting an instrument into the wound and measuring how far it penetrated. This is only practical for wounds that are at least several millimetres deep.

2.9 Summary

Flying-fox species are listed as Vulnerable on the IUCN Red List of Threatened Species (Lunney, et al., 2008) and are frequently harmed by contact with artificial structures, often sustaining open wounds and bruises to the wings. Very little is known about flying-fox healing. Treatment for injuries is all too often euthanasia or permanent non-functional scarring (i.e. the flying-fox cannot fly).

Flying-fox wing membrane follows the basic mammalian skin pattern, see Figure 1. The wing is unusual (Swartz, et al., 1996) in that it is a large area of skin without a subdermis. The membrane is a pigmented, nearly hairless, highly compliant integument with visible bands of muscle and elastin (Church & Warren, 1968; Iversen, et al., 1974). The membrane (patagium) consists of three layers ï a dorsal and a ventral epidermis and a dermis of normal connective tissue (Crowley & Hall, 1994). Healing and treatment can be complicated by the reduction or absence of the subdermal structures.

Healing is a complex set of processes beginning immediately after the injury event and continuing for up to two years (Clark, 1996a). Healing was divided into four major stages: haemostasis, inflammation, granulation and scar remodelling. Each stage provides the initiators and mediators for subsequent stages. It is important to note that although considered as distinct stages for ease of analysis, the processes overlap in time and may occur in parallel throughout the injury.

Only a few studies of healing in Chiroptera have been carried out. These include full thickness open injuries, partial thickness open injuries and closed injuries. Full thickness open injury studies noted that the initial wound immediately increased by 40% due to the stretch on the wing membrane (Church & Warren, 1968) and healed by the ventral and dorsal epithelial layers fusing across the wound edge within three days (Church & Warren, 1968; Bhangoo, 1974; Bhangoo & Church, 1976). Infill of a 20 mm x 20 mm hole was complete in 28 days. Partial thickness open injuries (epidermal damage only) of a 12 mm x 50mm area (Iversen 1974) were re-epithelialised by 7-11 days. Closed injuries (crushing bruises) of 8 mm x 1 mm size (Church & Noronha, 1965; Church, et al., 1966) were reported as healed within 20 days.

Possible treatments were identified through literature review, current veterinary practice and rehabilitator experience. Moist wound healing has been shown to be more effective than dry since at least 1962 (Winter, 1962) and is today regarded as the gold standard (Jones & Harding, 2001). This may be achieved by a dressing from simple to complex (see Table 1) or by a topical application. Various dressings and topical applications were reviewed: occlusive or semi-occlusive adhesive film, saline, hydrogel / hydrocolloidal, silicon meshes, combination dressings, collagen dressings, barrier films, special dressings (such as alginates, amniotic membrane, and graft materials), silver (as a dressing or topical application), keratinolytic solutions, growth factors, honey, arnica, *Aloe vera*, and macadamia oil. The dressing or application must not only be suitable for healing it must be acceptable to the flying-fox and easy to use for the rehabilitator.

The objective of this study was to identify effective and efficient treatments. An effective treatment enables the injuries to heal so that full flight is achieved. Efficiency is assessed in terms of: time to complete healing, minimising stress to the flying-fox, ease of treatment, and, minimising cost for the rehabilitator.

The hypotheses are that:

- all treatments will achieve healing, and,
- some treatments will enable faster healing than others.

3Materials and Methods

3.1 Patient Source

It was expected that most flying-foxes would come into the study from normal rescues such as caught in nets (entangled in loose netting thrown over backyard fruit trees) or barbed-wire (e.g. paddock and security fences) and come into care during summer. This is when backyard fruit trees are fruiting and flying-fox numbers are highest in urban areas.

It was considered preferable that flying-foxes be transported from the rescuer to the research facility as soon as possible after the injury event, preferably within 24 hours. Not all flying-foxes were expected to show obvious injuries on arrival and not all were expected to develop observable injuries. Rescuers who provided animals to the study were asked to estimate the position on the wing (of the strands of the net) and severity of the entanglement (this information was patchy at best).

It was expected that the grey-headed flying-foxes would be the primary species as the research facility was based in the Greater Sydney region. Black flying-foxes (*P. alecto*) have reached the Sydney basin and little red flying-foxes (*P. scapulatus*) are found in the nearby Hunter region and so one or two were possible study animals. It was expected that the majority of the flying-foxes would be adult and that both genders would be represented.

3.2 Assessment of Injuries

3.2.1 Introduction

It was realised in the first year of the study that the classic definitions and assessments for healing are not entirely useful with reference to flying-fox wing injuries. Most flying-fox wound healing (and all wounds treated in this study) are conventionally classified as secondary intention wound healing. Healing is normally pictured as occurring in parallel with (or across) the underlying structures (e.g. dermis, bone, muscle,). In full thickness membrane wounds of flying-foxes the healing occurs at right angles to the underlying structures (which have been totally lost in the area of the wound). In a flying-fox wing the wound may heal by the dorsal and ventral epithelial layers fusing (similar to primary intention healing), see Figure 5, leaving a large

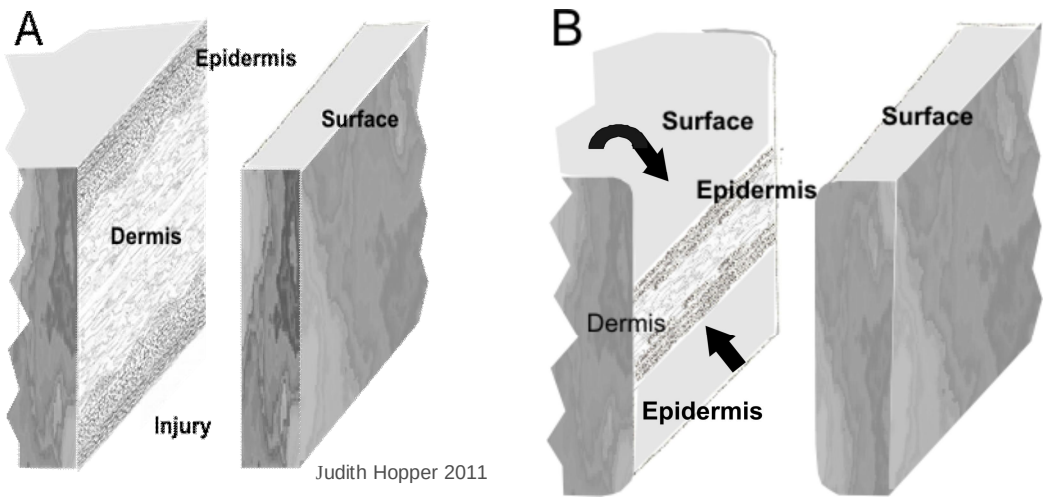


Figure 5 Schematic of flying-fox wing membrane full thickness healing by ñprimary intentionò A. Injury Site, cut through all membrane layers. B. Dorsal and ventral epidermal layers healing across the dermis. Arrows indicate direction of granulation growth.

hole, rather than a single epidermis meeting across the wound, see Figure 3. This process gives a sealed (i.e. healed) wound but a hole in the wing (which normally fills in during the scar-remodelling phase). The wound may have healed but the wing has not. This understanding required a change of approach in the assessment of healing. Three aspects of healing, therefore, were defined for this research: healing of the injury, healing of the wing and healing for restoration of flight capability. Healing of the injury is the focus of this research study. The latter two activities are described in section 224.2 Appendix B Healing for Release.

3.2.2 Assessment Approach

Flying-fox wings were examined and assessed on arrival and regularly until release or euthanasia. A two-part assessment was chosen:

1. assessing the state of the injury, i.e. determining progress through the phases of healing (see Table 3 Injury States), and,

2. the change in size of a wound.

It was initially intended to analyse the reduction in size of closed injuries and so all injuries were photographed to measure their size. In the analysis phase it became apparent that certain injuries had not been accurately recorded (for measurement analysis) due to the complexity of the injury,

the inability to determine precise edges on a dark wing or due to the position of the injury on the wing (e.g. wrapping around the wrist, see Figure 6).

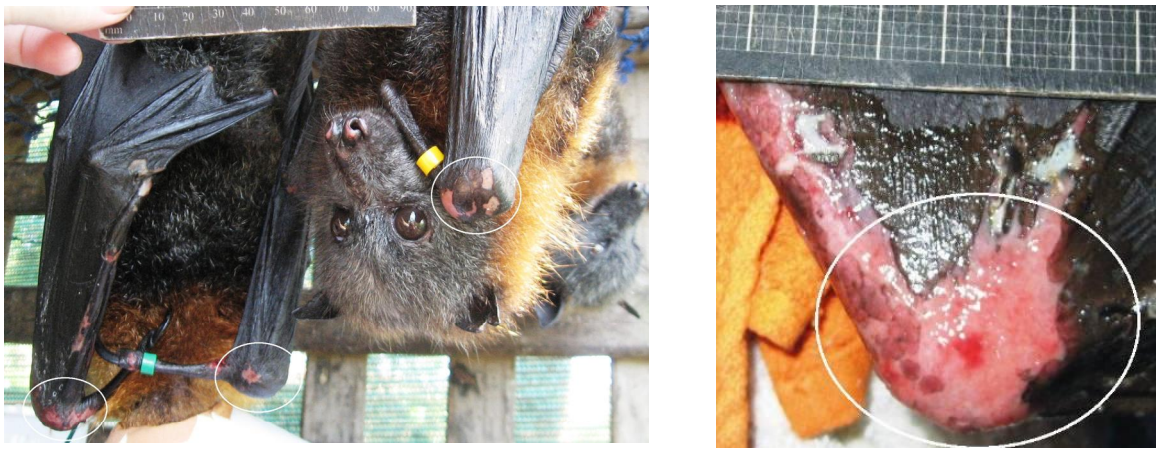
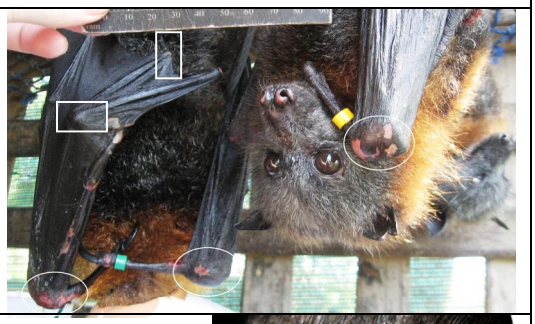
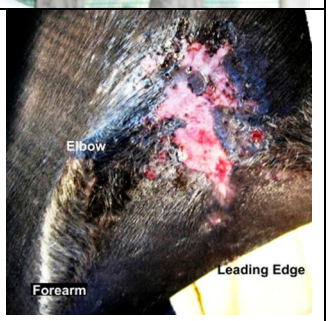


Figure 6 Wrist injuries. Circles indicate wrist areas.

3.2.3 Wing Site Classification

All injuries were classified and analysed by site, see Table 2 and Figure 2.

Table 2 Wing Injury Sites

Site Name	Description	Sample Image
Patagium	Any area of open membrane of the propatagium, dactylopatagium, uropatagium or plagiopatagium	
Leading Edge	The cranial edge of the propatagium that contains a major vein and a band of connective tissue	
Finger bone	The phalanges (digit bones) of the wing.	
Joint	Phalangeal joints (outlined with rectangles in the image). Carpus (wrist joint ï circled in the image). Elbow joint - not pictured.	
Arm	Forelimb bones not including the wrist or hand bones and not including elbow joint injuries.	

3.2.4 Injury States

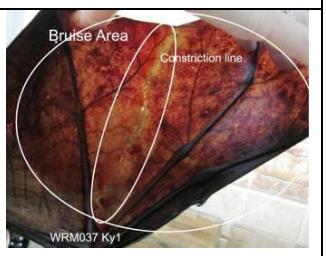
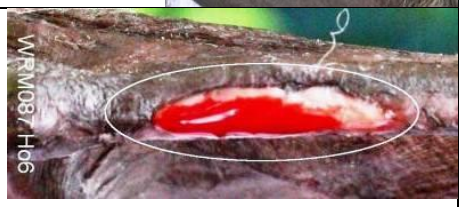
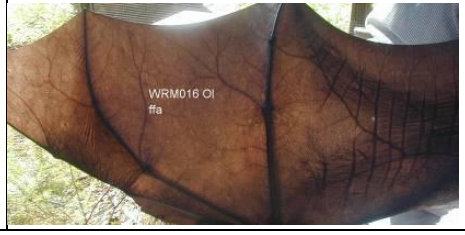
Two healing points and two main phases were defined for this research. ôResolution of Inflammationô point - which combines haemostasis and inflammation processes as one main phase (inflammation phase), ôCompletion of Granulationô point (i.e. reaching re-epithelialisation or functional scar) - which combines the processes of ECM deposition, angiogenesis and normal re-epithelialisation or scar formation as the second main phase (granulation phase). The ôResolution of Inflammationô point was defined as the point at which inflammation resolved and granulation began and was identified as the day half way between when new tissue was macroscopically visible and the last day on which it was not. ôComplete Healingô refers to both phases in combination. Scar remodelling was outside the scope of this research study but was required on welfare grounds (in order to ensure the flying-fox was fit for release) and is covered in Appendix B Healing for Release.

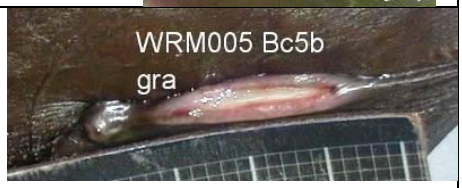
To map progress towards the three healing points a series of descriptive states was defined - injury states as described in Table 3. The following states were regarded as óhealedô: scar with re-epithelialisation (swr), full epithelialisation (fep) and full colour (fco).

Flying-fox wings were evaluated for injuries and the observed injuries were coded on arrival of the flying-fox and at each treatment or daily review. The state of the injury was recorded on the individual flying-foxôs care record. It should be noted that injuries were not always apparent on arrival and sometimes changed category during progression.

All injuries were photographed weekly or when a change of state was perceived. Unfortunately, this assessment allows bias on the part of the researcher to influence the outcome. To overcome this bias all images, stripped of identifying information, were examined by a trained assessor and each injuryôs state recorded. All conflicting state changes were reviewed and resolved, taking into account both assessments so that none of the wound states remained equivocal.

Table 3 Injury States

Observable State	Code	Description	Sample Image
Bruise	rbs	A red shadow seen when the membrane is trans-illuminated, or, a white shadow seen on the surface of the wing.	
Swelling	swe	Build up of fluid between the epidermis and dermisï e.g. a blister.	
Epithelial layer damage (erosion)	eld	Minor scrape of the top layers only, e.g. peeling.	
Dermal layer damage (ulcer)	dld	Moderate scrape involving damage through the epidermis but not yet a hole.	
Subdermal damage	sdd	Loss of dermal layers of the membrane ï to produce a hole or the exposure / damage of bone.	
Fully functional area	ffa	Normal appearance	

Observable State	Code	Description	Sample Image
Full epithelialisation	fep	Recovered injury with complete replacement by a normal epithelial layer.	
Full colour	fco	Return of normal pigmentation.	
Granulation	gra	Development of new tissue in the injury (therefore inflammation has resolved at the injury edge).	
Scar with re-epithelialisation	swr	The injury is functionally healed although scar remodelling, hole infilling and re-pigmentation may continue.	 
Bone loss	blo	Permanent loss of bone	
Wing loss	wlo	Loss of an area of wing membrane	

3.2.4.1 Membrane Structure

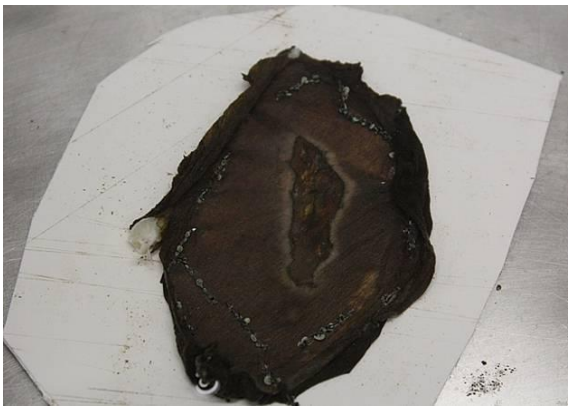


Figure 7 Mounting for tissue fixation.

One of the flying-foxes died in care, during the healing process and the opportunity was taken to obtain samples of tissue from three different healing areas. These were preserved in neutral buffered formalin and embedded in paraffin wax as outlined in Crowley and Hall (1994). As they experienced difficulty in keeping the tissue flat the entire wing area was stitched to a piece of stiff plastic prior to immersion in the formalin, see Figure 7. A cardboard frame was stapled to the sample of tissue required, prior to beginning the dehydration / clearing process, see Figure 8. Dehydration was by immersion in graded alcohols as is usual. Clearing however was with celloidin in a 1:1 absolute alcohol and ether mix (1:1 celloidin to mix). This was carried out overnight followed by three one-hour immersions in chloroform. The tissue was then: embedded in wax in the normal manner, sectioned (4 ï 6 µm thick) and stained using a haematoxylin and eosin stain and a Gömöri trichrome stain. The trichrome stain rendered collagen green, muscle, fibrin and erythrocytes red, and nuclei blue-black. Slides were reviewed under light microscopy and digitally imaged for analysis.

Figure 8 Preparation for histological sectioning.

The three areas of tissue were:

- normal wing dactylopatagium (state: ffa),
- breaking down patagium (state: dld Ÿ sdd), and,
- healing patagium (from the edge of a full thickness hole through the membrane - state: swr).

Digital images of these tissues were reviewed and analysed for composition and presentation. Identified structures and presentations were compared with known descriptions, see sections 2.3.3 Micro Anatomy ï Megachiropteran Wings and 2.5 Chiropteran Healing.

3.2.5 Measurement

Measuring wound depth was considered impractical for membrane wounds as measurement would require microscopy techniques. This would necessitate unacceptable restraint of the flying-fox and equipment which was impractical to use.

Measuring length and width with a rule was considered too inaccurate, as naturally occurring injuries are often irregular in shape.

The tracing method requires the subject to be immobile for some time. Further, it requires a firm base from which to trace ï such as a leg ï rather than a flexible base such as an elastic wing membrane.

Photography is relatively quick and suitable for a flying-fox (which cannot be completely immobilised) with multiple wounds and so was the method selected. As accuracy requires the injury to be parallel to the plane of the lens for each measurement, extreme care was needed to ensure that the camera was held correctly. Further, a closed injury (e.g. bruise) on a black wing is difficult to see. This was, in part, overcome by using a back lit wing. While blue light actually provided the best contrast (W. Corbridge, unpublished data, 2005), sunlight or normal fluorescent light was fully adequate and was used.

The size of the injury was calculated using a scale in the image. A millimetre scale was glued to a magnetic strip to form a small rule that was placed beside the wound prior to photography, see Figure 9. If necessary, e.g. when only one handler was available, another magnetic strip on the other surface of the membrane kept the scale in place. As printers and photocopiers can distort printed scales, each rule was checked with a micrometer. There was less than 1% error in length.

Figure 9 Rule used to measure injuries.

Occasionally the rule was not available, e.g. one of the flying-foxes took it, it became too wet or otherwise damaged or the imaging was carried out off-site. A substitute was then used, such as a graduated syringe or barcode and this was later measured and recorded for reference.

The software package Image J (Rasband) was used to calculate area. The image was calibrated using the included scale and the area calculated from a line drawn along the edge of the injury using a computer mouse. The process was checked against well-defined simple shapes (e.g. rectangles and circles) of known size and the accuracy was within 1%. Twenty real wounds (i.e. undefined complex shapes) were measured at least twice. Difference in area measurements of real wounds was less than 5%.

3.3 Allocating Injuries to Treatment Groups

3.3.1 Initial Randomisation

At least five and preferably 10 injuries were required per treatment for statistical power. Some treatment types did not fit a particular injury classification based on veterinary / welfare grounds e.g. *Aloe vera* on a severe open arm wound. The position of injuries relative to each other occasionally dictated treatment, e.g. two injuries a few centimetres apart would have to be treated in the same manner, as treatments could not be isolated. Occasionally the rescuer had begun treatment, particularly after the first year of the trials when the success with macadamia oil became apparent. Any treatment started was continued for that injury - which is why there are more injuries in the macadamia oil group than in the other treatment groups.

Subject to the above provisos, injuries were allocated to a treatment group on arrival of the flying-fox or on appearance of the injury until at least five injuries were allocated to a treatment and then the next treatment was begun. The cycle was repeated until the end of the study except for the high failure rate treatments, which were discontinued, see section 3.3.2 Change of Treatment.

Numbers of injuries per treatment group varied, due to the above qualifications, but none of the treatment groups had fewer than the desired number of five. In fact the lowest number in an active treatment group was 14 with nine in the no treatment (control) group.

A single treatment was maintained until the injury was healed or healing was deemed to have failed.

Overall the approach resulted in a minimum of ten injuries on ten individual flying-foxes per treatment. In practice, 30 flying-foxes had mixed treatments, with 26 of those due to the failure of an initial treatment requiring a change of treatment for a specific injury, see section 3.3.2 Change

of Treatment. The maximum number of different treatments per flying-fox was two in the case of non-failures and three in the case of failures.

3.3.2 Change of Treatment

Changes of Treatment due to Lack of Healing

The treatment of an injury was rated as having failed if an unacceptable animal welfare issue arose. This was determined by:

- the injury worsening beyond an acceptable animal welfare state e.g. major bone exposed,
- an injury phase stalled and did not progress, creating an animal welfare issue,
- other serious event according to veterinary advice.

Three failures within the same treatment group would mean that no further injuries would be treated with that application. In practice, due to the number of injured flying-foxes being received, considerably more than three injuries were usually treated with the same treatment in parallel so that if a treatment consistently failed it was across more than three injuries. When a treatment was discontinued due to a failure of healing, the data set was included in the non-healing analysis ï see section 4.3 Success or Failure.

If an injury was to become unacceptably severe based on veterinary / welfare assessment or the patient developed systemic problems then the flying-fox would be removed from the study and treated as dictated by welfare or veterinary requirements.

Changes of Treatments when Healing was Progressing

A treatment was sometimes changed although it was not considered to have failed, i.e. one of the defined healing points (see 3.2.4 Injury States) had been reached. This may have been due to inexperience (i.e. a belief that the treatment was failing when it is now known to be progressing), or accident (i.e. handler error in applying an incorrect treatment).

If a treatment was changed for either of the above reasons then it was not considered to have failed. The data from it was used as either a count of successful treatments and / or an estimate of time to reach the related healing point.

3.4 Treatment Selection

3.4.1 Initial Selection Considerations

The literature review, current veterinary practice and personal experience produced a list of possible treatments for injuries. Flying-fox rehabilitators were surveyed informally to identify current treatments but few treatments were identified and therefore the survey did not provide clear directions.

Certain environmental constraints had to be considered when selecting treatments. The first main constraint was that the technique required knowledge or facilities not practically available for wildlife and the second, if a thick dressing was required it would not likely be tolerated by a flying-fox.

Some treatments, e.g. addition of fibroblasts, while trialled successfully on animals (rabbits) (Sandulache, Zhou, Sherman, et al., 2003) were still not considered practical for wildlife treatment situations. Other applications, such as amniotic membrane, graft materials and growth factors were also rejected as impractical at this stage. Further, many of these treatments, as well as alginates and improved dry dressings require a thick covering dressing not likely to be tolerated by a flying-fox.

Alginate preparations were further contra-indicated as they are not suitable for dry wounds and it was considered that they may dehydrate the wing membrane. The solutions from alternative and complementary medicines listed under ñ2.7.4.4 Other Solutionsò were rejected due to lack of supporting evidence, cost and / or the difficulty in obtaining them.

Although there were positive indications for the use of laser therapy (Demir, Balay, & Kirnap, 2004) and a laser was available to the researcher, it was decided that these treatments are generally too expensive and too impractical for most rehabilitators and would only be trialled if the other treatments were unsuccessful.

3.4.2 Final Selection Considerations

The final selection process was influenced by the accepted standard of creating a moist wound healing environment. It was not feasible to trial all treatments reviewed and therefore certain constraints were applied. The treatment had to be:

- available over-the-counter, and
- usable on the flying-fox without excessively compromising normal behaviour during application or during use.

The first requirement, in particular was relaxed towards the end of the research for cases that were intransigent to other methods. This is discussed under **"**Hypotheses

Dressings

A dressing is only useful if it remains in place. When treating wildlife the removal of the treatment by the patient is a significant factor. Thick dressings are not likely to be tolerated by a flying-fox (pers. obs.). A thinner dressing, such as a polyurethane film, is better tolerated (or at least harder to remove), by the flying-fox (pers. obs.). A thin, transparent hydrocolloid dressing which enabled injury monitoring without removal was therefore considered desirable. For a list of dressing types considered see Table 1.

