CHAPTER II

LITERATURE REVIEW

2.1 MAGGOT THERAPY

Maggot therapy, also known as maggot debridement therapy (MDT), larval therapy, biodebridement (Jones and Wall 2008) or biosurgery (Jaklic 2008), is a type of biotherapy involving the intentional application of live, disinfected fly larvae or maggots into the non-healing wound of a human (Robinson and Norwood 1933; Thomas et al. 1999; Mumcuoglu et al. 2001; Husain and Fallat 2003) or animal (Sherman et al. 2007a; Sherman et al. 2007b; Jones and Wall 2008) to debride the necrotic wound (Ziffren et al. 1953; Vistnes et al. 1981; Mumcuoglu et al. 1999), reduce bacterial contamination of the wound (Simmons 1935; Thomas and Jones 2000) as well as enhance the formation of healthy granulation tissue (Jones and Wall 2008) and stimulate healing in non-healing wounds (Robinson 1935; Prete 1997). In addition, Jukema et al. (2002) reported that the use of the medicinal larvae as natural remover of necrotic and infected tissue had prevented amputation in 11 selected patients.

2.1.1 History of Maggot Therapy

Certainly, maggot therapy is not a new discovery; the therapeutic effects of maggots upon the healing of chronically infected wounds have been documented in the English literature for hundreds of years (Prete 1997; Sherman *et al.* 2000). In the late 1920s, Dr William Baer, a World War I battlefield orthopaedic surgeon accidentally observed that war wounds that had been infested by the larvae of blow fly healed remarkably well without complications (Baer 1931). As a result of these observations and also successful clinical trials in osteomyelitis patients (Baer 1931), maggot therapy was routinely and extensively used with high success rates in the management of chronic wounds and osteomyelitis in the 1930s and early 1940s (Mumcuoglu *et al.* 2001; Nuesch *et al.* 2002).

In 1940s, with the advent of antimicrobial drugs and the use of aggressive surgical debridement, the employment of maggot debridement therapy in treating intractable wounds soon declined (Thomas *et al.* 1999; Nuesch *et al.* 2002; Kerridge *et al.* 2005). However, in the late 1980s and 1990s, maggot therapy has been reintroduced for the treatment of untreatable wounds in United States (Sherman *et al.* 1995), Great Britain (Thomas *et al.* 1996) and Israel (Mumcuoglu *et al.* 1998, 1999), primarily in response to the emergence of antibiotics-resistant strains of bacteria and search for suitable method to treat chronic ulcers (Sherman *et al.* 1995; Kerridge *et al.* 2005). Sterile maggots of *L. sericata* are now being produced in and supplied by specialist centres in the USA, the UK, Germany, Israel, the Ukraine and Hungary (Thomas *et al.* 1999).

2.1.2 Maggot Therapy in Malaysia

In 2003, the Institute for Medical Research (IMR), Ministry of Health, Malaysia has established the sterilization procedures of *L.* cuprina eggs and first-instar larvae; as well as conducted the first clinical trial of maggot therapy in the Lumut Navy Hospital, Perak on a patient with intractable diabetic leg ulcer. After three days of *L. cuprina* larval therapy, appearance of fresh tissue can be noticed on the diabetic patient. The patient was discharged on the third week after three treatments. Since May 2006, 27 diabetic patients with intractable ulcers from Lumut Navy Hospital were also treated with classical application of *L. cuprina* larvae, and the results were very encouraging. All of them responded well after three to seven treatments, their ulcers were completely debrided and healed without complication. These outcomes have proven the therapeutic effect of *L. cuprina* larvae as well as the effectiveness of eggs and maggots sterilization methods used in the preparation of sterile, medical grade larvae.

Subsequently, a case controlled study was done from December 2005 to May 2007 by Dr. Aaron