Spray bandages were considered a potential practical alternative to polyurethane dressings in areas difficult to wrap in a dressing. Several different commercially available spray dressings were trialled on human wounds to assess the ñstingò factor (pers. obs.).

Topical Applications

Silver sulphadiazine (SSD) efficacy over other applications was not supported by the literature review. Further, it is very expensive and not available over the counter thus incurring added expense for the rehabilitator and stress to the flying-fox by requiring a visit to a veterinary clinic. Silver sulphadiazine was not, therefore, formally trialled, however two flying-foxes were treated with SSD by the rescuer but this was stopped and other treatments begun because of lack of healing progression.

Ilium Oticlean is a veterinary keratinolytic solution used, by the present researcher, over many years for wound healing in brushtail possums (*Trichosurus vulpecula*). The efficacy of this

particular product was not well documented in the literature; however many wildlife rehabilitators use it and so a formal trial was considered beneficial. While Ilium Oticlean is only obtainable from a vet, a prescription is not required and it was added to the trial.

Honey was supported by the outcome of the literature review, was cheap, readily available and relatively easy to apply so was added to the trial. It should be noted that some of the factors in honey contributing to its usefulness may be destroyed by the high temperatures of pasteurisation, by light, by dilution and by some forms of filtering (Molan, 1992b) commonly used in commercially edible honey manufacture. Unprocessed honey is reasonably easy to obtain direct from a reliable apiarist.

Aloe vera plants are easy to obtain and grow, so fit the criterion of ease of accessibility by rehabilitators. Further, the researcher had personal experience of the success of treating burns in a ringtail possum with *Aloe vera*. For these reasons and because there was not a clear mandate against using *Aloe vera* from the literature, it was added to the trial.

Arnica cream is readily obtainable and as such fits the criterion of ease of accessibility by rehabilitators. Further, it is a popularly accepted product likely to be used by rehabilitators. For these reasons, and because there was not a clear mandate against using arnica from the literature, it was included in the trial.

Macadamia oil benefits have little conclusive evidence in the literature but it was cheap, readily available and appeared to benefit flying-fox membranes (pers. obs.). For these reasons it was added to the trial.

Infection Treatments

As a dressing was going to be required for any antimicrobial topical application it was decided that if a wound became infected a self-adhesive antimicrobial impregnated dressing would be used as an antimicrobial. These dressings are freely available over the counter in a range of sizes. The most common non-antibiotic antimicrobial dressings are iodine or silver. Silver was preferred as iodine can dehydrate the skin (veterinary advice[11]). In these instances the dressing would be selected for its antimicrobial properties not its alleged healing properties.

[11] Wildlife Assistance & Information Foundationôs Wildlife Veterinary Clinic

More complex antimicrobial treatments were available, the most obvious being antibiotic creams. These, however, required a prescription and therefore an increase in cost and difficulty in a rehabilitation situation. Their efficacies are established so they were not included in the trial. This would not exclude their use on welfare grounds if required.

3.4.3 Final Treatments Selected for Application

3.4.3.1 Macadamia Oil

ñCookingò grade oil (Macadamia Oils of Australia, Wollongbar NSW) was selected, not the more highly processed ñcosmeticò oil. The two versions are functionally the same but the cooking grade is cheaper and more easily obtained.

3.4.3.2 Hydrocolloid Dressing

A semi occlusive hydrocolloid dressing was required. A non-adhesive hydrocolloid dressing such as DuoDerm® (a combined gel and dressing by ConvaTec, Skillman, NJ, USA) was desired. The adhesive on these dressings is inactivated by the fluid in the wound and therefore adheres only to the undamaged area of the epidermis.

Duoderm is a veterinary product with reported successful[12] use on flying-foxes. It was found, however, to have several problems:

- it is opaque so the injury was difficult to monitor,
- it stuck to the injured areas of the wing, despite its advertised non-adhesive properties and was difficult to remove without causing further damage, and,
- many flying-foxes seemed to find it obtrusive and constantly tried to remove it.

Combinations of adhesive transparent polyurethane film and a clear hydrocolloid gel were considered and various options were explored. Opsite® (Smith & Nephew, Australia) and Tegaderm® (3M Health Care, USA) are both thin polyurethane dressings. Although Opsite® is similar to Tegaderm® the backing made it extremely difficult to apply to a flying-foxôs wing size and shape. It is much cheaper than tegaderm® but application requires a large, flat and immobile

[12] by one very experienced rehabilitator working with a wildlife veterinarian

surface. TegadermÊ has a backing removed prior to application leaving only a border to be removed after application (see Figure 10). Neither dressing includes a hydrocolloid.

Solosite^{Ê} (Smith & Nephew, Australia), a carboxymethylcellulose wound gel is readily available in a tube. It is similar to the hydrocolloid incorporated in DuoDerm.

Figure 10 Solosite and Tegaderm dressing. Dressing cut in half.

The most suitable combination was TegadermÊ and SolositeÊ . SolositeÊ was also chosen because carboxymethylcellulose traps bacteria well (Tachi, et al., 2004).

3.4.3.3 Silver Dressings

The silver impregnated adhesive dressing Elastoplast® product ñFast SilverHealing^{Ê} ò (Beiersdorf, Hamburg, Germany) was selected for infected injuries as it is readily available in a range of sizes and shapes.

3.4.3.4 Spray Bandage

Spray bandages were originally envisaged for use where it was difficult to apply conventional dressings e.g. around a wrist. A range of spray bandages were tested on human wounds (pers. obs.) to test the extent of stinging when applied to an open injury. Leuko® spray bandage (BSN Medical, Hamburg, Germany)[13] was chosen as it was found to be the only painless product. There is considerable confusion in identifying this product as its name has changed over the last few years and similar names have been used for totally different products. The actual product used is the one pictured in Figure 11.

Figure 11 Leuko® spray bandage.

[13] Leuko was a brand name of BSN Medical, which was purchased by Beiersdorf AG (Germany) and renamed as Elastoplast spray bandage

3.4.3.5 Veterinary Wound Cleansing Solution

Ilium Oticlean (Troy Laboratories, Glendenning NSW) was selected as the veterinary wound cleansing solution due to its current use with wildlife and reported success (pers. obs.). This is a veterinary product for horses, dogs and cats containing Propylene glycol 40ml, malic acid 2.25g, benzoic acid 0.15g and salicylic acid 0.037g. per 100ml ("Ilium oticlean," 2002). It debrides necrotic tissue, and inhibits bacterial growth while promoting regeneration (A. Gallard, e-mail, April 8, 2009). It is recommended in the treatment of wounds and abrasions particularly in the presence of necrotic tissue or debris (A. Gallard, e-mail, April 8, 2009). Care is also required in the identification of this product. The actual product used is the one pictured in Figure 12.

Figure 12 Ilium Oticlean.

3.4.3.6 Aloe vera

Aloe vera plants were chosen as supplying a fresh and easily obtainable source of *Aloe vera* gel.

3.4.3.7 Honey

Unprocessed natural honey was required from the same source for the entire project. The honey obtained for use came from bees feeding on three different groups of eucalyptus trees: Iron Bark, Stringy Bark and Grey Gum (pers. comm.). The honey was cold filtered but was otherwise unprocessed.

3.4.3.8 Arnica

Topical arnica cream (*Arnica montana* 100mg/g) (Brauer, Tanunda, South Australia), see Figure 13, was selected as an easily obtainable and reliable source of arnica.

Figure 13 Arnica cream.

3.4.4 Summary

The literature review, current veterinary practice and personal experience were used to develop a list of possible treatments for injuries. Certain environmental constraints such as the availability of techniques and facilities for treating wildlife and the handling requirements of treating wild flying-foxes selected the following for trial:.

- a simple wet dressing: a hydrocolloid / hydrogel application (SolositeÊ and Tegaderm$^{\hat{E}}$),

- a spray dressing barrier film (Leuko® spray),
- veterinary topical applications for injuries: keratinolytic dermal wash (Ilium Oticlean®),
- alternative or complementary treatments: honey, *Aloe vera* plant, arnica cream and macadamia oil,
- infection response: silver dressings were the response of choice if wounds became infected,
- no treatment controls.

Treatments included in this research were for injury healing only. A healed injury does not mean a releasable flying-fox. Injury healing might result in scarring, reducing function, or wing loss. Such flying-foxes would then require physiotherapy and flight exercise; see Appendix B Healing for Release.

3.5 Treatment Protocols

3.5.1 General

Cleaning a wound to remove foreign bodies and sources of infection is a normal first step in treatment (Rodeheaver, 2001). Flying-foxes usually had attended to this step themselves and no other cleaning or debridement occurred except for obviously ódirtyô injuries which were simply washed with water.

3.5.2 Macadamia Oil

Macadamia oil was applied, at room temperature, as a light coating to the wound from a special squeeze-bottle, see Figure 14, which allowed a controlled application to the injury area. For a few flying-foxes who did not need close inspection or who became stressed when handled, a pump spray bottle was used while the flying-fox remained hanging in the pteropery. The oil was applied sufficiently often to keep the injured area from drying out. This was usually once a day but in two flying-foxes with severe wounds it was necessary to apply the oil more often.

Figure 14 Drip bottle with macadamia oil.

Flying-foxes often licked the oil and several seemed to like the taste but did not

remove all of the oil. Licking was usually by another flying-fox and applying macadamia oil to an uninjured area could distract them.

3.5.3 Hydrocolloid Dressing

TegadermÊ and SolositeÊ were used in combination. Dressings were changed every two to five days, depending on the state of the dressing and the injury, and lost dressings were replaced immediately. After the previous dressing was removed, any heavy exudate around the injury was patted dry. Normally the dressing lifted easily. If not, gently stretching the dressing at right angles to the direction of pull usually lessened contact and allowed removal.

A line of SolositeÊ was applied over the injury then covered by a dressing big enough to cover the injury with a reasonable overlap on dry healthy membrane. óReasonableô was a balance between opposing needs:

- adequate overlap for adhesion,
- a preference to avoid finger bones (covering óbumpsô such as fingers is difficult without tenting which traps air and makes the dressing more liable to lift),
- the need to avoid joints (which need to flex),
- the flying-foxôs temperament (what size will he or she leave alone, usually smaller was better), and,
- the need to stabilise the area with the dressing (i.e. to use the dressingôs mechanical properties to hold the wing together).

Very occasionally a length of Leukoplast® (BSN Medical, Hamburg) was added to the dressing on a wrist wound and then wrapped around the thumb. This was due to insufficient healthy membrane on the wrist to attach the dressing.

3.5.4 Silver Dressings

Infected wounds were treated with the most appropriate size of silver dressing for the wound. The TegadermÊ dressing could continue to be applied to the remainder of the wound area, such that the TegadermÊ may have overlapped the silver dressing.

3.5.5 Spray Bandage

Initially incoming injuries were allocated to the spray bandage trial regardless of site. It became apparent that use on large areas of wing was impractical. The entire surface became very sticky which trapped dust and pulled on the membrane. Use on large areas, therefore, was discontinued.

As per the product directions, the bandage was applied by spraying over the entire area from a short distance. Although the spray bandage appeared (where it was visible in the peri wound area) to remain in place for several days, it was re-sprayed (but not removed) each day to ensure the injury remained covered.

3.5.6 Veterinary Wound Cleansing Solution

Ilium Oticlean was applied daily, from the spray bottle, as per the directions, i.e. sprayed from a short distance to coat the wound. The application was allowed a minute or two to dry before the flying-fox was returned to the enclosure.

3.5.7 Aloe vera

Freshly picked *Aloe vera* leaves were cut with a sharp knife, the spines trimmed and the flat part of the leaf removed, see Figure 15. The mucilaginous gel was scraped from the centre with a spatula and applied directly to the wound. No covering was used, as the flying-foxes did not lick off the *Aloe vera*.

Figure 15 Cut *Aloe vera* leaf.

3.5.8 Honey

A thick layer of honey was applied to the injury surface using a clean spatula, then covered by a TegadermÊ dressing. The dressing kept the honey in place and prevented the flying-foxes eating it.

3.5.9 Arnica

The arnica cream was used daily and applied directly over the bruise area. No covering was used, as the flying-foxes did not lick off the arnica.

3.5.10 ORC / Promogran™

Figure 16 Promogran dressing.

For certain injuries it was considered that a dressing which could supply a physical matrix as well considerable enhancement of granulation was required. Oxidised regenerative cellulose dressings were considered a possibility, see section 5 Discussion - Future Directions. Promogran (Johnson and Johnson, New Brunswick, New Jersey USA) see Figure 16 for identification, is an extremely expensive dressing unavailable in Australia. A box was obtained from Germany to trial fast granulation over exposed finger bones. Because of its expense and limited availability, this treatment was not included in the trials using groups of flying-foxes. Instead, the dressing was applied under TegadermÊ to one flying-fox to explore its potential for future assessment.

3.6 Infection Management

During the early analysis of the results it became apparent that hypothesis one was going to be rejected and indeed there was a strong division between treatments which succeeded and treatments which failed. Honey is reportedly antimicrobial (Bergman, et al., 1983; Subrahmanyam, 1991; Molan, 1992b; Willix, et al., 1992; McCarthy, 1995; Zaghloul, et al., 2001). Macadamia oil has been reported to suppress the growth of gram positive bacteria (Wille & Kydonieus, 2003) and the carboxymethylcellulose of SolositeÊ was found to trap bacteria away from the injury (Tachi, et al., 2004). It was considered that antimicrobial activity might be contributing to the effectiveness of the successful treatments. Consequently, an early preliminary investigation was carried out on the effects of these treatments on bacterial growth.

Macadamia oil, SolositeÊ and honey were tested against pure cultures of *Staphylococcus aureus, Klebsiella aerogenes, Escherichia coli* and an environmental swab from a flying-fox wing on standard blood agar, 37^{o}C / 24 hour incubation.

Each set of plates (one of each pure culture and the environmental swab) received separate treatments which a) covered the entire surface of the plate or b) were placed in a well cut in the agar:

1. SolositeÊ gel was squeezed onto the plate and spread with a swab.

2. Macadamia oil - dripped over the surface to a desired depth. It is important not to tilt as this smears the bacterial growth over the plate.

3. Macadamia oil - added to a well.
4. Honey - added to a well.
5. Untreated control.

Treatment effects on growth may be actively bactericidal or bacteriostatic or passive, i.e. due to the exclusion of air. As nothing was known about macadamia oilôs effect on bacteria, the cover and well methods were both used. Four or five drops of oil from a sterile 1 mm syringe filled a normal hole. Honey did not drip and tended to coat the outside of the syringe and run into the wells from the outside as well as the inside of the sterile syringe. A total of 1.5 - 2 ml of each substance was tested.

Growth patterns on the plates were assessed visually for density and growth characteristics and recorded as light, moderate or heavy in line with standard microbiological practice and the following noted on the treated plates:

- reduced areas of growth, such as clear rings,
- changes in population type and differences in the proportion of gram positive and gram negative cocci and bacilli between treated plates and untreated control plates.

Digital images were taken of all plates.

All cultures were gram stained. Digital images were taken of selected slides with light microscopy at x 1000 magnification.

Later analysis of the results revealed that infections did not occur in either success or failure treatments so an in-depth study of infection control was not considered of value.

3.7 Handling and Housing

Flying-foxes were only held by trained handlers who had been vaccinated against rabies (and hence Australian Bat Lyssavirus). Gloves were not used by most handlers as they reduce dexterity and therefore ability to handle the flying-fox. Usually one person held the flying-fox and a second person applied the treatment. This minimised handling time which reduced stress to the flying-fox.

Each incoming flying-fox was inspected and all injuries recorded on a diagram, photographed and described. Details of age, gender, general health and the circumstances of the rescue were also recorded. Each flying-fox was identified, usually by a coloured thumb band. Flying-foxes were housed in a large open air aviary (a ñpteroperyò), a semi-enclosed closely monitored room (the ñhospital wardò) or a hospital cage (in the ñhospital wardò) depending on health. All flying-foxes had at least one companion at all times to reduce stress in this highly social species. On arrival this companion was one of the two permanent care flying-foxes accustomed to the environment and the handlers. Once accustomed to the process the companion may have been another wild flying-fox if separation from or within the pteropery was required. Flying-foxes confined to hospital conditions were moved to the pteropery as soon as they were well enough.

Flying-foxes were fed 350-400 g of fresh fruit per day with a nutrient supplement. This food was offered in the evening and available throughout the night. This is a typical rehabilitation diet used for decades (Jackson, 2003). While many laboratory investigations show significant effects on healing by adding various nutritional components these were not supported by clinical findings (Arnold & Barbul, 2006). The effects of dietary variation were not evaluated in this study. If the food was completely consumed on two consecutive nights then the amount was increased (and decreased if significant amounts remained). Fruit was cut, pureed and/or hung whole on wires depending on the health of the flying-foxes ï particularly with reference to mouth injuries (often damaged in attempts to escape wire or netting entanglement).

Each flying-fox was inspected daily (hospital cases more often) and treated as required by the specific protocol, see section 3.5 Treatment. Handling protocols were designed to ensure control of the flying-fox with minimal stress. The flying-fox was wrapped in a towel (600 mm x 600 mm) lifted from the pteropery / cage ceiling and hung on the handler. This mimicked wrapping by a motherôs wings, which helped minimise stress and handling. Each wing was inspected, photographed, treated and any other conditions monitored. The flying-foxôs head was kept uncovered, see Figure 17, because observations over many years showed, in contrast to other wild animals, flying-foxes tend to be calmer if they can see when being handled. Any flying-fox determined to bite could have the head covered with the holding cloth and be restrained.

Figure 17 Flying-fox wrapped for treatment.

If further control was required then diazepam could be given (Valium from Roche Products Pty Ltd, Spain1.67 ï 3.3 mg / kg) 10 minutes before treatment. Diazepam was chosen on the advice[14] that it is a calmant and amnesic and because it is easily administered in the field. Using an inhalant anaesthetic was not considered suitable as it did not provide any extra benefit to the handler and would require extra stress and risk to the flying-fox as it requires removal from the cage or pteropery, transporting to a veterinary clinic, and the general stress and risk of applying an anaesthetic.

If required an ñElizabethan collarò was available to prevent a flying-fox from disturbing the treatment. This was constructed from a stiff plastic sheet attached with a hook and loop fastener (e.g. Velcro® from Velcro Australia Pty Ltd, Hallam Victoria, Australia), see Figure 18. This collar was also used during the treatment process as it enabled the flying-fox to see without being able to bite even when only one handler was available.

The largest possible housing was chosen to allow as much movement, and preferably flight, as possible in order to minimise loss of muscle tone.

Figure 18 Elizabethan collar.

[14] Wildlife Assistance & Information Foundationôs Wildlife Veterinary Clinic

3.8 Statistical Analysis

3.8.1 Introduction

Two specific injury parameters were recorded and analysed:

- the state of the injury (see Table 3 Injury States) and
- the size of the injury.

From the data recorded for the states of every injury the following could be calculated:

- duration of the inflammation phase,
- duration of the granulation phase,
- time for non-healing,
- counts of injuries under each treatment which completed the defined inflammation phase (i.e. reached the healing point ôResolution of Inflammationõ),
- counts of injuries under each treatment which completed the defined granulation phase (i.e. reached the healing point ôResolution of Granulationõ),
- counts of treatments that failed in either phase.

Two outcomes were important in assessing treatment success:

- the success or failure of healing and
- the healing time.

From the data recorded for size, the rates of closure during tissue regeneration could be calculated.

Statistical modelling and transformations of the data followed the advice of a biostatistician (pers. comm.). A significance level of alpha 0.1 was selected. All analyses were conducted in ñRò version 2.11 (R Development Core Team, 2010).

3.8.2 State Analysis

The factors analysed for successful healing (i.e. healing pass / fail) were:

- gender,
- open or closed injury,
- injury site (arm, finger bone, joint and patagium and leading edge),
- severity, and,
- treatment type.

Results were analysed for the inflammation phase (377 injury / treatment combinations in 67 flying-foxes), the granulation phase (313 injury / treatment combinations in 67 flying-foxes) and for the phases combined (total healing). A Fischerôs exact test (Bickel & Doksum, 1977) was used to maximise the robustness.

3.8.3 Time Analysis

3.8.3.1 Inflammation Phase Time Analysis

When more than one treatment was applied to an injury during the inflammation phase the healing time could not be attributed to a specific treatment and so these injuries were not included in the time analysis. Data was analysed on 219 injury / treatment combinations in 59 flying-foxes.

A mixed model was used with error terms for the injury and for each flying-fox. The factors included in the model were: site of injury, injury category (open or closed), severity of injury and treatment method. The model tested was:

$h(y_{if}) = d + t_{if} + c_{if} + s_{if} + v_{if} + m_{if} + d_f + e_{if}$

where

- y_{if} is the time taken to reach resolution of inflammation after treatment has commenced on injury (i) on flying-fox (f),
- t_{if} is the time that treatment commenced,
- c_{if} is the category of injury (i) on flying-fox (f) (open or closed),
- s_{if} is the site of injury (i) on flying-fox (f,)

- v_{if} is the severity of injury (i) on flying-fox (f),
- m_{if} is the treatment applied to injury (i) on flying-fox (f),
- d_f is the error term for flying-fox (f),
- e_{if} is the error term for injury (i) on flying-fox (f),
- e and d are assumed to be normally distributed, (as n > 100),
- $h(y) = y^b$ is the transformed value of y.

Box-Cox power transformations (Stuart, Ord & Arnold, 2004) were used, prior to analysis, to standardise the variance and ensure it was independent of the mean. 0.3 was selected for b. The mixed model above was used and an analysis of variance carried out.

3.8.3.2 Granulation Phase Time Analysis

When more than one treatment was applied to an injury during the granulation phase the healing time could not be attributed to a specific treatment and so these injuries were not included in the time analysis.

Data was analysed on 265 injury/treatment combinations for 59 flying-foxes. The healing times for the granulation phase were logarithmically transformed to normalise the data before the model was fitted. The model used was a mixed model with error terms for the injury and for the individual flying-fox. The model included site of injury, injury category (open or closed), severity of injury and treatment method. Age and gender were not statistically significant at the 5% level in the mixed model:

$\log(h_{ifsvmc}) = a_s + l_v + k_m + g_c + d_f + e_{if}$

where:

- h_{ifsvmc} = healing time of injury (i)on flying-fox (f) at site s with severity v, treatment m and injury category (c),
- a_s = effect of site (s),
- l_v = effect of severity (v),
- k_m = effect of treatment (m),
- g_c = effect of injury category ©,

- d_f = error term due to flying-fox (f),
- e_{if} = error term due to injury (i)on flying-fox (f),

The mixed model above was fitted and an analysis of variance carried out. Predictions were calculated for combinations of factors.

3.8.4 Size Analysis

For analysis, at least three data points were required in the straight-line section of the plotted curve.

A log of the wound area against day was graphed and an exponential decay model fitted:

$A = s\, e^{-r\, d}$

- A is the wound area,
- s is the initial wound area,
- r is the instantaneous decay rate as a proportion of the area,
- d is the time in days since the injury.

P-values for the ANOVA and for Kruskal-Wallis test (Lehmann & DôAbrera, 2006) were calculated for treatment and for severity.

3.8.5 Family Error Rate

It was considered that as several comparisons were being made at once on the data it was possible that a significant result could occur by chance - family error rate. There are several ways of making adjustments for this in multiple comparisons if needed.

4 Results

4.1 Introduction

Flying-foxes came into the study over a period of six years. Flying-foxes were admitted to the study whether or not they seemed injured when rescued and were obtained as soon as possible after the injury event, usually within 24 hours.

Study animals were primarily sourced from the Sydney Basin, Illawarra and Central Coast regions and were grey-headed flying-foxes. The remainder were rescued from other areas of New South Wales.

A total of 92 flying-foxes were registered for the research[15] and 67 flying-foxes were included in the study with 300 injuries. Overall, 91% of the flying-foxes in the study successfully reached full flight capability.

Rarely (on three occasions) a wound became infected and was successfully treated with a silver dressing.

It should be noted that due to the significance level of the statistical results obtained, the family error rate was not considered a factor for the primary conclusions. Caution, however, should be applied to any marginal results, particularly any small data sets.

4.2 Injury Assessment

4.2.1 Wing Observations

The average time between injury event and injury appearance was three days. Closed injuries accounted for one third of the total injury count with most of these being bruises. Most bruises (55%) appeared by day two. Approximately 15% did not appear for more than five days and the

[15] Many more animals were transferred from rescue groups to the scientific license issued for this research. At the start of the research these were registered in the study on notification of the rescue. When it became apparent that not all flying-foxes being submitted would be suitable then a registration number was not issued until arrival. Those not issued a research number were tracked separately under the scientific licence - all were treated and released.

latest bruise appeared after 14 days. While this bruise may have occurred due to an injury event within the pteropery, its position and presentation on the wing are consistent with the injury event (net entanglement). Most bruises (54%) degenerated to open wounds before healing.

The healed state most usually recorded was swr (scar). These scars were visible as either a white area on the open membrane or a thickened rolled edge of a hole. In the latter situation the ñscar[16]ò was black in appearance. If a flying-fox was in care for a sufficient time for remodelling then full colour (fco) was reached and recorded after swr. Occasionally, with small wounds or closed injuries fep / fco (full re-epithelialisation without colour / full colour) may be reached without apparent scar.

4.2.2 Membrane Observations

Histological sections from three different membrane conditions were reviewed from one flying-fox:

- normal wing dactylopatagium (state = ffa)
- breaking down patagium (state - eld Ÿ dld), and,
- healing patagium (from the edge of a full thickness hole through the membrane, state = swr).

All three examples showed varying thicknesses of dermis between two epithelial layers with normal structures expected in mammalian skin - see below for details.

Normal Membrane

The dorsal epidermis was thicker than the ventral epidermis and contained more melanin granules. The strata corneum layers had separated and lifted away to a varying extent[17]. The dorsal epithelium thickness typically 16.5 µm, not including

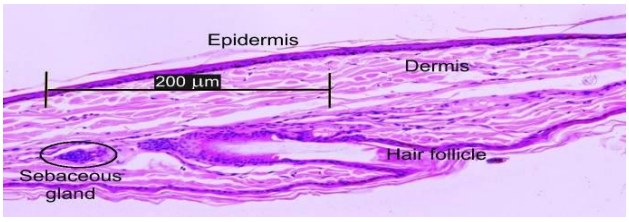


Figure 19 Cross-section of normal wing membrane. Histology of normal wing membrane showing the dermis between two layers of epidermis and a hair follicle. Haematoxylin and eosin stain, x 400.

[16] While this thickened edge may not technically be a scar the resulting contraction due to the thickening and the need to infill the hole meant that remodeling was still required.

[17] This was probably due to the paraffin wax embedding (Crowley & Hall, 1994).

the separated layers, compared with typically 7 µm on the ventral surface. The separated layers of keratinised corneum were very thin making them difficult to measure but ranged about 8 µm to 14 µm. The epidermal layer contained very few hairs and those observed were on the ventral surface. The wing membrane was thicker around the hair follicles by up to 60%, see Figure 19. The few hairs seen had a diameter of up to 20 µm[18]. Sebaceous glands were associated with the hair shafts.

The epidermis consisted of four layers, see Figure 20:

- stratum basale of low columnar cells,
- stratum spinosum of flattened, spiny shaped cells occurring infrequently,
- stratum granulosum, a layer clearly distinguishable although individual cells were not clear,
- stratum corneum of at least three to six and occasionally more layers.

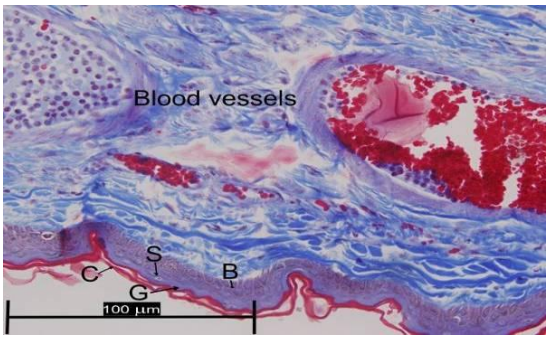


Figure 20 Normal wing membrane - high magnification image. Section is showing ventral epidermal layers: stratum corneum (C), stratum spinosum (S), stratum granulosum (G), stratum basale (B) and dermis. Trichrome stain, x 1000.

The normal dermis averaged 170 µm thick and contained blood vessels, fibroblasts, macrophages, nerves and collagen. The main structures were grouped towards the centre of the dermis but distinctive papillary and reticular layers were not apparent.

Breaking down Membrane

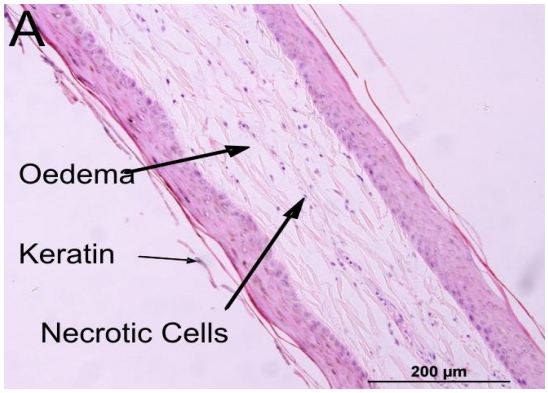
Where damage to the wing resulted in full thickness necrosis of the membrane this mostly appeared to involve all layers simultaneously. Occasionally damage to the wing resulted in more gradual necrosis of the layers over a wide area. Macroscopically the tissue in this area appeared white and nearly transparent, see Figure 21. Microscopically all

Figure 21 Wing patagium with membrane breaking down.

[18] An insufficient amount of any hair remained to measure length

dermal layers were basically present but evidence of tissue breakdown was clearly visible, such as:

- breakdown or separation across cell layers,
- separation between cell layers,
- increasing loss of cell layers, particularly close to the centre of the damaged area,
- presence of necrotic cells,
- increasing oedema in the dermal layers (and corresponding thickening of the membrane, e.g. to approximately 300 µm) and
- the presence of many extravascular red blood cells throughout the dermis.



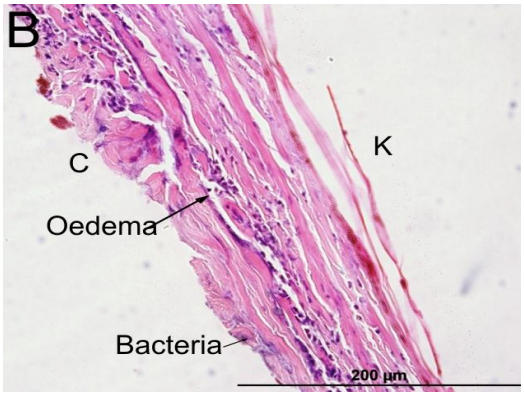


Figure 22 Wing membrane breakdown histology. (A) with oedema, necrotic cells and sloughing keratin layers. Haematoxylin and eosin stain. (B) increased breakdown with cellular layers breaking down (C), Keratin lifting away (K) and bacteria. Haematoxylin and eosin stain.

Many of these features are visible in Figure 22.

Healing Membrane

Macroscopically the tissue appeared as an enlarged, rolled edge around the hole, see Figure 23. Microscopically all normal layers were present, see Figure 24. At the healing edge (first 2 mm) the tissue had:

- greatly thickened, up to 460 µm just behind the tip (375 µm in from the edge) and 265 µm at the tip itself; by 2 mm from the tip this was reduced to 220 µm,

Figure 23 Thickened edge of a patagium hole injury. This image shows a typical patagium wound which has healed around the edge leaving a hole to be in-filled. There is a thumb claw showing beneath the hole on the left side.

- increased epithelium, an average of 82 µm in the first millimetre from the tip,
- no difference in thickness between the two epithelial layers,
- increased number of small blood vessels and dense collagen in the dermis from the tip for 1 mm, beyond that fewer blood vessels and more space (presumably fluid) between the collagen fibres,
- macrophages and fibrocytes throughout the dermis.

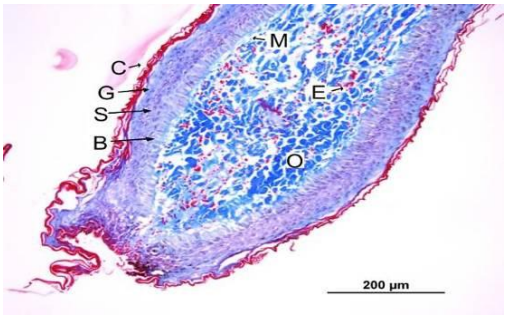


Figure 24 Thickened edge of healing hole histology. Increased epithelium on both surfaces meeting across the dermis at the tip, basement membrane (M), stratum basale (B), stratum spinosum (S), stratum granulosum (G), stratum corneum (C), erythrocytes indicating small vessels (E), note also dense collagen (O). Trichrome stain.

Most of the increase in the epithelium appeared to be from an increased stratum spinosum layer. The stratum corneum was clearly discernable. In this tissue section the basement membrane was visible at the enlarged healing tip but was too thin to obtain an accurate measurement.

4.3 Success or Failure of Healing

Not all treatment events resulted in successful healing. This was indicated by an injury deteriorating to the point where the injury was reclassified to a different state, see Table 3 Injury States. The three main state events that induced a change in treatment (i.e. the previous treatment was considered to be failing), are:

- dermal layer damage (dld) failing to resolve after 11 days (average) and / or the area extending,
- injury progressing to sub-dermal damage (sdd) after 10 days (average) and / or sdd area extending,
- granulation (gra) failing to progress for 11 days (average).

Under these conditions a failure was recorded and the treatment changed. Not all the factors analysed affected the success of healing, see Table 4. Treatment type was highly significant ($p = 0.005$) in healing success, injury site (e.g. injury over the arm, patagium or joint) was also significant ($p = 0.09$) but gender, injury category (open or closed) and injury severity were not.

Table 4 Factors affecting Inflammation and Complete Healing

The effects of gender, injury category (open or closed) and injury severity were not significantly different. Treatment was highly significant. alpha = 0.1

Factor	p-values for Resolution of Inflammation	p-values for Completing Healing (inflammatory and granulation phases combined)
Gender	0.34	0.44
Category	0.65	1
Severity	0.91	0.55
Site	0.09	0.09
Treatment	0.01	0.01

4.3.1 Success or Failure of Healing due to Site

Site affected the probability of healing (p = 0.09). Leading edge injuries failed the most, across all treatment types, with failures occurring during the inflammation phase, see Table 5 and Table 6. There was no significant difference in healing success between arm, finger bone and joint during the inflammation phase. The probability of failing during the granulation phase, however, was higher for injuries over the finger bone and joints than other areas, see Table 7.

Table 5 Effect of Site on Probability of Healing

Significance levels of contrasts in the effect of site on the probability of healing to the Resolution of Inflammation (inflammation phase)

Site	Arm	Finger bone	Joint	Patagium	Wing leading edge
Arm		0.52	0.11	0.01	0.01
Finger bone	0.52		0.31	0.02	0.01
Joint	0.11	0.31		0.21	0.01
Patagium	0.01	0.02	0.21		0.01
Wing leading edge	0.01	0.01	0.01	0.01	

Table 6 Effect of Injury Site on the Probability of Failure during the Inflammation Phase
with 95% confidence intervals

Site	Number treated	Proportion failed	Confidence Interval
Arm	53	0.02	0.00 - 0.10
Finger bone	61	0.02	0.00 - 0.09
Joint	30	0.03	0.00 - 0.17
Patagium	72	0.04	0.01 - 0.12
Wing leading edge	21	0.14	0.03 - 0.36

Table 7 Effect of Injury Site on the Probability of Failure during the Granulation Phase
with 95% confidence intervals

Site	Number treated	Proportion failed	Confidence Interval
Arm	50	0.00	0.00 - 0.07
Finger bone	60	0.05	0.01 - 0.14
Joint	29	0.07	0.01 - 0.23
Patagium	69	0.03	0.00 - 0.10
Wing leading edge	18	0.00	0.00 - 0.19

Complete Failure

Failure to achieve healing before substantial loss of bones - multiple phalanges on multiple digits - such that flight could never be achieved and euthanasia was required occurred in six cases. Where there was some vital membrane remaining along the bone and therefore supporting a vascular system it was usually possible to maintain the bone by keeping a moist dressing in place. When large areas of membrane around the bone were damaged it became extremely difficult to maintain vital bone. It was observed that the epithelium died, followed by the subdermal layer and this effect ñrippled backò along the wing (dieback). It would appear, from knowledge of membrane anatomy and the healing processes, that the corneum layer of the epidermis died, the basal / cellular layers below were then exposed and so dehydrated and died. This then exposed the dermis, which disintegrated removing the vascular system. This in turn killed the opposing epidermis and exposed more of the edge of the wing and the cycle repeated. The only blood flow

to exposed finger bones that have lost the surrounding vessels is through the bone medulla. Complete loss of membrane also left the bones without any stabilising physical support. The combination of a compromised blood supply and frequent separation at joints due to movement resulted in necrosis of the bones of affected digit(s).

4.3.2 Success or Failure of Healing due to Treatment

Some treatments resulted in healing failure, particularly during the inflammation phase. For a definition of failure see section 3.3.2 Change of Treatment. Treatments fell into two groups for effectiveness during the inflammation phase. Macadamia oil, hydrocolloid (SolositeÊ) / dressing and honey / dressing had significantly higher chances of success than no treatment, spray bandage, *Aloe vera*, arnica or Ilium Oticlean ($p < 0.1$), see Table 8 and Figure 25.

Table 8 Significant Difference between Treatments

Significant difference between treatments for success of completion of inflammation phase (P values)

	No treatment	**Spray bandage**	***Aloe vera***	**Arnica**	**Ilium Oticlean**	**Macadamia oil**	**Hydro-colloidal**	**Honey**
No treatment		---	---	---	---	---	---	---
Spray bandage	1.00		---	---	---	---	---	---
Aloe vera	1.00	1.00		---	---	---	---	---
Arnica	0.36	0.21	0.22		---	---	---	---
Ilium Oticlean	0.36	0.14	0.14	1.00		---	---	---
Macadamia oil	< 0.01	< 0.01	< 0.01	0.10	0.11		---	---
Hydro-colloidal	< 0.01	< 0.01	< 0.01	0.05	0.06	0.51		---
Honey	< 0.01	< 0.01	< 0.01	0.01	0.01	0.12	0.50	

The grouping of treatments for the effectiveness in enabling complete healing (inflammation and granulation phases combined) was similar to the inflammation phase, see Table 9, although the hydrocolloid dressing was less effective in the granulation phase than in the inflammation phase.

Table 9 Significant Difference between Treatments for Success of Completion of Full Healing
i.e. inflammation and granulation phases combined (P values). The effectiveness of treatments for enabling successful completion of healing fell into two groups: macadamia oil, hydrocolloid dressing and honey dressing had a significantly higher chance of success than no treatment, spray bandage, *Aloe vera*, arnica or Ilium Oticlean

	No treatment	**Spray bandage**	***Aloe vera***	**Arnica**	**Ilium Oticlean**	**Macadamia oil**	**Hydro-colloidal**	**Honey**
No treatment		---	---	---	---	---	---	---
Spray bandage	1.00		---	---	---	---	---	---
Aloe vera	1.00	1.00		---	---	---	---	---
Arnica	0.36	0.53	0.37		---	---	---	---
Ilium Oticlean	0.68	0.56	0.56	1.00		---	---	---
Macadamia oil	0.01	<0.01	<0.01	0.03	0.01		---	---
Hydro-colloidal	0.03	<0.01	<0.01	0.21	0.11	0.41		---
Honey	<0.01	<0.01	<0.01	0.01	<0.01	0.29	0.07	

The failure ranges and averages, as a percent of the total injuries in a treatment group, are shown in Figure 25. Treatments fell into three groups for the likelihood of full healing; two groups had unacceptable failure levels. No treatment, spray bandage, and *Aloe vera* were the first group of failures (> 43% failure), arnica and Ilium Oticlean were the second group (approximately 30% failure) and macadamia oil, hydrocolloid (SolositeÊ) under a dressing, and honey under a dressing were the most successful group (< 10% failure). If an injury reached granulation then it

had a high probability of healing regardless of treatment, but the time required for granulation phase healing varied with the treatment.

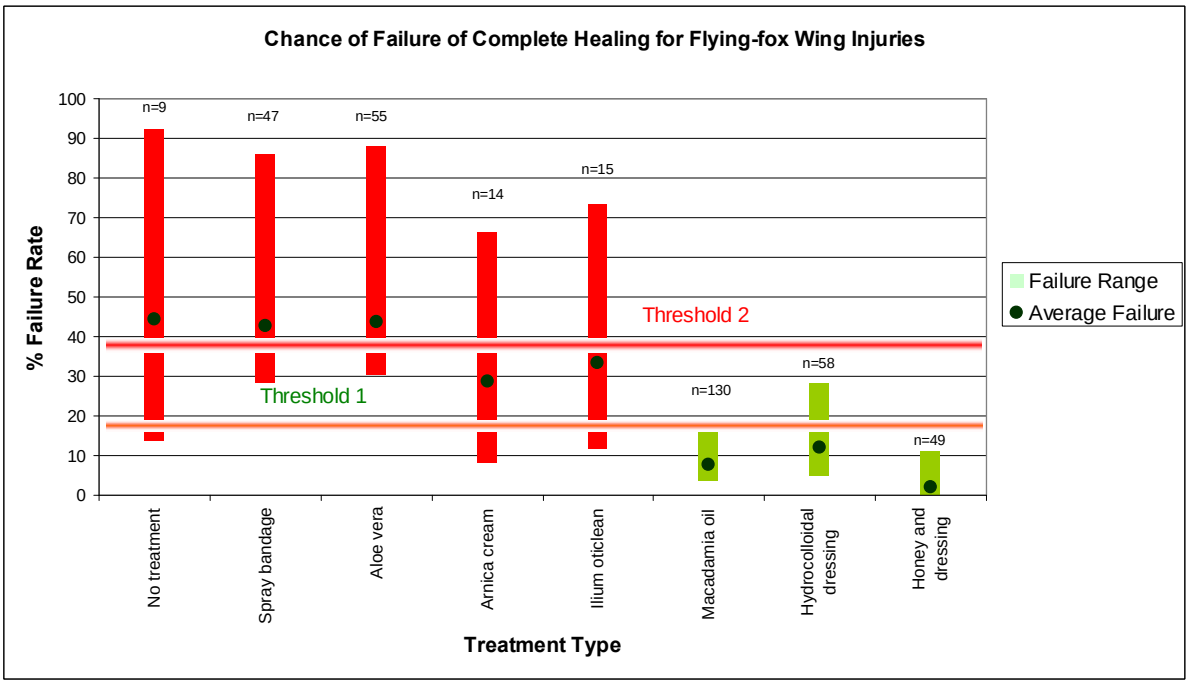


Figure 25 Chance of treatment failure.

4.3.2.1 Bacterial Growth

The investigation of bacterial growth was inconclusive. The untreated environmental control swabs grew mixed moderate to heavy cultures with some haemolytic colonies, see Figure 26. The mixed cultures were mixed gram positive cocci with some gram negative bacilli.

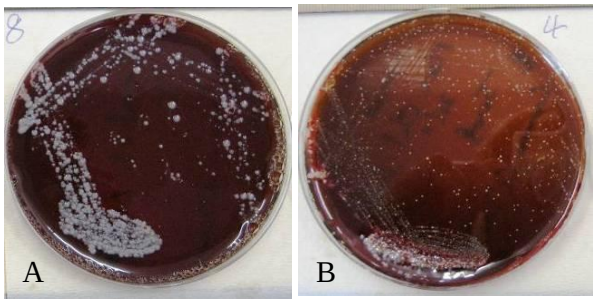


Figure 26 Culture from a swab from a healthy flying-fox wing. A- untreated plate, B ï plate flooded with macadamia oil.

SolositeÊ did not have an observable impact on bacterial growth either negative or positive.

Growths of the pure cultures and the environmental swab were markedly reduced when flooded with macadamia oil and the haemolytic rings were smaller. The gram stain confirmed the presence of mixed organisms. Growth was not reduced when the oil was placed in a well. It should be noted that most of the oil did not disperse into the surrounding agar.

All plates showed markedly less growth in a ring around the well containing honey, see Figure 27. Most of the honey had disappeared from the well.

Figure 27 Honey treated plate. Reduced growth around the wells is clearly visible.

4.4 Healing Time

Healing time during the inflammation phase was not affected by any factor (category, site, severity or treatment) but the granulation phase healing time was significantly affected by site and treatment.
Gender was not significant in either phase.

4.4.1 Inflammation Phase

No significant effects were found for injury category, site, severity and treatment on time for injuries with a single successful treatment during the inflammation phase. Treatments that failed to heal were not included in this analysis.

Similar results were found in analyses based on the time from injury rather than the start of treatment. The longer the delay in starting treatment the longer the time to reach granulation regardless of site, category, severity or treatment.

The model predicts time for the inflammation phase (time from injury) as 10.6 ± 1.0 days.

4.4.2 Granulation Phase

Injury category, site, severity and treatment all significantly affected healing time. Granulation times varied from just over 2 days to over 60 days see Figure 28.

Effect of factors

Category: open injuries had a significantly longer healing time than closed injuries ($p < 0.005$).

Site: site split into two significantly different groups. Injuries on the arm, patagium and elbow joint healed faster than injuries involving the membrane of finger bone, finger joint, wrist joint, or wing leading edge, ($p < 0.05$)., see Table 10.

Severity: granulation phase time increased with severity level ($p < 0.01$) regardless of treatment or site.

Treatment Group: analysis of the three successful treatments (those with an average failure to reach granulation of less than 10%) demonstrated that macadamia oil treatment results in significantly faster healing times than either honey and dressing or hydrocolloid (SolositeÊ) and dressing ($p < 0.05$), see Table 11.

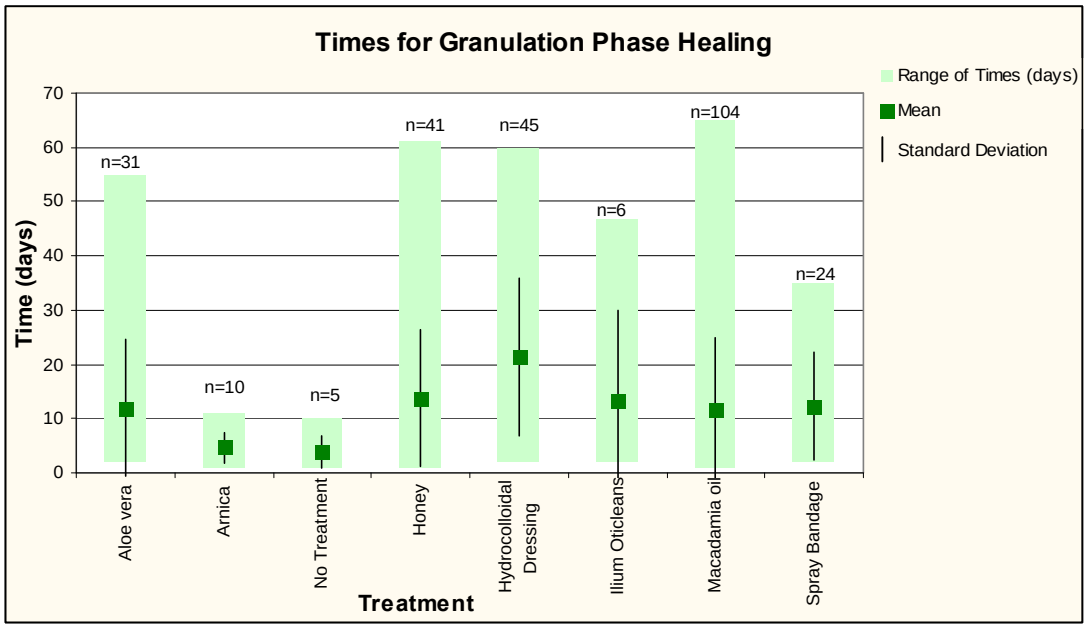


Figure 28 Granulation phase healing times.

Table 10 Comparisons of Granulation Phase Healing Times based on Site $p < 0.05$ indicates a significant difference between the two sites. Injury sites: arm, elbow and patagium are significantly faster than the other sites

Site	Finger bone	Finger joint	Elbow joint	Wrist joint	Patagium	Wing leading edge
Arm ratio	2.12	1.86	0.94	1.74	0.97	1.62
95% confidence interval	1.55-2.89	1.21-2.84	0.51-1.72	1.04-2.93	0.73-1.29	1.03-2.54
p value	0.00	0.00	0.83	0.04	0.83	0.04
Finger bone ratio		0.88	0.44	0.82	0.46	0.76
95% confidence interval		0.57-1.34	0.24-0.82	0.49-1.37	0.33-0.62	0.48-1.21
p value		0.54	0.01	0.45	0.00	0.25
Finger joint ratio			0.50	0.94	0.52	0.87
95% confidence interval			0.26-0.97	0.54-1.63	0.34-0.80	0.51-1.50
p value			0.04	0.82	0.00	0.62
Elbow joint ratio				1.86	1.04	1.73
95% confidence interval				0.90-3.8	0.57-1.90	0.87-3.44
p value				0.09	0.91	0.12
Wrist joint ratio					0.56	0.93
95% confidence interval					0.33-0.93	0.50-1.72
p value					0.03	0.81
Patagium ratio						1.67
95% confidence interval						1.05-2.66
p value						0.03

Table 11 Comparisons of Granulation Healing Times based on Treatment. Wounds treated with macadamia oil heal faster than the other two successful treatments: honey and SolositeÊ with dressings. N.B. the p values for the remaining treatments are based on small sample sizes

Treatment	Arnica	No treatment	Honey & dressing	Hydro-colloidal dressing	Ilium Oticlean	Macadamia oil	Spray bandage
Aloe vera ratio	1.37	1.08	1.66	1.72	0.78	1.02	1.33
95% confidence interval	0.70-2.68	0.47-2.48	0.97-2.85	1.04-2.85	0.34-1.81	0.66-1.56	0.77-2.31
p value	0.35	0.85	0.07	0.04	0.56	0.93	0.30
Arnica ratio		0.79	1.21	1.25	0.57	0.74	0.97
95% confidence interval		0.33-1.86	0.62-2.35	0.67-2.35	0.23-1.43	0.41-1.33	0.51-1.83
p value		0.58	0.57	0.48	0.23	0.31	0.93
No treatment ratio			1.54	1.59	0.72	0.94	1.23
95% confidence interval			0.68-3.49	0.71-3.57	0.25-2.07	0.44-2.00	0.54-2.81
p value			0.30	0.26	0.54	0.88	0.62
Honey and dressing ratio				1.04	0.47	0.61	0.80
95% confidence interval				0.64-1.68	0.20-1.09	0.41-0.92	0.46-1.39
p value				0.88	0.08	0.02	0.43
Solosite™ dressing ratio					0.45	0.59	0.77
95% confidence interval					0.20-1.00	0.42-0.82	0.48-1.24
p value					0.05	0.00	0.29
Ilium Oticlean ratio						1.31	1.71
95% confidence interval						0.61-2.82	0.74-3.93
p value						0.49	0.21
Macadamia oil ratio							1.31
95% confidence interval							0.85-2.02
p value							0.22

A selection of predicted times, based on the model, for some typical injuries are shown in Table 12 and Table 13.

Table 12 Granulation Phase Healing Times for Open Injuries
Predicted times for different open injury severities and treatments for completing the granulation phase

Treatment	**Moderate Open Injury Patagium** (days)	**Severe Open Injury Patagium** (days)	**Moderate Open Injury Finger bone** (days)	**Severe Open Injury Finger bone** (days)
Honey and dressing	11	18	24	39
Hydrocolloid dressing	11	18	25	40
Macadamia oil	7	11	15	24

Table 13 Granulation Phase Healing Times for Closed Injuries
Predicted times for different closed injury severities and treatments for the granulation phase

Treatment	**Moderate Closed Injury Patagium** (days)	**Severe Closed Injury Patagium** (days)	**Moderate Closed Injury Finger bone** (days)	**Severe Closed Injury Finger bone** (days)
Honey and dressing	7	12	16	26
Hydrocolloid dressing	8	12	17	27
Macadamia oil	4	7	10	16

4.5 Healing Quality

Most treatments that resulted in final healing produced a fully regenerated membrane or a smooth pliable scar, see Figure 29. Some injuries in the unsuccessful treatment groups healed with a scar that was hard and rough rather than flexible, see Figure 30. This meant that not only did these treatments (*Aloe vera*, spray bandage and Ilium Oticlean) frequently fail to heal effectively, the result was often suboptimal. This qualitative outcome supports the categorisation of these treatments as ónot recommendedô.

Figure 29 Smooth pliable healing with macadamia oil.

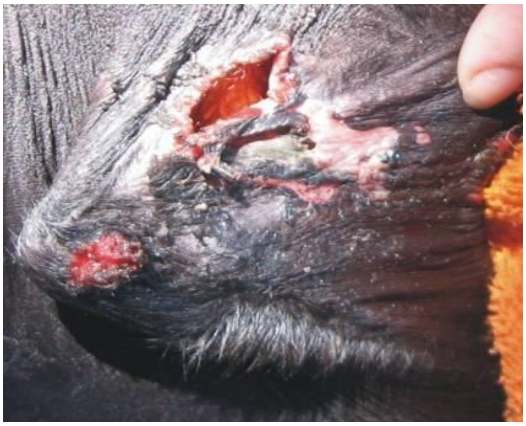


Figure 30 Poor quality healing with *Aloe vera*.

Some injuries developed over-granulation. This was either smooth granulation tissue see Figure 31 and Figure 32 or poor quality granulation tissue see Figure 33.

Figure 31 Over-granulation along a finger bone.

Figure 32 Over-granulation at a joint.

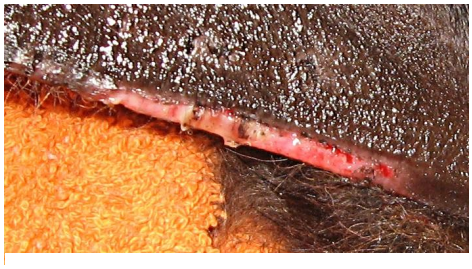


Figure 33 Over-granulation at a leading edge. Showing rough texture as the granulation develops.

4.6 Wound Size Results

In hindsight it became apparent that wounds healed quickly, and weekly measurements often provided too few data points per wound. Other issues were:

- many wounds were highly irregular in shape which made accurate measurement extremely difficult, particularly with extensive wounds. Different healing stages within many wounds further complicated the analyses,
- some extensive wounds spread and coalesced, see Figure 34 and,
- minor differences in the camera angle or in stretching the wing could add significant error to the measurements (correcting this would have damaged the wound and stressed the flying-fox).

Figure 34 Two simple injuries coalescing into a complex injury.

These issues and the protocol for recording size meant that only 29 data points from 100 injuries could be used for analysis. There were too few suitable data sets for *Aloe vera*, arnica and Ilium Oticlean so they were excluded from the size analysis. There were sufficient data sets for honey, macadamia oil, hydrocolloid dressing and spray bandage to analyse the effect of treatment on size of injury during healing.

Results were:

- severity did not significantly affect the rate of size reduction ($p > 0.05$),
- treatment did not significantly affect the rate of size reduction ($p > 0.05$),
- usually the hole size initially increased (for up to a few days) even without further damage,
- once the initial increase stabilised, hole size decreased at a generally steady average rate of 9.1% (of remaining size) per day, regardless of severity or treatment until the hole was nearly filled. This was regardless of the initial hole size
- as complete closure approached, the closure rate appeared to slow and the steady decrease model no longer applied.

Table 14 Rate of Decrease of Hole Size
Closure rate for hole size after initial increase stabilised. This rate does not apply once the hole size approaches zero[19].

Treatment	Number of Observations	Rate of Closure (%/day)	95% Confidence Interval
Hydrocolloid (SolositeÊ) and dressing	8	11.6	9.0, 12.1
Honey	5	10.6	8.6, 12.5
Macadamia oil	12	8	6.7, 9.3
Spray bandage	4	5.2	2.9, 7.5

4.7 Handling

As expected flying-foxes proved to be easy patients to handle and in most cases nothing more than the wrap was needed to restrain them during treatment. A few very stressed, or severely injured flying-foxes who required extreme stillness during treatment, were given diazepam before treatment. A few cases were also given diazepam on arrival, on veterinary advice, to minimise the impact of capture stress.

While most flying-foxes did not lick their injuries once treatment commenced some did try to interfere with their injuries/treatments. These flying-foxes were ñcollaredò to prevent them removing their treatments. The collar was usually removed after an hour or two. Adding a collar during handling of uncooperative flying-foxes also proved useful, particularly for a single handler, and removed the need for chemical restraint or excessive physical restraint. The style of collar used, see Figure 18 - was developed to be easy to put on or remove by a single handler - experienced or otherwise - and to not interfere with the flying-foxesô ability to eat or drink. Most of the flying-foxes appeared to ignore the collar, after an initial head shake and hung in the normal relaxed position.

A few flying-foxes removed the dressings every day but most tolerated them. Removal usually occurred overnight and the dressing would be replaced in the morning.

[19] see section 3.8.4 Size Analysis for information on included wounds

Dressings that were not applied completely flat - i.e. with tenting or with crinkles at the edges tended to lift easily. This also occurred if the underlying topical medication spread to the edges of the dressing and prevented it from adhering to the membrane.

Applying a dressing meant that the injury area had to be held still, necessitating constraint of the patient, and care had to be taken to apply the dressing correctly. Both of these factors meant that a dressing treatment required more time by the handler, and more stress to the flying-fox, than a non-dressing treatment. In fact for many non-dressing applications it was possible to apply them without removing the flying-fox from its hanging position or constraining it in any way. Although the flying-fox was not removed it was necessary to unwrap both wings as the flying-foxes kept the injured wing wrapped closest to the body, regardless of which wing was injured.

Treating an injury area which required a dressing (or dressings) could take from two to five minutes whereas the administration of a single topical application would typically take less than half a minute.

5 Discussion

The aim of this research project was to find treatments that can be used by rehabilitators to heal wing injuries such that the flying-fox is releasable, and the project has succeeded in achieving this for most injury types. The objective was to identify effective and efficient treatments. An effective treatment enables the injuries to heal so that full flight is achieved. Efficiency is assessed in terms of time to complete healing, minimising stress to the flying-fox, ease of treatment, and, minimising cost for the rehabilitator. The efficiency factors were important in selecting treatments for the final recommendation.

The treatment hypotheses were that:

- all treatments will achieve healing, and,
- some treatments will enable faster healing than others.

As previously outlined the baseline treatment approach to flying-fox healing by rehabilitators prior to this research usually resulted in euthanasia, see section 1 and Table 15 .

Table 15 Flying-fox Treatment Lore

Condition	Response
Tears from the leading (and frequently trailing) edge of the wing membrane	Cannot be healed and therefore euthanise
Broken bone	Will not be strong enough to support flight and therefore euthanise
Large holes (greater than 60 mm radius ï with some rehabilitation groups 30 mm radius)	Cannot fly with these holes and the flying-fox will be in care too long while healing and therefore euthanise
Any injury likely to take more than 2 weeks to heal	The flying-fox will suffer too much stress while in captivity for that time and will lose too much muscle tone to sustain full flight and therefore euthanise

Wounds over the forelimb	Most of these would regress and the flying-fox would die so therefore euthanise

The recommendation of euthanasia in response to the above injury types has been negated as a result of this study. Effective and efficient treatments have been identified which are for successful treatment of these conditions as discussed below.

Tears from the Leading or Trailing edge - euthanise: superseded

The results did not support the belief that these injuries cannot heal such that the flying-fox should be euthanised. Although the leading edge of the wing is the most difficult site to treat in terms of practical application, particularly when severe, all leading edge injuries eventually healed (with honey dressing, macadamia oil or hydrocolloidal dressing). Trailing edge injuries (which were part of the patagium injury analyses) were generally simple to treat and healed well. The results did not support routine euthanasia for these injuries.

Contraction of the leading edge, e.g. due to scarring, can severely limit flight capability and result in a lengthy rehabilitation (physiotherapy) time to recover flight after the initial injury has healed. It is therefore critical to treat injuries to this site effectively so that scaring is minimised. Unlike the trailing edge of the wing, the leading edge contains a major vein and a band of connective tissue. The area is also under extreme stretch between the at rest position of the wing and extension of the wing[20]. Physical stress is, therefore, applied to the injury every time the flying-fox reaches out with its thumb when climbing, feeding or grooming. This constantly disrupts the granulation process, see Healing Problems below. Treatment is also often complicated by the nature of the injury. Injuries typically resulted from constriction e.g. by strands of a fruit tree net. Consequently, damage to the surrounding propatagium and across the arm may be too severe to attach a dressing. These wounds must nevertheless be kept moist. Macadamia oil was highly successful in this but severe cases required more frequent applications than once per day. In the most extreme case macadamia oil was applied every two hours for a week and then every three hours during the day and four hourly overnight, for seven weeks. The flying-fox was successfully released. The severity of this wound was at least partly due to the initial unsuccessful use of *Aloe vera* gel. This emphasises the need to both treat injuries and commence treatment immediately. When a moist healing environment was successfully maintained, full healing was achieved. Full

[20] full extension of the wing is 180 degrees at the elbow

healing occurred even when the leading edge was totally disrupted. In a severe case over 90% of the propatagium was destroyed including the leading edge. The membrane regenerated when treated with macadamia oil and the flying-fox achieved full flight and was released.

While the time to heal could be extensive with leading edge injuries (e.g. nearly 1.5 months) all these flying-foxes healed, recovered their strength, full flight capability and were released. This research did not find any reason to euthanise flying-foxes resulting from leading edge injuries as has been past practice.

Large Holes (greater than 60 mm diameter – with some rehabilitation groups 30 mm diameter) - euthanise: superseded

There is not any evidence to support euthanasia based on hole size. Regardless of size, patagium holes heal easily and well. Nor is it necessary to wait until the hole has closed prior to release. If the flying-fox wing maintains the appropriate shape and has sufficient sail area overall to be aerodynamic then the flying-fox can fly and it can be released It would appear to be intuitively obvious (but apparently has not been) that, even without human interference, flying-foxes must sustain the occasional puncture wound on landing, playing and fighting and be able to recover. Certainly flying-foxes with large holes were observed to fly well and strongly in the pteropery during the course of the research. This research did not find any reason to euthanise flying-foxes based only on hole size.

Broken Bone – euthanise: superseded

Treating fractures was not a part of this research however during the course of the research three flying-foxes did present with finger fractures as part of the general wing damage. All three were treated (the dressing used on the open injury also stabilised the fracture) and released, following complete healing of injuries. Further, several flying-foxes presented at the Wildlife Assistance & Information Foundationôs Wildlife Veterinary Clinic (St Ives, Sydney) with wing (arm and finger bone) fractures. These were treated and released and many others with wing and leg fractures have been treated and released through the Austral Veterinary Clinic (Austral, Sydney, NSW). These successes counter those current practices which indicate euthanasia is required for all flying-foxes with wing or leg fractures.

Any Injury Likely to Take More Than 2 Weeks to Heal – euthanise: superseded

Most injuries required at least two weeks to heal completely. All flying-foxes treated and released were in care for at least 17 days and up to several months. All flying-foxes demonstrated strong flight prior to release and did not show any indicators of stress such as weight loss or poor pelage. All flying-foxes were soft released and therefore could be monitored. None returned showing any form of stress as is frequently the case when released animals are not coping.

There is no evidence to support a policy of euthanasia for injuries requiring more than two weeks for rehabilitation.

Wounds Over the Forelimb – Generally Regressed and the animal died / euthanised: superseded

Injuries involving the arm and forearm had a very high chance of successful healing when treated appropriately. Failures to achieve granulation did occur but a change of treatment to honey dressing, macadamia oil or hydrocolloidal dressing achieved 100% success. There are not any indications for euthanasia.

General Healing Outcomes

Inflammatory responses to open injuries (wounds) were macroscopically observable within 24 hours as was also reported by Iversen et al. (1974) for wounds in *P. giganteus*.

Wounds involving the epidermal layer only, re-epithelialised in 17 ± 11 days. The high variation is biased by a few extreme outliers. When these are removed the average time for these injuries to re-epithelialise is 13.7 ± 3.6 days which is closer to the Iversen et al. (1974) results (7 to 11 days). Importantly the Iversen et al. study (Iversen, et al., 1974) investigated ñclean woundsò, i.e. the boundaries of the injury were known. Injuries that occur in the field may have large areas of damage - not initially visible to the eye - from constriction or exposure to excessive sun or wind. In these circumstances the tissue clean-up phase will require longer and therefore healing would take longer. Further, this reinforces the need to treat injured areas appropriately from the moment of injury.

Closed injuries (bruises) presented a different picture from open injuries (wounds). Predicted time for bruise healing varies from 12 to 36 days with an average of 19 days which is comparable to Church and Noronha (1965) who reported a healing time for minor bruises of 20 days. Many

bruises (45%) took longer than 2 days to appear with the longest being 14 days. This supports the policy of many flying-fox rehabilitators of not releasing a flying-fox for two weeks after an entanglement event in order to allow time for bruise injuries to become visible. This is particularly important as the majority of bruises, from minor to very severe, regressed to open injuries that would then need additional treatment. Further, the flying-fox would be unable to fly during this time and so is unlikely to survive in the wild. Again, this situation emphasises the need to begin appropriate treatment early! Whether closed or open the more severe the injury the longer the time required to complete healing.

The significantly longer time required to heal open injuries than closed injuries is probably due to several factors. One of the factors is that open wounds are subject to contamination: from the environment, from bacterial loading and from increased epidermal debris: all of which will increase the debridement time required. Another reason for the time increase is that time for re-epithelialisation is required only for open injuries.

Time for the inflammation phase did not vary with treatment being 10.6 ± 1 days. This is comparable to human wounds which take up to two weeks to resolve inflammation (Kirsner & Bogensberger, 2002). There was very little difference in granulation time between treating with honey or the hydrocolloid regardless of category, severity or site, but the macadamia oil granulation phase was clearly shorter than these two. This may indicate that a mixed protocol should be considered. The healing times for the ñfailedò treatments; topical *Aloe vera* and keratinolytic solutions (Ilium Oticlean), may be faster than the successful treatments but the data sets are small (bringing in the possibility of an effect from family error rates). It is possible, however, that one of these treatments may be of use for a part of the process and so useful in a mixed protocol.

In summary while time differences were observed, the practical significance of the results is determined by the success / failure rates of the various treatments.

Healing Problems

Finger bones, non-elbow joints and leading edge injuries were not only slower to heal but the sites were prone to granulation quality issues. This negatively affected the duration of healing and sometimes the quality of healing as described in the Results. Finger bone injuries had a tendency to excessive granulation and the leading edge to disrupted granulation. This could be due to a combination of ongoing disruption to the injury (e.g. bending of joints and stretching of the

leading edge) and lack of tissue volume to provide physical support and healing components (e.g. keratinocytes, vascular supply).

Ponies and horses also sustain injuries in sites that lack tissue support. Excessive or poor granulation has been studied in horses and ponies (Jacobs, Leach, Fretz, et al., 1984; Wilmink, Van weeren, Stolk, et al., 1999; Theoret, Barber, Moyana, et al., 2001; Wilmink & van Weeren, 2005). This poor granulation was attributed to an inadequate or extended inflammatory phase (Wilmink, Stolk, Van weeren, et al., 1999; Wilmink & van Weeren, 2005). The reasons are not entirely clear but appear to relate to the anatomic environment, e.g. greater muscle tissue in body wounds which may supply nutrients, oxygen and chemokines faster and / or to unbalanced levels of TGF_{β} (lower levels of TGF_{β} in the initial inflammatory response but persisting for longer which maintains fibroblast proliferation and stimulates inflammation) (Wilmink & van Weeren, 2005). Histological examination of overgranulated horse wounds has shown increased and non-resolving inflammation, slowed re-epithelialisation and decreased apoptosis (Lepault, Céleste, Doré, et al., 2005). Excessive ECM may also be caused by microvascular occlusion (Lepault, et al., 2005). Wilmink et al. (2005) described exuberant granulation as "typically unhealthy in appearance, its surface is riddled with many grooves and clefts, and it is produced above the wound margins".

Grooves and clefts were typical of poor healing in leading edge wounds in this study but not of the over-granulation observed over joints, see Figure 32 or along finger bones, see Figure 33, where the granulation tissue continued to grow without proper epithelialisation. Excessive joint granulation was usually resolved by the growth of epithelial tissue, then by reduction of the underlying tissue mass. A similar finding was observed in flying-foxes flying in a pteropery. Occasionally an injury developed on the wing tip with over-granulation believed to be due to repeatedly hitting the top of the pteropery with the wing tip during flight. The situation rarely resolved and the current practice is to release the affected individual as soon as possible to prevent continued re-injury. This situation indicates that over-granulation in this research may have been due to incomplete resolution of the inflammation phase (in this case due to repeated injury maintaining the inflammatory process). Leading edge granulation tissue also eventually resolved with a normal appearing structure.

Effectiveness

The honey treatment had a higher chance of success than SolositeÊ and TegadermÊ during the inflammation phase ($p = 0.05$) and overall ($p = 0.07$). This contrasts to the Ingle et al. (2006) trial

conducted on goldmine workers, which did not record any failures for either honey or the wound gel. The success of the honey treatment supports the results of the Subrahmanyam trials (Subrahmanyam, 1991, 1993, 1997, 2001). The reason why honey is successful remains unclear. Its antimicrobial properties were not believed to be the primary contribution in this research as very few injuries appeared to be infected regardless of treatment, indicating that infection was not a major factor in flying-fox wing injuries. Presumably, honeyôs healing capacity is due to other properties e.g. providing a moist wound environment, debridement, and facilitating re-epithelialisation. The success of SolositeÊ also could not be attributed to any antimicrobial property (as none was demonstrated) but to its ability to maintain a moist wound environment and absorb wound exudate.

Macadamia oil was also highly effective in terms of likelihood of success and reduced time to healing. Macadamia oil was associated with a lower success rate than the hydrocolloid for completing the acute inflammation phase but was associated with a much higher success rate for healing during the granulation phase. Treatment protocol may influence the outcome for the inflammation phase. Two flying-foxes with severe open membrane damage could not be treated with a dressing, which was normal medical practice for such extensive injuries. Both were treated with macadamia oil but much more frequently than daily and both recovered and were released. This suggests the success rate could be improved by increasing the frequency of application. Treating every two hours for six weeks is not practical for most rehabilitation situations but it can be followed, and may need to be implemented when the flying-fox populations reach ñCritically Endangeredò. This situation is likely to occur soon (for the grey-headed flying-fox in Australia) with a wild population that has a half-life equal to 6.5 years and where higher survival rates are needed to stabilise the wild population (Divljan 2008).

Aloe vera results were unexpected with a failure rate of 44% (the same rate as not treating the injury) with most injuries failing to resolve inflammation. The failure rate was low during the granulation phase. It may be suitable as a treatment once inflammation has resolved. It should, however, be noted that most injuries that continued through to granulation during treatment with *Aloe vera* were minor and the quality of repair was often poor. There is, therefore, very little indication for its use.

Keratinolytic dermal wash solution (Ilium Oticlean) had a high failure rate (33%) with failures in both healing phases. It may have a faster healing time during granulation than the hydrocolloid dressing however the sample size was small (due to the high failure rate during the inflammation

phase). It was not easier to apply than macadamia oil and was more expensive than macadamia oil or honey. Its use, therefore, is not recommended.

Spray bandage group had a comparable failure rate (43%) to the no treatment group with most of the injuries failing to resolve inflammation. Spray bandage had a low failure rate during the granulation phase, but most injuries that continued to granulation under a spray bandage were minor. There is, therefore, very little indication for its use.

Arnica topical application had a high failure rate (29%) with most injuries deteriorating to open wounds. The thin strands of fruit tree netting wrapped around a flying-fox wing can act like a tourniquet with consequent damage to the blood vessels. A similar effect is seen where the wing is wrapped tightly around the strands of barbed wire. Substantial areas of wing can be lost requiring long periods in rehabilitation or, in the worst cases, euthanasia. It was hoped Arnica would prevent or minimise the consequent bruising and therefore lessen the effect of constriction damage. Arnica as a highly successful treatment in preventing bruising and promoting healing is not supported by this research. Only minor injuries progressed to the granulation phase under arnica treatment and most of those developed into an open wound before healing. A different application method (e.g. systemic) may have a higher success rate as is claimed by Brett (2007, August 5-9) and could perhaps be trialled. Currently it is not recommended as a topical treatment.

Despite the large number of injuries only three minor infections occurred, regardless of treatment. The infected injuries resolved with a silver dressing, however the number was too small to support or contradict any of the findings of other researchers on the efficacy of silver (except Vermeulen 2007 in the Cochrane report which concluded that there was insufficient evidence).

Macadamia oil, honey with a dressing (TegadermÊ) and hydrocolloid (SolositeÊ) with a dressing (TegadermÊ) were all consistently successful and are the treatments of choice. All of them provide a moist wound environment and all of them contain, or are reputed to contain, healing factors, albeit different factors. Interestingly some of the factors in honey and macadamia oil overlap (many of the B vitamin group and many of the minerals (Bergman, et al., 1983; Akhtar, et al., 2006)) .

Holes

Holes in the patagium have several interesting outcomes other than time to close. The pattern of change in hole size in the patagium, initially increasing then decreasing steadily until the hole

almost closed, is similar to that in previous studies (Church & Warren, 1968; Bhangoo, 1974; Bhangoo & Church, 1976), suggesting that the increase occurs even when the surrounding membrane is undamaged, i.e. the injury is not necessarily becoming worse. It should be noted that comparisons with standard hole models is complicated by the nature of the wounds. Most wound size models assume a certain simple, symmetric shape for the hole e.g. round, oblong. The shapes of holes in flying-fox wings are frequently not symmetric or simple, particularly where structures other than the open membrane, such as fingers or arms, are involved or where surrounding bruising has occurred. Increases in the size of a hole continuing after a few days may be regarded as a problem e.g. bruises regressing to open wounds. These observations are important to rehabilitation practices such as deciding when to treat and when to release.

Reducing hole size was not generally considered important unless the hole was compromising flight ï which was rare. The most interesting observation with reference to holes was that the cut edge of holes in the patagium healed by tissue growth between the ventral and dorsal epithelial surfaces, see Figure 5 and Figure 24, and the hole then filled in as part of the scar remodelling phase. Once the hole healed, the significant factor was the remaining wing area available to support flight. If the missing membrane area encompasses finger bones (i.e. a complex hole) then flight might be compromised.

Efficiency

The objectives were to not only find an effective treatment but to select an efficient treatment (minimising application time, flying-fox stress, difficulty of application protocol and purchase cost). None of the treatments considered successful are, in the Australian context, overwhelmingly expensive or difficult to obtain. The options of a pharmacy ñover the counterò treatment and other easily obtainable treatments should provide wildlife professionals with a useful choice which balances effectiveness, cost and ease of use for their particular situation.

The honey and hydrocolloidal gel both require a dressing. Applying a dressing successfully requires more time and more skill than applying a topical medication and requires greater restraint of the flying-fox. It is therefore less efficient (increased time, stress and difficulty) than not using a dressing. An application of macadamia oil is the least time consuming and stressful treatment. If it is critical to maintain a constantly moist environment, e.g. a large wound exposing bone, then the repeated applications of oil will be necessary. In this case the oil treatment becomes more time consuming and more stressful to the flying-fox, than using a dressing based treatment. Some

welfare considerations may also dictate the use of a dressing over the injury, e.g. pain reduction. Selection between honey and gel is a balance between decreased healing time (honey) and decreased handling (gel). Honey needed to be applied daily, therefore daily handling of the flying-fox, whereas the wound gel could be left for several days, thereby decreasing time and stress.

Cost consideration will depend on the resources available to the rehabilitator and the number of injuries to be treated. In this research the honey was provided free by the apiarist and the macadamia oil bought in bulk - which is very inexpensive. Further, the number of injuries was high so shelf life was not a consideration - all products were used before expiration dates were reached.

Unprocessed honey is not usually an expensive item but may be difficult to obtain - particularly at short notice. In this situation a specialist medical honey may be the only suitable option and this can be expensive. Macadamia oil is relatively inexpensive and easily obtained from any grocery store including small country corner stores. Unprocessed honey and macadamia oil are also human food items which may lessen the impact of expiration dates.

The wound gel must be bought via a pharmacy (or similar) and does not have an alternative use. The dressing used (TegadermÊ) is the more expensive of the over-the-counter options but also the easiest and quickest to apply. There is an online source for this dressing which is inexpensive compared with the over-the-counter costs.

Hypotheses

The **first hypothesis,** that all treatments will provide healing was rejected. There were distinct differences in success between treatments.

The **second hypothesis** that some treatments would result in faster healing than others was accepted.

Future Directions

Remaining Issue

The treatment of complex, severe injuries involving major damage to large areas of the membrane but which leave the supporting finger bones, at least initially alive, remains unresolved. This

situation often occurs in net caught injuries that result in massive bruising. If the finger bones can be kept viable, the flying-fox can be saved. The faster the membrane regenerates, providing a vascular supply and physical stability to the bones, the higher the chance of maintaining vital bone and regenerating the area.

Oxidised regenerative cellulose (ORC) / collagen matrix can protect endogenous growth factors and has been shown to improve re-epithelialisation in acute wounds when used in conjunction with a hydrocolloid (Cullen, Smith, McCulloch, et al., 2002; Cullen, Watt, Lundqvist, et al., 2002; Jeschke, Sandmann, Schubert, et al., 2005). It was hoped that Promogran (see section 3.5.10) would provide stability and allow faster granulation. In a single flying-fox treated with Promogran it did appear that granulation was occurring faster than would otherwise be expected. Unfortunately, the product structure did not provide physical support to stabilise the finger bones during granulation and several phalanges over multiple digits were lost and the flying-fox had to be euthanised.

Alginates, which promote granulation (Myer, 2002), may also assist extreme cases as may other passive and interactive biological dressings. Some of these also provide structural support and should be trialled in future research. The need to stabilise the wing remains the biggest hurdle in the treatment of these flying-fox wing injuries.

Improvements from Mixed or Changed Protocols

Mixed treatments may be a better approach for two reasons:

1. some may only be useful during part of the healing process e.g. targeting the inflammation phase and therefore may only be effective for that phase and not for other phases, and

2. some treatments are easier to use than are others and therefore are indicated to decrease handling and time; e.g. a simple topical application versus a dressing.

Some treatments that failed inflammation healing may be useful as a granulation phase treatment in a mixed protocol. For example, *Aloe vera* may be effective during the granulation phase but the failure rate during the inflammation phase was too high to make an assessment.

Honeyôs effectiveness in promoting healing of the inflammatory phase makes it the treatment of choice for that phase however its effectiveness was not as clear for the granulation phase where macadamia oil may be better. Macadamia oil was easier to apply so a mixed protocol has merit.

The indication that increasing the frequency of macadamia oil application improves results could be investigated to determine the optimal application frequency for different injuries.

Histology and Biochemistry

Healing is a dynamic and complex chemical and cellular process and it is reasonable to assume that a highly effective treatment (e.g. honey, wound gel and macadamia oil) would also be chemically complex at the biochemical level and address more than one aspect of the healing environment. While the antimicrobial effects of the treatments may have been active, the results of the microbiological testing were inconclusive. A logical interpretation would be that the effect of the successful treatments was due to a combination of moisture and some positive factor or factors, possibly nutritional factors. It would be, therefore, interesting to study the effects of the constituents of the successful treatments on the phases of the healing process and to carry out a comparison between them.

The change from primarily neutrophils to monocytes / macrophages marks the change from early to late phase inflammation (Clark, 1996b). Failure to change may result in chronic wounds and also excessive granulation (Wilmink & van Weeren, 2005). It would therefore be useful to determine the chemical and cellular activities of the over-granulating and slow healing injuries in flying fox wing injuries.

Other Species

This work was primarily carried out on the grey-headed flying-fox. The principles of wound healing and treatment are generally applicable to any mammal study and largely to any vertebrate study and therefore the results of this study should be applicable to many other injury healing situations. Extending the study to include other chiropteran species (and indeed other wildlife species) would assist in validating the results and expanding the usefulness of the protocols.

These treatment protocols were developed with both cost and availability issues as part of the selection process. In most countries, cost is a major consideration in the treatment of wildlife (and frequently for the treatment of domestic animals and indeed humans). The honey and macadamia oil treatments have the potential to provide excellent healing for very low cost.

6 Conclusion

The aim of the research, to identify a treatment or treatments for rehabilitators to use on injured flying-fox wings, was achieved. Three effective and efficient treatment protocols were developed: honey under dressing (TegadermÊ), a wound gel (SolositeÊ) under a dressing (TegadermÊ), and macadamia oil.

Kane (2001) wrote that ñeach wound and each host is an individual with unique problems and potential to healing and productivityò. In Chiropteran terms óproductivityô translates to the ability to fly sufficiently well to survive in the wild. This research clearly showed that rehabilitation policies requiring euthanasia for wing injuries cannot be supported. The most that should be stated is that an injury that severely compromises phalanges of multiple digits currently has a poor prognosis for release back to the wild. Apart from this, suitable treatments for virtually all wing injury types are available.

It is recommended that each patient and each injury be assessed individually and a course of treatment defined and implemented. Although the underlying biochemical processes have not yet been elucidated, three successful treatments were identified and are recommended for membrane injuries. These are honey under a polyurethane dressing (e.g. TegadermÊ), a wound gel (e.g. SolositeÊ) under a polyurethane dressing (e.g. TegadermÊ) or macadamia oil.

7 Bibliography

Akhtar, N., Ahmad, M., Madni, A., & Sattar, M. (2006). Evaluation of basic properties of macadamia nut oil. *Gomal University Journal of Research, 22*, 21-27.

Alonso, D., Lazarus, M. C., & Baumann, L. (2002). Effects of topical arnica gel on post-laser treatment of bruises. *Dermatologic Surgery, 28*, 686-688.

Arnold, M., & Barbul, A. (2006). Nutrition and wound healing. *Plastic and Reconstructive Surgery, 117*(7S), 42S-58S.

Atkins, J. (2010). Humans battle fruit bats for bumper mango crop Retrieved 21/3/12, 2012, from http://www.abc.net.au/local/stories/2010/01/19/2795865.htm

Banks, V., Bale, S., Harding, K., & Harding, E. (1997). Evaluation of a new polyurethane foam dressing. *Journal of Wound Care, 6*(6), 266-269.

Banks, V., & Harding, K. (1994a). Comparing two dressings for exuding pressure sores in community patients. *Journal of Wound Care, 3*(4), 175-178.

Banks, V., & Harding, K. (1994b). The use of two dressings for moderately exuding pressure sores. *Journal of Wound Care, 3*(3), 132-134.

Bannenberg, G. L., Chiang, N., Ariel, A., Arita, M., Tjonahen, E., Gotlinger, K. H., Hong, S., et al. (2005). Molecular circuits of resolution: Formation and actions of resolvins and protectins. *Journal of Immunology, 174*(7), 4345-4355.

Bar-Shavit, R., Kahn, A., Fenton, J. [n.], & Wilner, G. (1983). Chemotactic response of monocytes to thrombin. *Journal of Cell Biology, 96*(1), 282-285.

Bartholomew, G., Leitner, P., & Nelson, J. (1964). Body temperature, oxygen consumption and heart rate in three species of Australian flying-foxes. *Physiological Zoology, 37*, 179-198.

Bashir, S. J., Dreher, F., Chew, A. L., Zhai, H., Levin, C., Stern, R., & Maibach, H. I. (2005). Cutaneous bioassay of salicylic acid as a keratolytic. *International Journal of Pharmaceutics, 292*(12), 187-194.

Bergman, A., Yanai, J., Weiss, J., Bell, D., & David, M. (1983). Acceleration of wound healing by topical application of honey, an animal model. *The American Journal of Surgery, 145*(3), 374-376.

Bhangoo, K. S. (1974, April). *Wound contraction.* Paper presented at the International Symposium on Wound Healing, Rotterdam, the Netherlands.

Bhangoo, K. S., & Church, J. (1976). Elastogenesis in healing wounds in bats. *Plastic and Reconstructive Surgery, 57*(4), 468-479.

Bickel, P., & Doksum, K. A. (1977). *Mathematical statistics: Basic ideas and selected topics*. San Francisco: Holden-Day.

Bradley, D. (1998). *The effects of topically-applied acemannan on the healing of wounds with exposed bone.* Unpublished PhD Thesis, Auburn University, Alabama.

Brett, D. S. F. (2007, August 5-9). *Complementary therapies for wildlife*. Paper presented at the National Wildlife Rehabilitation Conference, Fremantle, Western Australia.

Broadbent, E., Petrie, K., Alley, P., & Booth, R. (2003). Psychological stress impairs early wound repair following surgery. *Psychosomatic Medicine, 65*(5), 865.

Brown, G., Nanney, L., Griffen, J., Cramer, A., Yancey, J., Curtsinger, L., Holtzin, L., et al. (1989). Enhancement of wound healing by topical treatment with epidermal growth factor. *New England Journal of Medicine, 321*(2), 76-79.

Caldwell, M. D. (1990). Topical wound therapy-an historical perspective. *Journal of Trauma-Injury, Infection, and Critical Care, 30*, 116-121.

Campbell, R. M., & Cuthbertson, D. P. (1967). Effect of environmental temperature on the metabolic response to injury. *Experimental Physiology, 52*(2), 114-129.

Carrier, E. B. (1926). Observation of living cells in the bat's wing. In J. Linhard (Ed.), *Physiological papers, dedicated to Prof. August Krogh* (pp. 1-9). Copenhagen: Levin & Munksgaard Publishers.

Chaby, G., Senet, P., Vaneau, M., Martel, P., Guillaume, J.-C., Meaume, S., Teot, L., et al. (2007). Dressings for acute and chronic wounds: A systematic review. *Archives of Dermatology, 143*(10), 1297-1304.

Chithra, P., Sajithlal, G. B., & Chandrakasan, G. (1998). Influence of *Aloe vera* on collagen characteristics in healing dermal wounds in rats. *Molecular and Cellular Biochemistry, 181*, 71-76.

Chithra, P., Sajithlal, G. B., & Gowri, C. (1998). Influence of *Aloe vera* on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *Journal of Ethnopharmacology, 59*(3), 179-186.

Choi, Y. S., Lee, S. B., Hong, S. R., Lee, K. W., & Park, M. H. (2001). Studies of gelatin-based sponges. Part III: A comparative study of cross-linked gelatin/alginate, gelatin/hyaluronate and chitosan/hyaluronate sponges and their application as a wound dressing in full-thickness skin defect of rat. *Journal of Materials Science: Materials in Medicine, 12*, 67-73.

Church, J. (Ed.). (1970). *Cell quantitation in regenerating bat web muscle*. Amsterdam: Excerpta Medica.

Church, J., & Griffin, E. (1968). *Mycobacterium buruli* lesions in the fruit bat web. *The Journal of Pathology and Bacteriology 96*, 508-512.

Church, J., & Noronha, R. (1965). Use of the fruit bat in surgical research. *East African Medical Journal, 42*(7), 348-354.

Church, J., Noronha, R., & Allbrook, D. (1966). Satellite cells and a skeletal muscle regeneration. *British Journal of Surgery, 53*(7), 638-642.

Church, J., & Warren, D. J. (1968). Wound healing in the web membrane of the fruit bat. *British Journal of Surgery, 55*(1), 26-31.

Church, M., & Levi-Schaffer, F. (1997). The human mast cell. *Journal of Allergy and Clinical Immunology, 99*(2), 155-160.

Clark, R. A. F. (1985). Cutaneous tissue repair: Basic biologic considerations. I. *Journal of the American Academy of Dermatology, 13*(5 Pt 1), 701-725.

Clark, R. A. F. (1990). Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. *Journal of Investigative Dermatology, 94*(s6), 128s-134s.

Clark, R. A. F. (1996a). *The molecular and cellular biology of wound repair* (Vol. 1). New York: Plenum Press.

Clark, R. A. F. (1996b). Wound repair - overview and general considerations. In R. A. F. Clark (Ed.), *The molecular and cellular biology of wound repair* (Vol. 1, pp. 3-37). New York: Plenum Press.

Clark, R. A. F., Tonnesen, M., Gailit, J., & Cheresh, D. (1996). Transient functional expression of alphavbeta 3 on vascular cells during wound repair. *The American Journal of Pathology, 148*(5), 1407-1421.

Cochrane reviews (2011). Retrieved 29/2/12, 2012, from http://www.cochrane.org/cochrane-reviews

Connell, K. A., Munro, U., & Torpy, F. R. (2006). Daytime behaviour of the grey-headed flying fox *Pteropus poliocephalus* Temminck (Pteropodidae: Megachiroptera) at an autumn/winter roost. *Australian Mammalogy, 28*, 7-14.

Cooper, R., Molan, P., & Harding, K. (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine, 92*(6), 283.

Creager, J. G. (1992). *Human anatomy and physiology*. Arlington, Virginia: Wm. C Brown Publishers.

Crivellato, E., & Ribatti, D. (2005). Involvement of mast cells in angiogenesis and chronic inflammation. *Current Drug Targets - Inflammation and Allergy, 4*, 9-11.

Crowley, G. V., & Hall, L. S. (1994). Histological observations on the wing of the grey-headed flying fox (*Pteropus poliocephalus*) (Chiroptera : Pteropodidae). *Australian Journal of Zoology, 42*, 215-231.

Cullen, B., Smith, R., McCulloch, E., Silcock, D., & Morrison, L. (2002). Mechanism of action of promogran, a protease modulating matrix, for the treatment of diabetic foot ulcers. *Wound Repair and Regeneration, 10*(1), 16-25.

Cullen, B., Watt, P. W., Lundqvist, C., Silcock, D., Schmidt, R. J., Bogan, D., & Light, N. D. (2002). The role of oxidised regenerated cellulose/collagen in chronic wound repair and its potential mechanism of action. *International Journal of Biochemistry & Cell Biology, 34*(12), 1544-1556.

Davidson, J. M. (2002). Growth factors, extracellular matrix, and wound healing. In L. C. Kloth & J. M. McCulloch (Eds.), *Wound healing: Alternatives in management* (3rd ed., pp. 129 - 148). Philadelphia: F.A. Davis Co.

Davis, R., Donato, J., Hartman, G., & Haas, R. (1994). Anti-inflammatory and wound healing activity of a growth substance in *aloe vera*. *Journal of the American Podiatric Medical Association, 84*(2), 77-81.

Dayan, D., Barr-Nea, L., Sandbank, M., Binderman, I., Mechanic, G. L., & Ishay, J. S. (1983). Oriental hornet venom enhances wound healing and repair in rat skin, possibly through its collagenolytic activity. *Acta Tropica, 40*(2), 147-153.

Demir, H., Balay, H., & Kirnap, M. (2004). A comparative study of the effects of electrical stimulation and laser treatment on experimental wound healing in rats. *Journal of Rehabilitation Research and Development, 41*(2), 147-153.

Desmouliere, A., Chaponnier, C., & Gabbiani, G. (2005). Tissue repair, contraction, and the myofibroblast. *Wound Repair and Regeneration, 13*(1), 7-12.

DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research, 89*(3), 219-229.

Dover, R., Otto, W., Nanchahal, J., & Riches, D. (1995). Toxicity testing of wound dressing materials in vitro. *British Journal of Plastic Surgery, 48*(4), 230-235.

Dyson, M., Young, S., Pendle, C. L., Webster, D. F., & Lang, S. M. (1988). Comparison of the effects of moist and dry conditions on dermal repair. *Journal of Investigative Dermatology, 91*(5), 434-439.

Dyson, M., Young, S. R., Hart, J., Lynch, J. A., & Lang, S. (1992). Comparison of the effects of moist and dry conditions on the process of angiogenesis during dermal repair. *Journal of Investigative Dermatology, 99*(6), 729-733.

Eijkelkamp, N., Engeland, C. G., Gajendrareddy, P. K., & Marucha, P. T. (2007). Restraint stress impairs early wound healing in mice via [alpha]-adrenergic but not [beta]-adrenergic receptors. *Brain, Behavior, and Immunity, 21*(4), 409-412.

Falanga, V. (1998). Wound healing and chronic wounds. *Journal of Cutaneous Medicine and Surgery, 3*(Supplement one), S1-1 to S1-5.

Falanga, V., Zitelli, J. A., & Eaglestein, W. H. (1988). Wound healing. *Journal of the American Academy of Dermatology, 19*, 559-563.

Feily, A., & Namazi, M. (2009). *Aloe vera* in dermatology: A brief review. *Giornale italiano di Dermatologia e Venereologia, 144*(One), 85-91.

Feldman, D., Rogers, A., & Karpinski, R. (1991). A prospective trial comparing biobrane, duoderm and xeroform for skin graft donor sites. *Surgery, Gynecology & Obstetrics, 173*(1), 1-5.

Feng, X., Clark, R. A. F., Galanakis, D., & Tonnesen, M. G. (1999). Fibrin and collagen differentially regulate human dermal microvascular endothelial cell integrins: Stabilization of ∝v/ϐ3 mrna by fibrin. *The Journal of Investigative Dermatology, 113*(6), 913-919.

Fernandez, H. N., Henson, P. M., Otani, A., & Hugli, T. E. (1978). Chemotactic response to human c3a and c5a anaphylatoxins, I. Evaluation of c3a and c5a leukotaxis in vitro and under simulated in vivo conditions. *Journal of Immunology, 120*, 109-115.

Fisher, P., Mathie, R., van Haselen, R., & Ernst, E. (2003). Homeopathic arnica: Author's reply. *Journal of the Royal Society of Medicine, 96*(4), 206-207.

Gabbiani, G. (2003). The myofibroblast in wound healing and fibroconnective diseases. *The Journal of Pathology, 200*(4), 500-503.

Gabbiani, G., Le Lous, M., Bailey, A. J., Bazin, S., & Delaunay, A. (1976). Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural and immunologic study. *Virchows Archiv - B: Cell Pathology, 21*(2), 133-145.

Gabbiani, G., Ryan, G. B., & Majno, G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Cellular and Molecular Life Sciences, 27*(5), 549-550.

Gajiwala, K., & Gajiwala, A. L. (2004). Evaluation of lyophilised, gamma-irradiated amnion as a biological dressing. *Cell and Tissue Banking, 5*, 73-80.

Ge, L., Gordon, J. S., Hsuan, C., Stenn, K., & Prouty, S. M. (2003). Identification of the [delta]-6 desaturase of human sebaceous glands: Expression and enzyme activity. *The Journal of Investigative Dermatology, 120*(5), 707-714.

Goss, R. (1980). Prospects of regeneration in man. *Clinical Orthopaedics and Related Research*(151), 270.

Graig Farm Organics. (2009). Rejuvenating cream - earthbound. (1/10/2009). Retrieved from http://80.247.2.193/product-rejuvenating_cream_50g_earthbound.html

Gray, G., & Yardley, H. (1975). Lipid compositions of cells isolated from pig, human, and rat epidermis. *Journal of Lipid Research, 16*(6), 434-440.

Grazul-Bilska, A., Johnson, M. L., Bilski, J. J., Redmer, D. A., Reynolds, L. P., Abdullah, A., & Abdullah, K. (2003). Wound healing: The role of growth factors. *Drugs of Today, 39*(10), 787-800.

Gupta, B. B. (1967). The histology and musculature of the plagiopatagium in bats. *Mammalia, 31*, 313-321.

Hall, S., Savill, J., Henson, P., & Haslett, C. (1994). Apoptotic neutrophils are phagocytosed by fibroblasts with participation of the fibroblast vitronectin receptor and involvement of a mannose/fucose-specific lectin. *Journal of Immunology, 153*(7), 3218-3227.

Hartop, P. J., & Prottey, C. (1976). Changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats. *British Journal of Dermatology, 95*(3), 255-264.

Haslett, C., & Henson, P. (1996). Resolution of inflammation. In R. A. F. Clark (Ed.), *The molecular and cellular biology of wound repair* (pp. 143-166). New York: Plenum Press.

Hebda, P. A., Klingbeil, C. K., Abraham, J. A., & Fiddes, J. C. (1990). Basic fibroblast growth factor stimulation of epidermal wound healing in pigs. *Journal of Investigative Dermatology, 95*(6), 626-631.

Heggers, J. P., Kucukcelebi, A., Stabenau, C. J., Ko, F., Broemeling, L. D., Robson, M. C., & Winters, W. D. (1995). Wound healing effects of *Aloe* gel and other topical antibacterial agents on rat skin. *Phytotherapy Research, 9*(6), 455-457.

Herndon, D., & Tompkins, R. g. (2004). Support of the metabolic response to burn injury. *Lancet, 363*(9424), 1895-1902.

Hinman, C., & Maibach, H. (1963). Effects of air exposure and occlusion on experimental skin wounds. *Nature, 200*, 377.

Holbrook, K., & Odland, G. (1978). A collagen and elastic network in the wing of a bat. *Journal of Anatomy, 126*, 21-36.

Houtsmuller, U. M. T., & van der Beek, A. (1981). Effects of topical application of fatty acids. *Progress in Lipid Research, 20*, 219-224.

Hunt, T. K. (1990). Basic principles of wound healing. *The Journal of Trauma, 30*(12 Supplement), S122-S128.

Ilium oticlean (2002, 31/1/02). Retrieved 29/2/12, 2012, from <http://troylab.com.au/products.php?tid=1&iid=23&pid=127>

Ingle, R., Levin, J., & Polinder, K. (2006). Wound healing with honey ï a randomised controlled trial. *South African Medical Journal, 96*(9), 831-835.

Iversen, O. H., Bhangoo, K. S., & Hansen, K. (1974). Control of epidermal cell renewal in the bat web. *Virchows Archiv - B: Cell Pathology, 16*, 157-179.

Jacinto, A., Martinez-Arias, A., & Martin, P. (2001). Mechanisms of epithelial fusion and repair. *Nature Cell Biology, 3*, E117-E123.

Jackson, S. (2003). Bats. In S. Jackson (Ed.), *Australian mammals biology and captive management* (1st ed.). Collingwood: CSIRO Publisher.

Jacobs, K. A., Leach, D. H., Fretz, P. B., & Townsend, H. G. G. (1984). Comparative aspects of the healing of excisional wounds on the leg and body of horses. *Veterinary Surgery, 13*(2), 83-90.

Jeffrey, S., & Belcher, H. (2002). Use of arnica to relieve pain after carpal-tunnel release surgery. *Alternative Therapies in Health and Medicine, 8*(2), 66-68.

Jeschke, M. G., Sandmann, G., Schubert, T., & Klein, D. (2005). Effect of oxidised regenerated cellulose/collagen matrix on dermal and epidermal healing and growth factors in an acute wound. *Wound Repair and Regeneration, 13*(3), 324-331.

Jones, V., & Harding, K. (2001). Moist wound healing. In D. L. Krasner, G. T. Rodeheaver & R. G. Sibbald (Eds.), *Chronic wound care: A clinical source book for healthcare professionals* (3rd ed., pp. 245-252).

Jørgensen, B., Price, P., Andersen, K., Gottrup, F., Bech-Thomsen, N., Scanlon, E., Kirsner, R., et al. (2005). The silver-releasing foam dressing, contreet foam, promotes faster healing of critically colonised venous leg ulcers: A randomised, controlled trial. *International Wound Journal, 2*(1), 64.

Joshi, S. P. (1998). Chemical constituents and biological activity of *Aloe barbadensis* - a review. *Journal of Medicinal and Aromatic Plant Sciences, 20*(3), 768-773.

Jull, A. B., Rodgers, A., & Walker, N. (2008). Honey as a topical treatment for wounds. *Cochrane Database of Systematic Reviews 2008,* (4). doi:10.1002/14651858.CD005083.pub2

Kane, D. P. (2001). *Chronic wound healing and chronic wound management* (3rd ed.): Wayne, PA: HMP Communications.

Katsuta, Y., Iida, T., Inomata, S., & Denda, M. (2005). Unsaturated fatty acids induce calcium influx into keratinocytes and cause abnormal differentiation of epidermis. *Journal of Investigative Dermatology, 124*(5), 1008-1013.

Kiecolt-Glaser, J. K., Marucha, P. T., Mercado, A. M., Malarkey, W. B., & Glaser, R. (1995). Slowing of wound healing by psychological stress. *Lancet, 346*(8984), 1194-1196.

Kirsner, R. S., & Bogensberger, G. (2002). The normal process of healing, chapter 1. In L. C. Kloth & J. M. McCulloch (Eds.), *Wound healing: Alternatives in management* (3rd ed., pp. 1-29). Philadelphia: F. A. Davis Company.

Konrad, D., Tsunoda, M., Weber, K., Corney, S. J., & Ullmann, L. (2002). Effects of a topical silver sulfadiazine polyurethane dressing (mikacure) on wound healing in experimentally infected wounds in the pig. A pilot study. *Journal of Experimental Animal Science, 42*(1), 31-43.

Kouzi, S. A., & Nuzum, D. S. (2007). Arnica for bruising and swelling. *American Journal Health-System Pharmacy, 64*(23), 2434-2443.

Krawczyk, W. S. (1971). A pattern of epidermal cell migration during wound healing. *Journal of Cell Biology, 49*(2), 247-263.

Kurkinen, M., Vaheri, A., Roberts, P. J., & Stenman, S. (1980). Sequential appearance of fibronectin and collagen in experimental granulation tissue. *Laboratory Investigation, 43*(1), 47-51.

Kweon, D.-K., Song, S. B., & Park, Y. Y. (2003). Preparation of water-soluble chitosan/heparin complex and its application as a wound healing accelerator. *Biomaterials, 24*, 1595-1601.

Lampe, K. (2002). Methods of wound evaluation, chapter 6 In L. C. Kloth & J. M. McCulloch (Eds.), *Wound healing: Alternatives in management* (pp. 153-197). Philadelphia: F A Davis Company.

Lansdown, A. B. G., Sampson, B., Laupattarakasem, P., & Vuttivirojana, A. (1997). Silver aids healing in the sterile skin wound: Experimental studies in the laboratory rat. *British Journal of Dermatology, 137*(5), 728-735.

Larjava, H., Salo, T., Haapasalmi, K., Kramer, R., & Heino, J. (1993). Expression of integrins and basement membrane components by wound keratinocytes. *Journal of Clinical Investigation, 92*(3), 1425.

Leicht, P., Siim, E., & Sorensen, B. (1989). Treatment of donor sites - duoderm or omiderm? *Burns, 15*(1), 7-10.

Lepault, É., Céleste, C., Doré, M., Martineau, D., & Theoret, C. L. (2005). Comparative study on microvascular occlusion and apoptosis in body and limb wounds in the horse. *Wound Repair and Regeneration, 13*(5), 520-529.

Lewis, J. H. (1977). Comparative haematology: Studies on chiroptera: *Pteropus giganteous*. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 58*(1), 103-107.

Lim, Y., Levy, M., & Bray, T. M. (2004). Dietary zinc alters early inflammatory responses during cutaneous wound healing in weanling cd-1 mice. *Journal of Nutrition, 134*(4), 811-816.

Lunney, D., Richards, G., & Dickman, C. (2008). *Pteropus poliocephalus*. *IUCN 2010. IUCN Red List of Threatened Species.* Retrieved from http://www.iucnredlist.org/

Lynch, S. E., Nixon, J. C., Colvin, R. B., & Antoniades, H. N. (1987). Role of platelet-derived growth factor in wound healing: Synergistic effects with other growth factors. *Proceedings of the National Academy of Sciences of the United States of America, 84*(21), 7696-7700.

Macadamia Oils of Australia (2006). *Macadamia oil MSDS and specifications (brochure)*. Alstonville, New South Wales, Australia: Author.

Madri, J. A., & Marx, M. (1992). Matrix composition, organization and soluble factors: Modulators of microvascular cell differentiation in vitro. *Kidney International, 41*(3), 560-565.

Madri, J. A., Marx, M., Merwin, J. R., Basson, C., Prinz, C., & Bell, L. (1991). Effects of soluble factors and extracellular matrix components on vascular cell behavior in vitro and in vivo: Models of de-endothelialization and repair. *Journal of Cellular Biochemistry, 45*(2), 123-130.

Madri, J. A., Pratt, B., & Tucker, A. (1988). Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the extracellular matrix. *Journal of Cell Biology, 106*(4), 1375-1384.

Madri, J. A., Sankar, S., & Romanic, A. M. (1996). Angiogenesis. In R. A. F. Clark (Ed.), *The molecular and cellular biology of wound repair* (2 ed., pp. 355-371). New York: Plenum Press.

Maenthaisong, R., Chaiyakunapruk, N., Niruntraporn, S., & Kongkaew, C. (2007). The efficacy of *Aloe vera* used for burn wound healing: A systematic review. *Burns, 6*, 713-718.

Majno, G., & Palade, G. E. (1961). Studies on inflammation: I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study. *Journal of Cell Biology, 11*(3), 571-605.

Martin, P. (1997). Wound healing - aiming for perfect skin regeneration. *Science, 276*, 75-81.

Martin, P., D'Souza, D., Martin, J., Grose, R., Cooper, L., Maki, R., & McKercher, S. R. (2003). Wound healing in the pu.1 null mouse - tissue repair is not dependent on inflammatory cells. *Current Biology, 13*(13), 1122-1128.

Martin, P., Hopkinson-Woolley, J., & McCluskey, J. (1992). Growth factors and cutaneous wound repair. *Progress in Growth Factor Research, 4*, 25-44.

Martini, F. (1998). *Fundamentals of anatomy and physiology* (4th ed.). Upper Saddle River, New Jersey: Prentice Hall Inc.

McCallion, R. L., & Ferguson, M. W. J. (1996). Fetal wound healing and the development of antiscarring therapies for adult wound healing. In R. A. F. Clark (Ed.), *The molecular and cellular biology of wound repair* (pp. 561-600). New York: Plenum Press.

McCarthy, J. (1995). The antibacterial effects of honey: Medical fact or fiction? *American Bee Journal, 135*(5), 341-342.

Mickleburgh, S., P, Hutson, A. M., & Racey, P. A. (1992). *Old world fruit bats: An action plan for their conservation* (1st ed.). Gland, Switzerland: IUCN.

Mickleburgh, S., P, Hutson, A. M., & Racey, P. A. (2002). A review of the global conservation status of bats. *Oryx, 36*(Part 1), 18-34.

Misirlioglu, A., Eroglu, S., Karacaoglan, N., Akan, M., Akoz, T., & Yildirim, S. (2003). Use of honey as an adjunct in the healing of split-thickness skin graft donor site. *Dermatologic Surgery, 29*(2), 168-172.

Molan, P. (1992a). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World, 73*(1), 5-28.

Molan, P. (1992b). The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. *Bee World, 73*(2), 59-76.

Morgan, J. E., & Partridge, T. A. (2003). Muscle satellite cells. *The International Journal of Biochemistry and Cell Biology, 35*(8), 1151-1156.

Mori, L., Bellini, A., Stacey, M. A., Schmidt, M., & Mattoli, S. (2005). Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. *Experimental Cell Research, 304*, 81-90.

Mulder, G. D., Altman, M., Seeley, J. E., & Tintle, T. (1993). Prospective randomized study of the efficacy of hydrogel, hydrocolloid, and saline solution moistened dressings on the management of pressure ulcers. *Wound Repair and Regeneration, 1*(4), 213-218.

Murphy, R. C. (1960). Fluorescence studies in the wing of the living bat. *The Anatomical Record, 136*, 127-135.

Myer, A. (2002). Dressings. In L. C. Kloth & J. M. McCulloch (Eds.), *Wound healing: Alternatives in management* (pp. 232-259). Philidelphia: F A Davis Company.

Newman, S. L., Henson, J. E., & Henson, P. M. (1982). Phagocytosis of senescent neutrophils by human monocyte-derived macrophages and rabbit inflammatory macrophages. *Journal of Experimental Medicine, 156*(2), 430-442.

Odland, G., & Ross, R. (1968). Human wound repair: I. Epidermal regeneration. *Journal of Cell Biology, 39*(1), 135-151.

Oryan, A., & Zaker, S. R. (1998). Effects of topical application of honey on cutaneous wound healing in rabbits. *Journal of Veterinary Medicine Series A, 45*(3), 181-188.

Ovington, L. (1999). Dressings and ajunctive therapies: AHCPR guidelines revisited. *Ostomy / Wound Management, 45*(1a), 14-35.

Paget, J. (1850). Lectures on inflammation. *The London Medical Gazette, 45*, 965-975.

Parks, W. C. (1999). Matrix metalloproteinases in repair. *Wound Repair and Regeneration, 7*(6), 423-432.

Pavletic, M. (1999). Basic principles of wound healing. In W. Saunders (Ed.), *Atlas of small animal reconstructive surgery* (2 ed., Vol. 1, pp. 11-63). Philadelphia.

Peacock, E. (1984). *Wound repair* (3rd ed.). Philadephia: WB Saunders.

Plendl, J., Snyman, C., Naidoo, S., Sawant, S., Mahabeer, R., & Bhoola, K. (2000). Expression of tissue kallikrein and kinin receptors in angiogenic microvascular endothelial cells. *Biological Chemistry, 381*, 1103-1115.

Qu, Z., Liebler, J. M., Powers, M. R., Galey, T., Ahmadi, P., Huang, X. N., Ansel, J. C., et al. (1995). Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *The American Journal of Pathology, 147*(3), 564-573.

Quay, W. (1970). *Integument and derivatives* (Vol. II). New York: Academic Press Inc.

Quinn, J., Lowe, L., & Mertz, M. (2000). The effect of a new tissue-adhesive wound dressing on the healing of traumatic abrasions. *Dermatology, 201*(4), 343-346.

Rasband, W. Image j (Version 1.40g). USA: National Institutes of Health.

Rayner, T. E., Cowin, A. J., Robertson, J. G., Cooter, R. D., Harries, R. C., Regester, G. O., Smithers, G. W., et al. (2000). Mitogenic whey extract stimulates wound repair activity in vitro and promotes healing of rat incisional wounds. *American Journal of Physiology, 278*(6), R1651-R1660.

Riches, D. W. H. (1996). Macrophage involvement in wound repair, remodeling, and fibrosis. In R. A. F. Clark (Ed.), *The molecular and cellular biology of wound repair* (2nd ed., pp. 95-141). New York: Plenum Press.

Ricks, D. J. (1991). Functional natural oils. *Cosmetics and Toiletries, 106*, 77-80.

Roberts, B., Catterall, C., Eby, P., & Kanowski, J. (2012). Long-distance and frequent movements of the flying-fox *Pteropus poliocephalus*: Implications for management. *PLoS ONE 7*(8). DOI:10.1371/journal.pone.0042532

Rodeheaver, G. T. (2001). Wound cleansing, wound irrigation, wound disinfection. In D. Krasner, G. T. Rodeheaver & R. G. Sibbald (Eds.), *Chronic wound care: A clinical source book for healthcare professionals* (3rd ed., pp. 369-383). Wayne, PA: HMP Communications.

Romer, A. S., & Parsons, T. (1977). *The vertebrate body* (5 ed.). Philadelphia London Toronto: WB Saunders Co.

Rubin, J. S., Osada, H., Finch, P. W., Taylor, W. G., Rudikoff, S., & Aaronson, S. A. (1989). Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America, 86*(3), 802-806.

Ruszczak, Z., Schwartz, R., Joss-Wichman, E., Wichman, R., & Zalewska, A. (2009). Surgical dressings. *emedicine*. Retrieved from http://emedicine.medscape.com/article/1127868-overview

Ryan, S. (November 2006). *Queensland nut tree Macadamia integrifolia*.

Saarialho-Kere, U., Chang, E., Welgus, H., & Parks, W. (1992). Distinct localization of collagenase and tissue inhibitor of metalloproteinases expression in wound healing associated with ulcerative pyogenic granuloma. *Journal of Clinical Investigation, 90*, 1952-1952.

Sandulache, V. C., Zhou, Z., Sherman, A., Dohar, J. E., & Hebda, P. A. (2003). Impact of transplanted fibroblasts on rabbit skin wounds. *Archives of Otolaryngology, 129*(3), 345-351.

Santoro, M., & Gaudino, G. (2005). Cellular and molecular facets of keratinocyte reepithelization during wound healing. *Experimental Cell Research, 304*(1), 274-286.

Schmidt, J., & Greenspoon, J. (1991). *Aloe vera* dermal wound gel is associated with a delay in wound healing. *Obstretrics and Gynecology, 78*(1), 115-117.

Schultz, G., White, M., Mitchell, R., Brown, G., Lynch, J., Twardzik, D., & Todaro, G. (1987). Epithelial wound healing enhanced by transforming growth factor-alpha and vaccinia growth factor. *Science, 235*(4786), 350-352.

Schuren, J., Becker, A., & Sibbald, R. G. (2005). A liquid film-forming acrylate for peri-wound protection: A systematic review and meta-analysis (3mÊ cavilonÊ no-sting barrier film). *International Wound Journal, 2*(3), 230-238.

Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J. A., Fiddes, J. C., & Gospodarowicz, D. (1987). Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. *Nature, 325*(6101), 257-259.

Serhan, C. N., Brain, S. D., Buckley, C. D., Gilroy, D. W., Haslett, C., O'Neill, L. A. J., Perretti, M., et al. (2007). Resolution of inflammation: State of the art, definitions and terms. *FASEB Journal, 21*(2), 325-332.

Sherratt, J. A., & Dallon, J. C. (2002). Theoretical models of wound healing: Past success and future challenges. *Comptes Rendus Biologies, 325*, 1-8.

Sidhu, G.-S., Singh, A.-K., Thaloor, D., Banaudha, K.-K., Patnaik, G.-K., Srimal, R.-C., & Maheshwari, R.-K. (1998). Enhancement of wound healing by curcumin in animals. *Wound Repair and Regeneration, 6*(2), 167-177.

Siedler, S., & Schuller-Petrovic, S. (2002). Wound healing enhancement in leg ulcers: A case report. *Cell and Tissue Banking, 3*, 25-28.

Simbirtsev, A., Konusova, V., McHedlidze, G., Paramonov, B., & Chebotarev, V. (2002). Pine resin and biopin ointment: Effects of water-soluble fractions on functional activity of peripheral blood neutrophils. *Bulletin of Experimental Biology and Medicine, 134*(1), 50-53.

Simpson, D., & Ross, R. (1972). The neutrophilic leukocyte in wound healing: A study with antineutrophilic serum. *Journal of Clinical Investigation, 51*, 2009-2023.

Smith, E. L. (1983). *Principles of biochemistry: Mammalian biochemistry*: McGraw-Hill.

Steenfos, H. H. (1994). Growth-factors and wound-healing. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery, 28*(2), 95-105.

Stephens, P., & Thomas, D. W. (2002). The cellular proliferative phase of the wound repair process. *Journal of Wound Care, 11*(7), 253-261.

Stevinson, C., Devaraj, V. S., Fountain-Barber, A., Hawkins, S., & Ernst, E. (2003). Homeopathic arnica for prevention of pain and bruising: Randomized placebo-controlled trial in hand surgery. *Journal of the Royal Society of Medicine, 96*(2), 60-65.

Strahan, R. (Ed.). (1995). *The mammals of Australia* (2nd ed.). Sydney: Reed New Holland.

Studier, E. (1972). Some physical properties of the wing membrane of bats. *Journal of Mammalogy, 53*, 623-625.

Subrahmanyam, M. (1991). Topical application of honey in treatment of burns. *British Journal of Surgery, 78*(For), 497-498.

Subrahmanyam, M. (1993). Honey impregnated gauze versus polyurethane film (opsite) in the treatment of burns - a prospective randomised study. *British Journal of Plastic Surgery, 46*, 322-323.

Subrahmanyam, M. (1997). A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulphadiazine. *Burns, 24*, 157-161.

Subrahmanyam, M. (2001). Effects of topical application of honey on burn wound healing. *Annals of Burns and Fire Disasters, XIV*(3).

Swartz, S., Groves, M., Kim, H., & Walsh, W. (1996). Mechanical properties of bat wing membrane skin. *Journal of Zoology, 239*, 357-378.

Sylvia, C. J. (2003). The role of neutrophil apoptosis in influencing tissue repair. *Journal of Wound Care, 12*(1), 13-16.

Tachi, M., Hirabayashi, S., Yonehara, Y., Suzuki, Y., & Bowler, P. (2004). Comparison of bacteria-retaining ability of absorbent wound dressings. *International Wound Journal, 1*(3), 177-181.

Tan, S., Roberts, R., & Sinclair, S. (1993). A comparison of zenoderm with duoderm e in the treatment of split skin graft donor sites. *British Journal of Plastic Surgery, 46*(1), 82-84.

Theoret, C. L. (2005). The pathophysiology of wound repair. *Veterinary Clinics of North America, Equine Practice, 21*, 1-13.

Theoret, C. L., Barber, S. M., Moyana, T. N., & Gordon, J. R. (2001). Expression of transforming growth factor ϐ$_1$, ϐ$_3$, and basic fibroblast growth factor in full-thickness skin wounds of equine limbs and thorax. *Veterinary Surgery, 30*(3), 269-277.

Thomas, D. R., Goode, P., LaMaster, K., & Tennyson, T. (1998). Acemannan hydrogel dressing vs saline dressing for pressure ulcers: A randomised controlled trial. *Advances in Wound Care, 6*, 273-276.

Thomas, S. (2008). Hydrocolloid dressings in the management of acute wounds: A review of the literature. *International Wound Journal, 5*(5), 602-613.

Thomas, S., Banks, V., Bale, S., Fear-Price, M., Hagelstein, S., Harding, K., Orpin, J., et al. (1997). A comparison of two dressings in the management of chronic wounds. *Journal of Wound Care, 6*(8), 383-386.

Tonnesen, M. G., Feng, X., & Clark, R. A. F. (2000). Angiogenesis in wound healing. *The Society for Investigative Dermatology, 5*(1), 40-46.

Tsuboi, R., & Rifkin, D. (1990). Recombinant basic fibroblast growth factor stimulates wound healing in healing-impaired db/db mice. *Journal of Experimental Medicine, 172*(1), 245-251.

van Rijswijk, L. (2001). Wound assessment and documentation. In D. Krasner, G. Rodeheaver & R. Sibbald (Eds.), *Chronic wound care: A clinical source book for healthcare professionals* (Vol. 3, pp. 101-115). Wayne, PA: HMP Communications.

Vermeulen, H., van Hattem, J., Storm-Versloot, M., & Ubbink, D. (2007). Topical silver for treating infected wounds. *Cochrane Database of Systematic Reviews, 1*. DOI:10.1002/14651858.CD005486.pub2

Viciano, V., Castera, J. E., Medrano, J., Aguiló, J., Torro, J., Botella, M. G., & Toldrá, N. (2000). Effect of hydrocolloid dressings on healing by second intention after excision of pilonidal sinus. *European Journal of Surgery, 166*(3), 229-232.

Vloemans, A. F. P. M., Soesman, A. M., Suijker, M., Kreis, R. W., & Middelkoop, E. (2003). A randomised clinical trial comparing a hydrocolloid-derived dressing and glycerol preserved allograft skin in the management of partial thickness burns. *Burns, 29*(7), 702-710.

Wahl, S. M., Hunt, D. A., Wakefield, L. M., McCartney-Francis, N., Wahl, L. M., Roberts, A. B., & Sporn, M. B. (1987). Transforming growth factor type ɓ induces monocyte chemotaxis and growth factor production *Proceedings of the National Academy of Sciences of the United States of America, 84*(16), 5788-5792.

Wang, M.-M., Thomson, M., Ali, M., Pace-Asciak, C. R., & Al-Hassan, J. M. (2001). Catfish gel extract causes increase in vascular permeability in the rat skin. *Inflammopharmacology, 8*(4), 397-403.

Wasiak, J., Cleland, H., & Campbell, F. (2008). Dressings for superficial and partial thickness burns. *Cochrane Database of Systematic Reviews, 4*. DOI:10.1002/14651858.CD002106.pub3

Webb, R., & Nicoll, P. (1944). Behaviour of lymphatic vessels in the living bat. *The Anatomical Record, 88*, 351-368.

Welch, M. P., Odland, G. F., & Clark, R. A. (1990). Temporal relationships of f-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *Journal of Cell Biology, 110*(1), 133-145.

Werner, S., Peters, K. G., Longaker, M. T., Fuller-Pace, F., Banda, M. J., & Williams, L. T. (1992). Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proceedings of the National Academy of Sciences of the United States of America, 89*(15), 6896-6900.

Wille, J. J., & Kydonieus, A. (2003). Palmitoleic acid isomer (c16:1d6) in human skin sebum is effective against gram-positive bacteria. *Skin Pharmacology and Physiology, 16*(3), 176-187.

Willix, D. J., Molan, P. C., & Harfoot, C. G. (1992). A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *Journal of Applied Microbiology, 73*(5), 388-394.

Wilmink, J. M., Stolk, P., Van weeren, P., & Barneveld, A. (1999). Differences in second-intention wound healing between horses and ponies: Macroscopic aspects. *Equine Veterinary Journal, 31*(1), 53-60.

Wilmink, J. M., Van weeren, P., Stolk, P., Van Mil, F., & Barneveld, A. (1999). Differences in second-intention wound healing between horses and ponies: Histological aspects. *Equine Veterinary Journal, 31*(1), 61-67.

Wilmink, J. M., & van Weeren, P. R. (2005). Second-intention repair in the horse and pony and management of exuberant granulation tissue. *Veterinary Clinics of North America, Equine Practice, 21*, 15-32.

Winter, G. D. (1962). Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature, 193*(4812), 293-294.

Winter, G. D. (1963). Effect of air exposure and occlusion on experimental human skin wounds. *Nature, 200*(4904), 378-379.

Winter, G. D., & Scales, J. T. (1963). Effect of air drying and dressings on the surface of a wound. *Nature, 197*(4862), 91-92.

Wu, M., Bao, B., Yoshii, F., & Makuuchi, K. (2001). Irradiation of crosslinked, poly (vinyl alcohol) blended hydrogel for wound dressing. *Journal of Radioanalytical and Nuclear Chemistry, 250*(2), 391-395.

Wyatt, D., McGowan, D., & Najarian, M. (1990). Comparison of a hydrocolloid dressing and silver sulfadiazine cream in the outpatient management of second-degree burns. *The Journal of Trauma: Injury, Infection, and Critical Care, 30*(7), 857.

You, Y., Park, W. H., Koh, B. M., & Min, B.-M. (2004). Effects of pva sponge containing chitooligosaccharide in the early stage of wound healing. *Journal of Materials Science: Materials in Medicine, 15*, 297-301.

Zaghloul, A. A., El-Shattawy, H. H., Kassem, A. A., Ibrahim, E. A., Reddy, I. K., & Khan, M. A. (2001). Honey, a prospective antibiotic: Extraction, formulation, and stability. *Pharmazie, 56*(8), 643-647.

Zheng, Q., Choi, J., Rouleau, L., Leask, R. L., Richardson, J. A., Davis, E. C., & Yanagisawa, H. (2006). Normal wound healing in mice deficient for fibulin-5, an elastin binding protein essential for dermal elastic fiber assembly. *Journal of Investigative Dermatology, 126*(12), 2707-2714.

8 Appendices

8.1 Appendix A Glossary and Abbreviations

8.1.1 Glossary

Term	Definition
Complete Healing	Refers to both the inflammation and granulation phases in combination - when the Completion of Granulationô point is reached.
Cytokines	Proteins secreted by cells and act on that cell or other cells to initiate or increase repair
Full flight	Flight capable of sustaining life in the wild e.g. the ability to gain height, manoeuvre and cope with strong winds.
Granulation Phase	Defined in this thesis to be the process following resolution of inflammation until functional repair (regeneration or scar)
Growth Factors	Biologically active polypeptides which enhance growth, differentiation or altered metabolism of the target cell
Completion of Granulation Point	Defined as the point at which the injury had either reached re-epithelialisation or functional scar.
Inflammation Phase	Defined in this thesis to be the processes from the time of injury to resolution of inflammation
Injury	Damaged tissue
Provisional ECM or Provisional Matrix	The initial ECM created during haemostasis and defined by Clark (1985)

Pteropery	Large aviary for bats
Soft release	A wildlife release method which provides shelter and food until the animal is able to sustain itself in an appropriate wild situation. Typically, and for this research, the animal is familiarised with a cage and then the door is opened. Food continues to be supplied until the animal is no longer returning. Monitoring is by visual check of the cage and / or by recording device.
Resolution of Inflammation Point	Defined as the point at which inflammation resolved and granulation began and was identified as the day half way between when new tissue was macroscopically visible and the last day on which it was not.
Wound	Open injury (broken epithelial layer)

8.1.2 Abbreviations and Acronyms

Abbreviation	Meaning
bFGF	Basic fibroblast growth factor
blo	Injury state: bone loss, see Table 3 Injury States
dld	Injury state: dermal layer damage, see Table 3 Injury States
ECM	Extra cellular matrix
eld	Injury state: epithelial layer damage, see Table 3 Injury States
fco	Injury state: full colour, see Table 3 Injury States
fep	Injury state: full epithelisation, see Table 3 Injury States
ffa	Injury state: fully functional area, see Table 3 Injury States
GC	Gas chromatography
gra	Injury state: granulation, see Table 3 Injury States

Abbreviation	Meaning
IL-1	Interleukin 1
KGF	Keratinocyte growth factor
ORC	Oxidised regenerative cellulose
PDGF	Platelet derived growth factor
rbs	Injury state: bruise, see Table 3 Injury States
sdd	Injury state: subdermal damage, see Table 3 Injury States
SSD	Silver sulphadiazine
swe	Injury state: swelling, see Table 3 Injury States
swr	Injury state: scar with re-epithelialisation, see Table 3 Injury States
$TGF_{\breve{U}}$	Transforming growth factor type Ŭ
$TGF_{ƀ}$	Transforming growth factor type ƀ
VGF	Vaccinia growth factor
wlo	Injury state: wing loss, see Table 3 Injury States

8.2 Appendix B Healing for Release

For flying-foxes three aspects of healing are required to achieve the aim of rehabilitation which is to release an animal to the wild with a high chance of survival.

These three aspects are:

- injury healing,
- wing healing, and,
- systemic readiness.

Injury Healing

Injuries consist of open (e.g. cuts, burns, abrasions, etc) and closed e.g. (bruises, haematomas, blisters) injuries and fractures. This research presented results from open and closed injuries. Fractures were attended to during the course of the research and have also been routinely managed by others e.g. Wildlife Assistance & Information Foundationôs Wildlife Veterinary Clinic (St Ives, Sydney) and the Austral Veterinary Clinic (Austral, Sydney, NSW). Fracture treatment for flying-foxes is well established.

Wing Healing

Once the injury has healed the whole wing must be healed. Most injuries healed without compromising the wing functionally. Occasionally an injury healed with scarring or membrane contraction that compromised flight. This must be corrected. An appropriate treatment protocol was developed by a specialist animal physiotherapist, Dr Helen Nicholson of Animal Physiotherapy Services at the Animal Referral Hospital - Strathfield, NSW Australia, see section 224.2.1 Physiotherapy Protocol.

Systemic Readiness

A flying-fox must be flight capable and fit to survive post-release. Injuries may result in problems to joints e.g. due to contraction of the wing membrane. Captivity may result in unacceptable loss of muscle fitness or willingness to fly. Joint and muscle damage were treated by exercises and physiotherapy as defined on a per individual basis by Dr Helen Nicholson of

Animal Physiotherapy Services. Muscle development was achieved by flight training see section 224.2.1 Physiotherapy Protocol.

8.2.1 Physiotherapy Protocol

A healed injury is necessary but not sufficient for a flying-fox to return to the wild. Normal wing membrane is strong and elastic (see section 2.3.3 Micro Anatomy ï Megachiropteran Wings). Sometimes healing produced a scar causing non-functional contraction of the wing. This required physiotherapy including special exercises, massage and / or stretching.

The physiotherapy protocol was started as soon as granulation was stable. Two main exercises were: a rolling massage of the healing edge, contraction area or scar and a pulling stretch across the contraction area. Both exercises were carried out for two minutes per session. Further, even with functional healing, when a flying-fox lost too much condition to be able to fly it required flight exercise until an acceptable level of strength returned.

The most common form of scarring occurred at a wing edge and caused contraction of the wing, see Figure 35. Occasionally this contraction caused two digits to fuse requiring minor surgery to correct. Also occasionally, when contraction caused problems around a joint special joint exercises were carried out under the supervision of animal physiotherapist Dr Helen Nicholson (see above).

Figure 35 Scar contraction. White oval indicates the contraction of the wing membrane due to a scar

For most cases a simple rolling massage and a two minute stretch at least daily were sufficient. Macadamia oil was used when massaging. Flying-foxes were restrained by wrapping as previously described and generally tolerated the massage well.

Flight exercise consisted of forcing the flying-fox to fly. Holding up a flying-fox and expecting it to fly is ineffective. The flying-fox had to learn what was expected and be confident it could perform. To achieve this, the flying-fox was thrown either to another handler or to a hanging hessian bag. This gave the flying-fox sufficient momentum to remain airborne, see Figure 36, even when the wings and muscles functioned poorly. As the flying-foxôs strength and competency improved the distance and the number of throws per session were increased and the amount of ñpushò given to the flying-fox was decreased until all the power was supplied by the

wings. At this point the handler was merely releasing the grip on the flying-fox held at a sufficient height to gain lift before hitting the ground. These exercises occurred at least daily. Sometimes a collar and lead were put on the flying-fox and it was flown from the lead. Note: Not all wild flying-foxes will fly with a collar and lead attached.

Occasionally a flying-fox which appeared fully flight capable would not fly. They seemed afraid to fly, possibly associating flight with pain. In these cases it was necessary to ensure all flights ended well to build self confidence. Moving the flying-fox to another situation, preferably with other flying individuals also assisted the resumption of flight.

To be considered ready for release a flying-fox must be able to gain height, manoeuvre and fly strongly.

Figure 36 Flying-fox in flight training. This flying-fox is in flight between two handlers shortly before final release. She had originally lost all of one pro-patagium and had extensive membrane damage to other areas of the patagium on both wings. She was treated with macadamia oil.

8.3 Appendix C Full Plate Photography

Table 16 Plates of Photographs Included in the Thesis

The following photographs are large size versions of images in the body of the document. They are, therefore captioned with the originating figure or table label.

Original Table / Figure Reference	Plate Number
Table 2	
Patagium	I
Leading Edge	I
Finger bone	II
Joint	II
Arm	I
Table 3	
Bruise	III
Swelling	V
Epithelial layer damage (erosion)	III
Dermal layer damage (ulcer)	IV
Subdermal damage	VI
Fully functional area	VII
Full epithelialisation	V
Full colour	VII
Granulation	IV
Scar with re-epithelialisation	VIII and VI
Bone loss	IV
Wing loss	VIII
Figure 19	IX
Figure 20	X
Figure 21	XI
Figure 22A	XI
Figure 22B	IX
Figure 24	X
Figure 29	XII
Figure 30	XII
Figure 31	XIII
Figure 32	XIII
Figure 33	XIII
Figure 37	XII


Table 2 Patagium


Table 2 Leading Edge
Elbow
Propatagium
Leading Edge

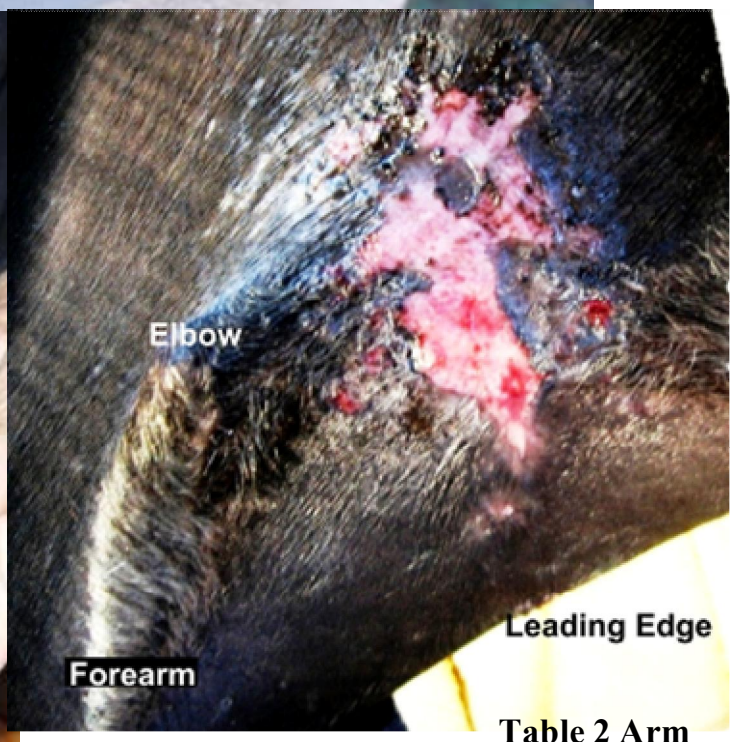

Elbow
Leading Edge
Forearm
Table 2 Arm

Plate I

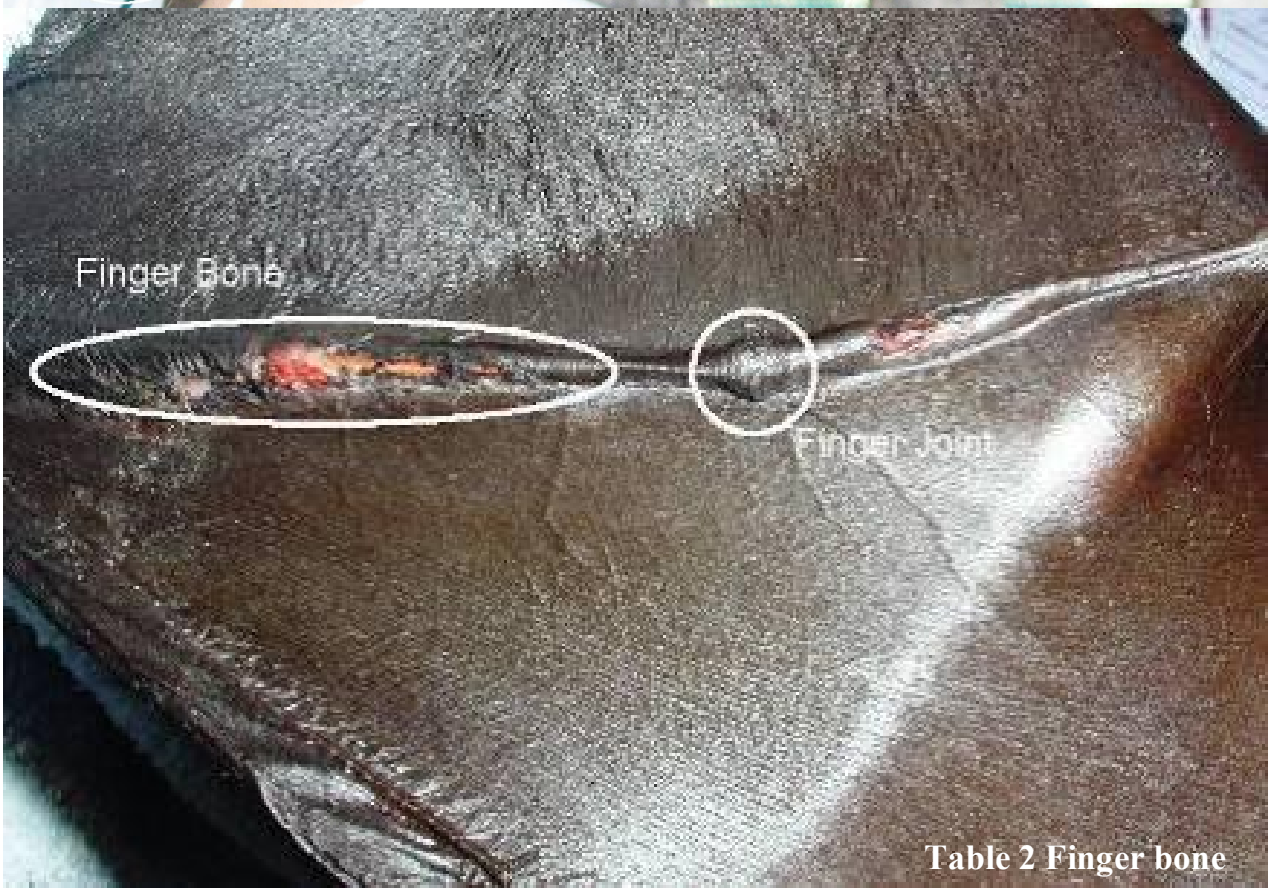


Plate II

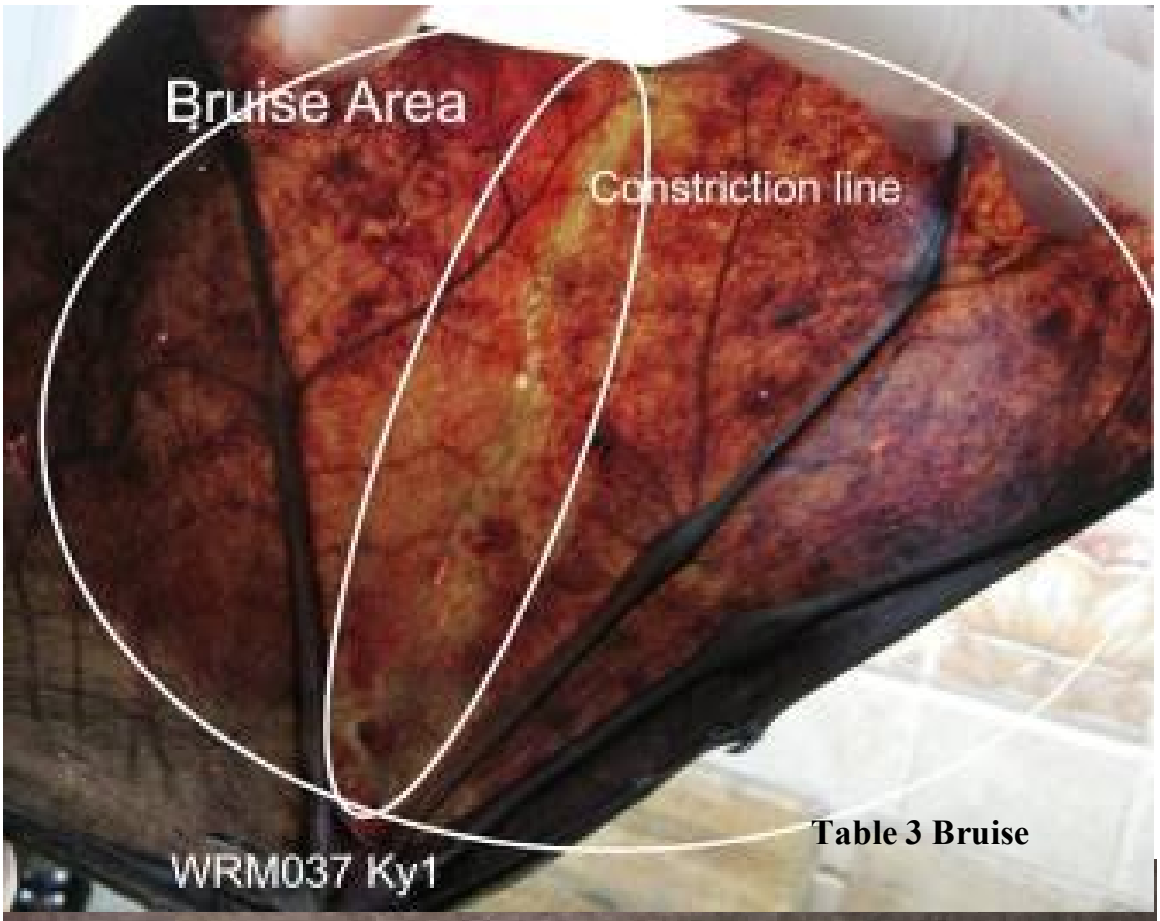


Table 3 Bruise

Table 3 Epithelial layer damage

Plate III

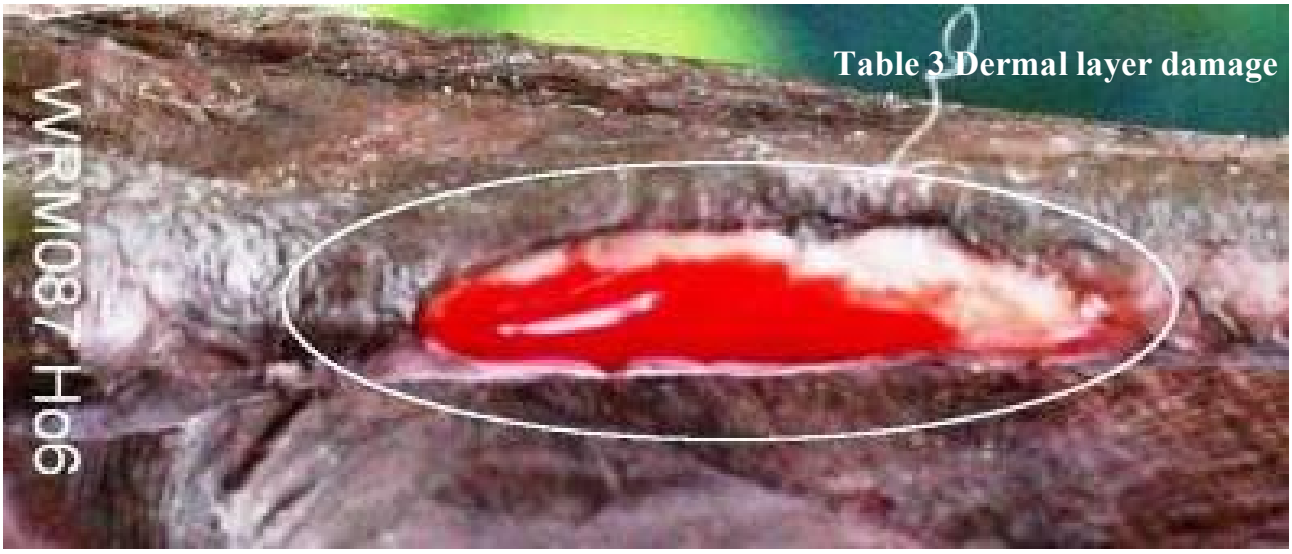


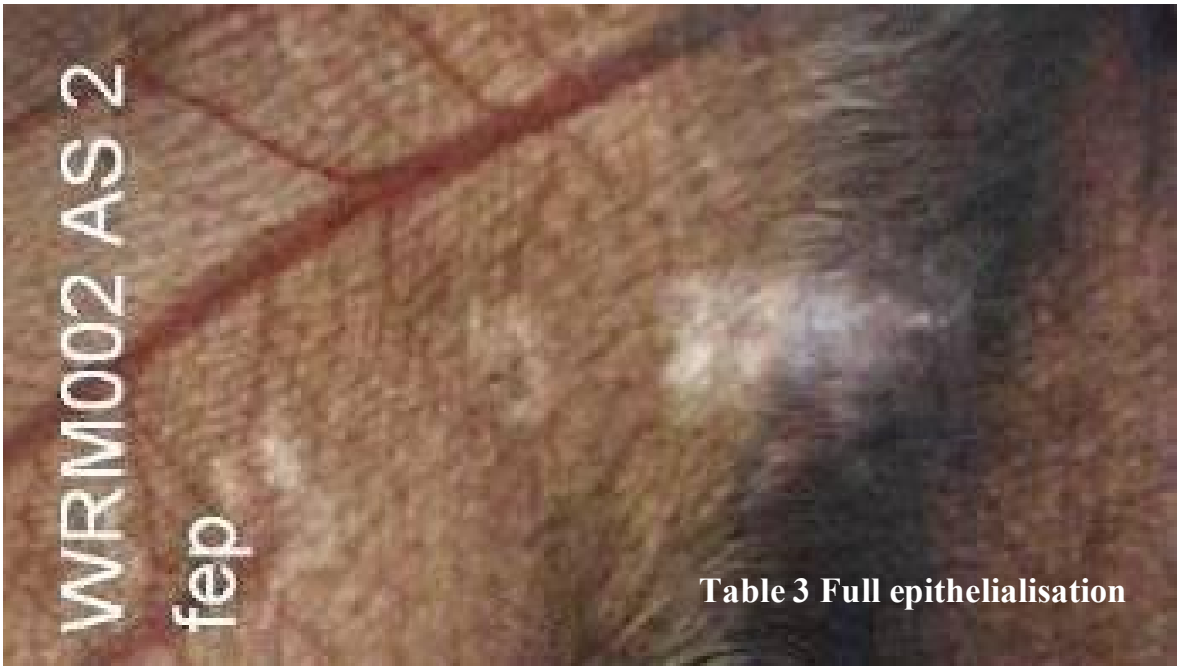
Table 3 Dermal layer damage
WRM087 Hp6


Table 3 Bone loss


WRM005 Bc5b
gra
Table 3 Granulati

Plate IV


WRM045 EI1 and EI2
Blisters
Table 3 Swelling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fep
Table 3 Full epithelialisation

Plate V

Table 3 Subdermal damage

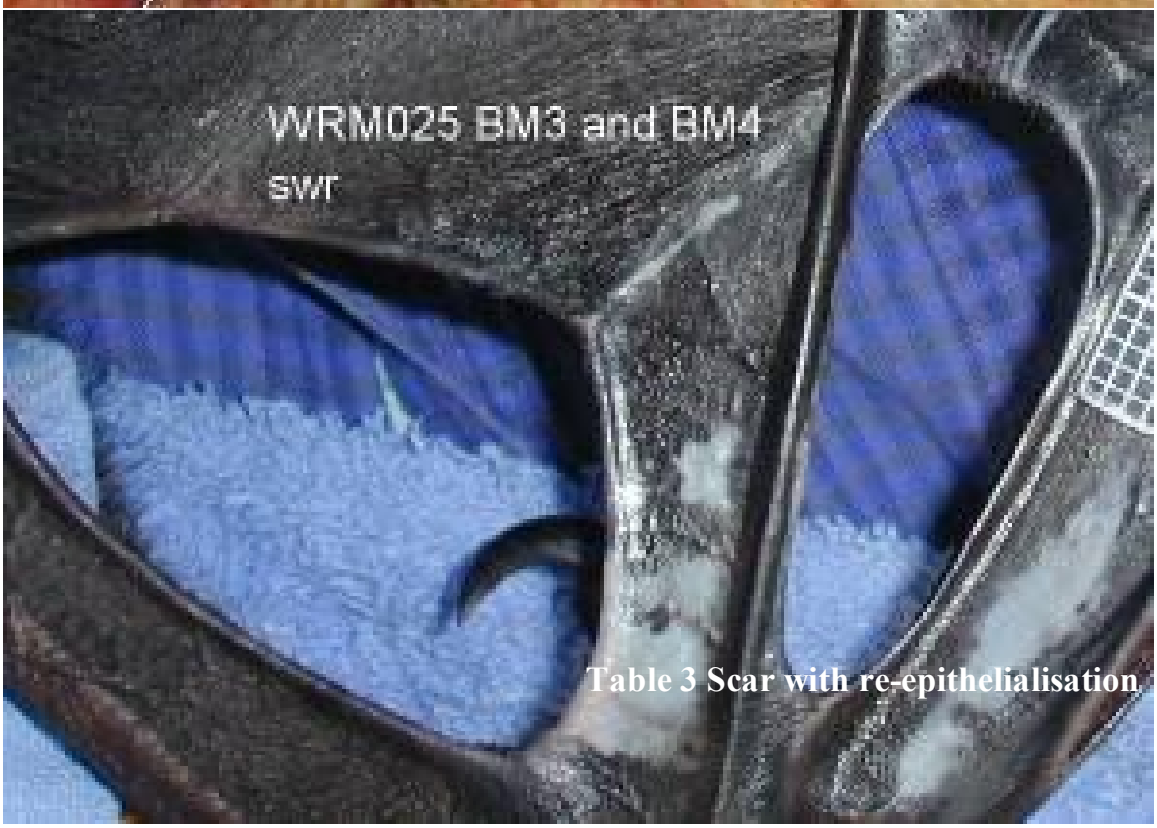

Table 3 Scar with re-epithelialisation

Plate VI


WRM073 Ge11
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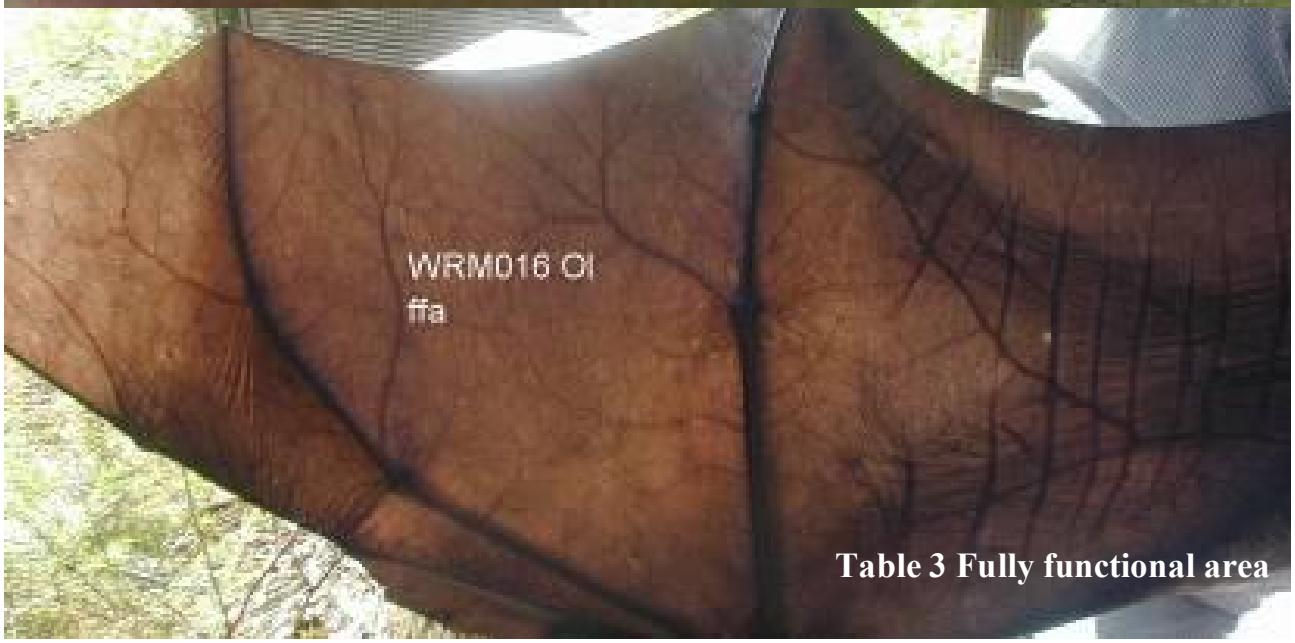
Table 3 Full colour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ffa
Table 3 Fully functional area

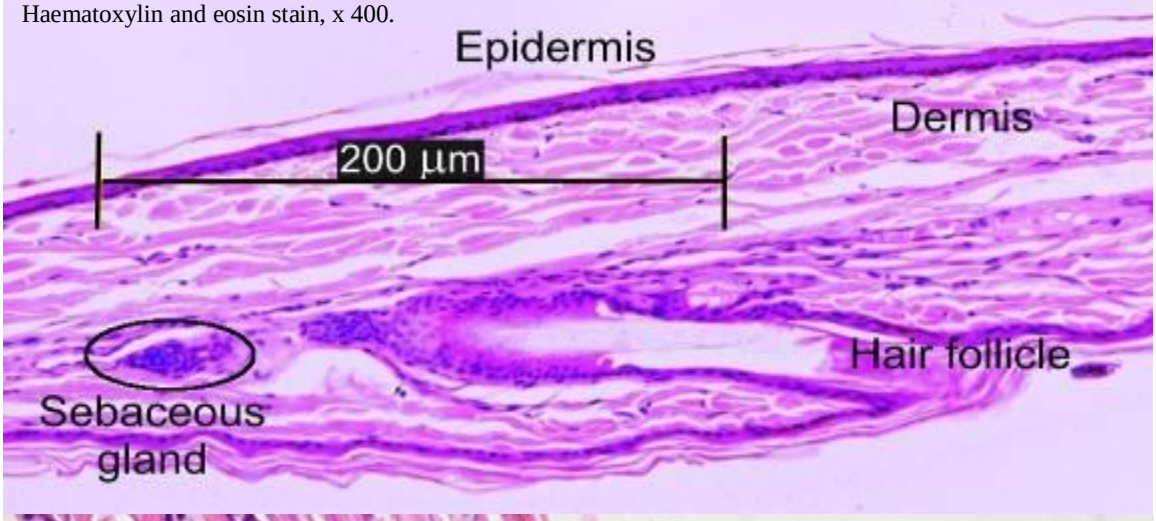
Plate VII

Table 3 Scar with re-epithelialisation

Table 3 Wing loss

Plate VIII

Figure 19 Cross-section of normal wing membrane.
Haematoxylin and eosin stain, x 400.



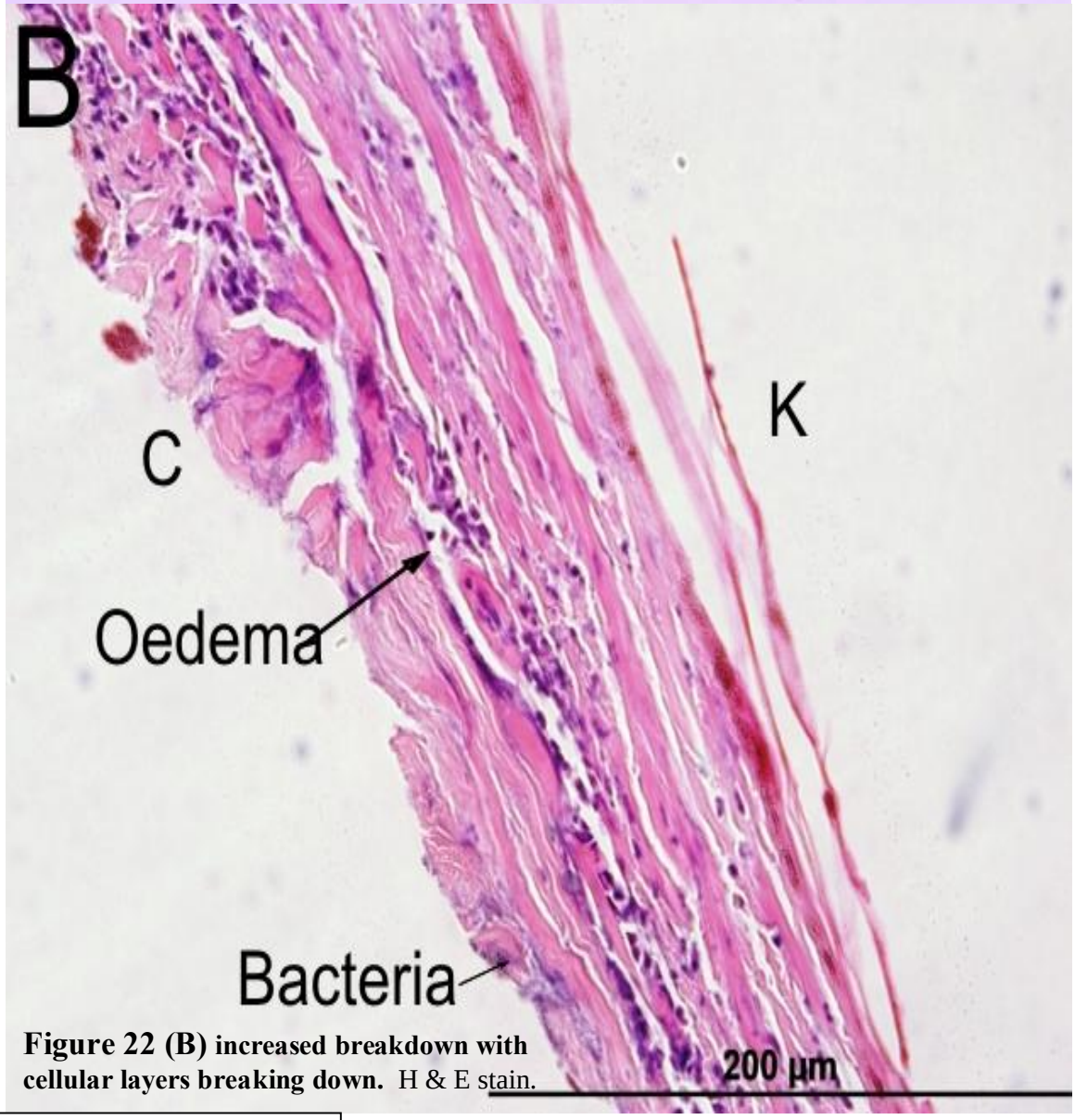


Figure 22 (B) increased breakdown with cellular layers breaking down. H & E stain.

Plate IX

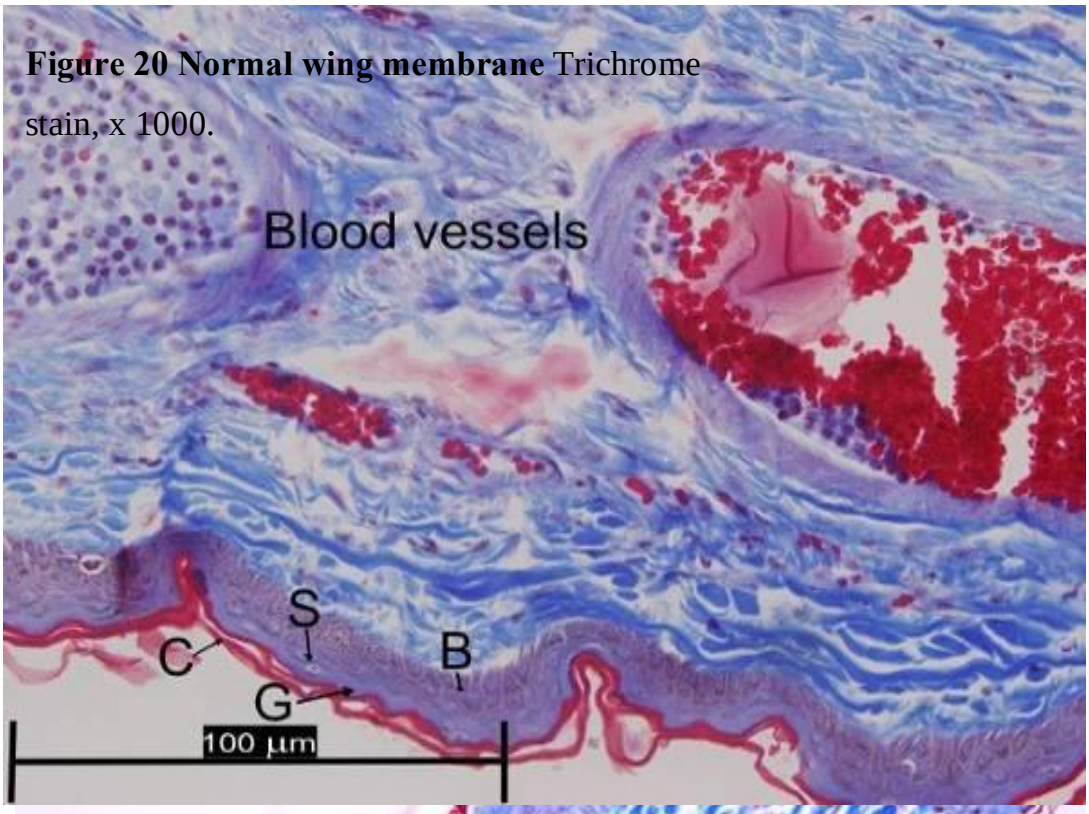


Figure 20 Normal wing membrane Trichrome stain, x 1000.

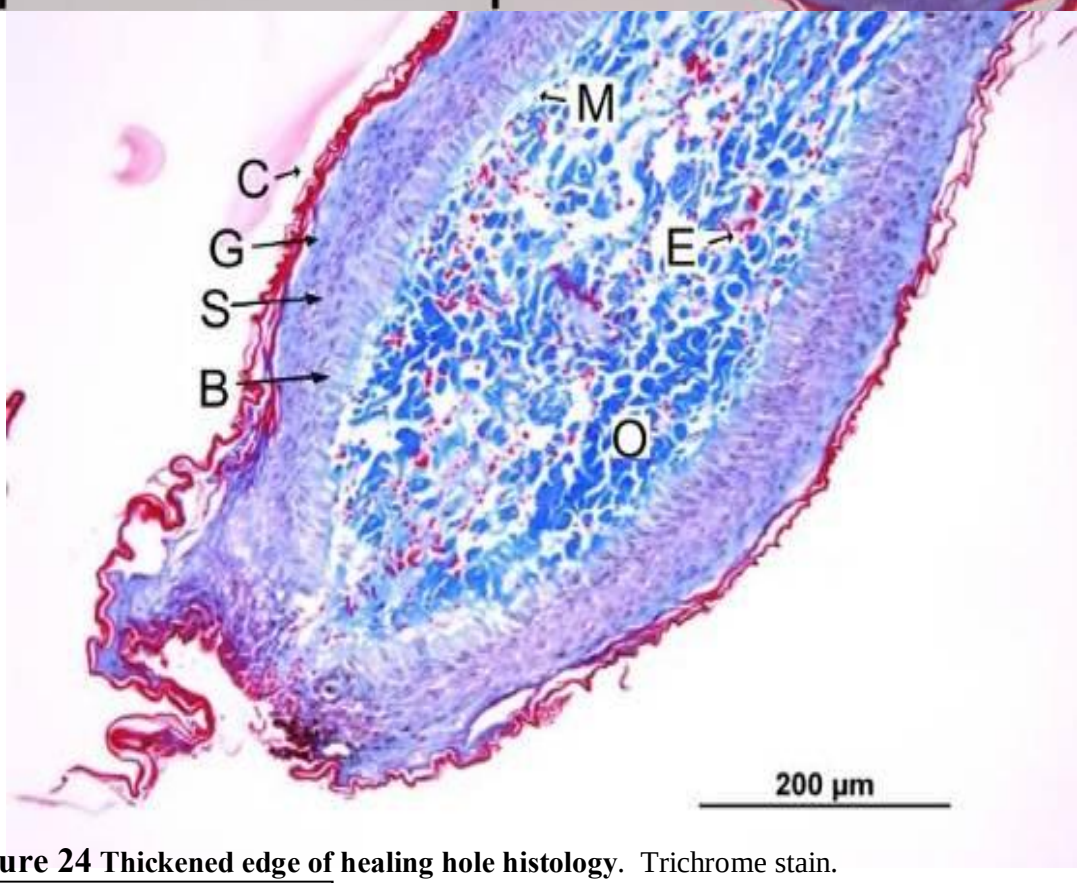


Figure 24 Thickened edge of healing hole histology. Trichrome stain.

Plate X

Figure 21Wing patagium with membrane breaking down.

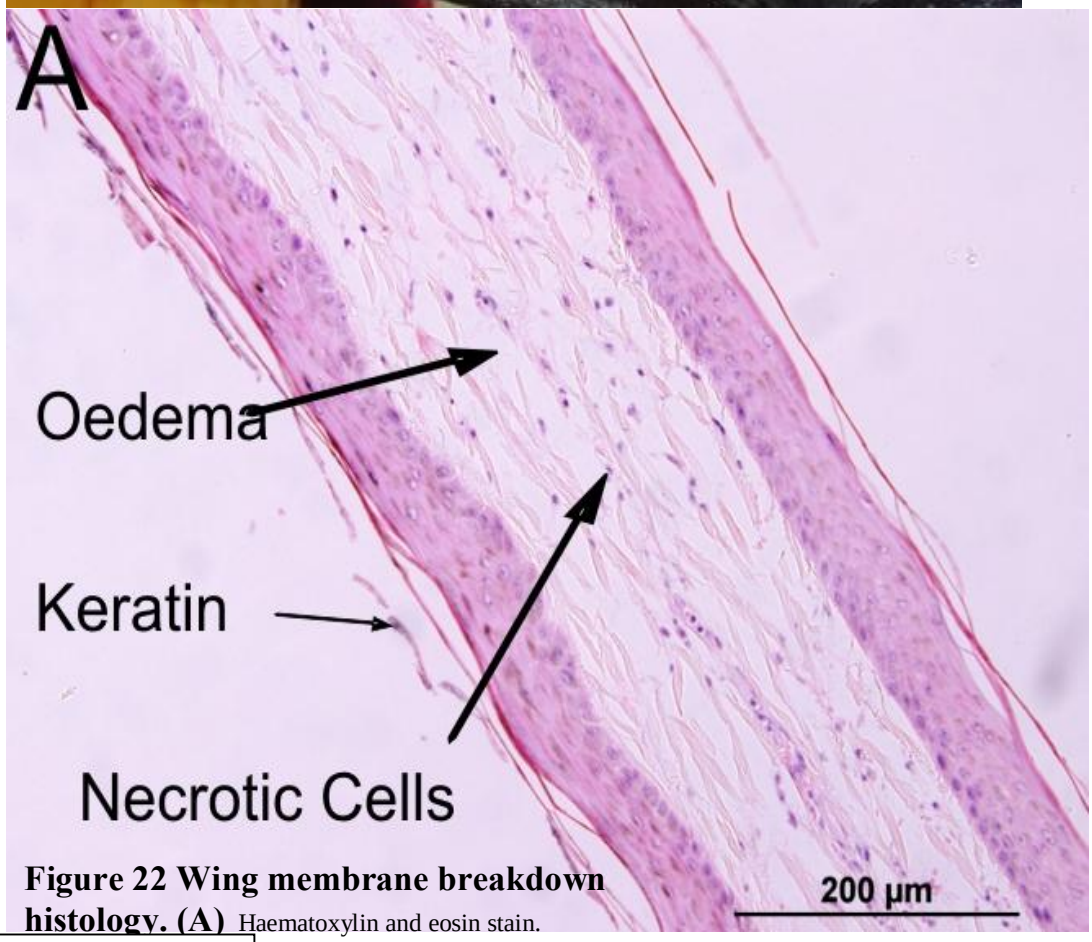


Figure 22 Wing membrane breakdown histology. (A) Haematoxylin and eosin stain.

Plate XI

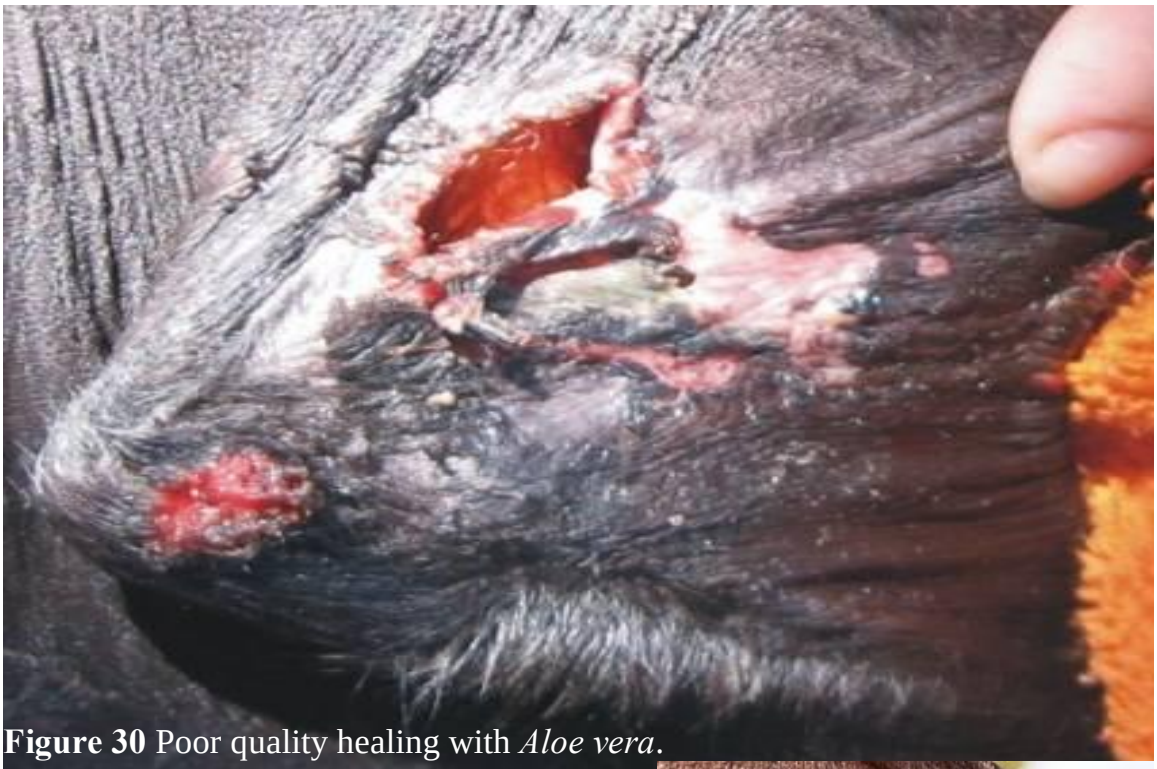


Figure 30 Poor quality healing with *Aloe vera*.

Figure 37 Two simple injuries coalescing into a complex injury.

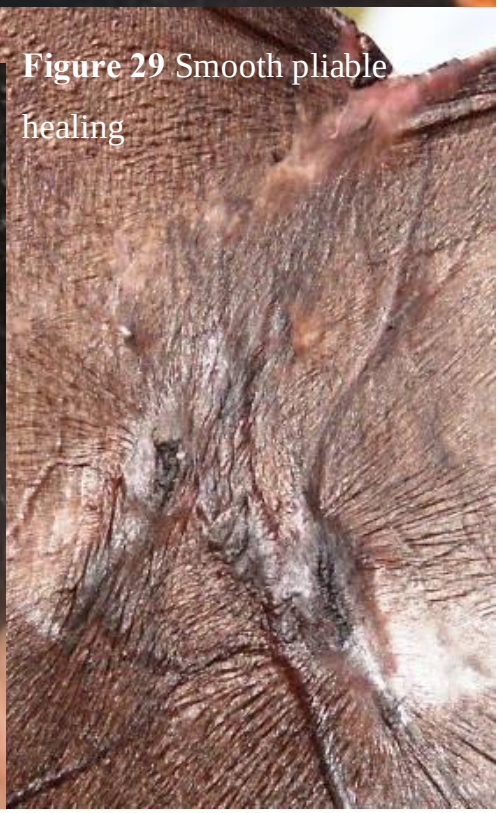


Figure 29 Smooth pliable healing

Plate XII

Figure 32 Over-granulation at a joint.

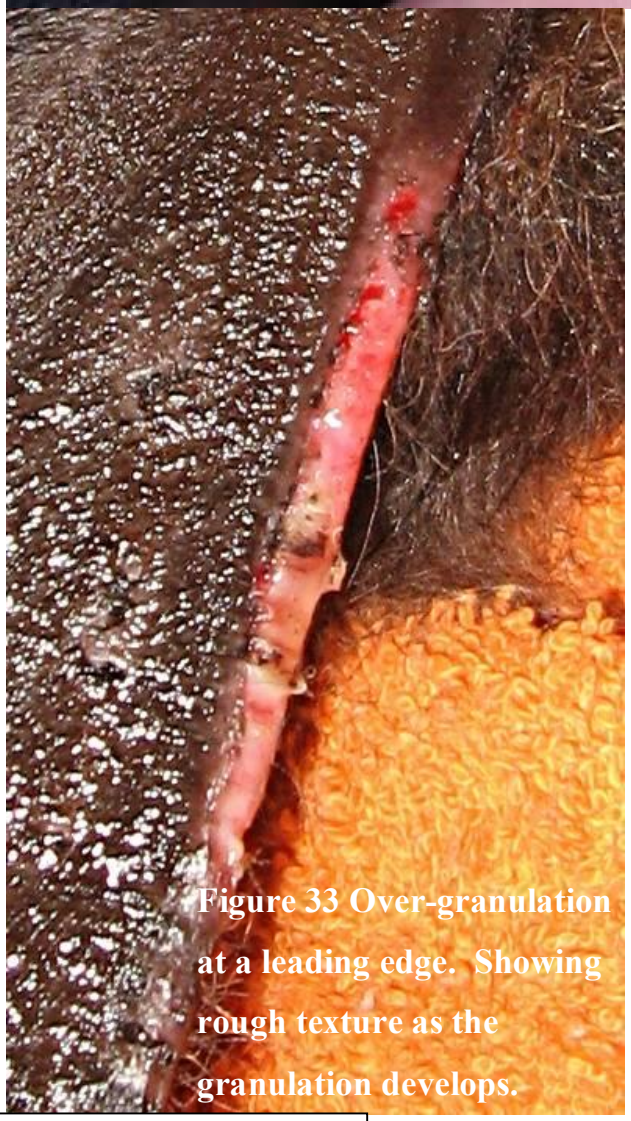


Figure 33 Over-granulation at a leading edge. Showing rough texture as the granulation develops.

Figure 31 Over-granulation along finger bone.

Plate XIII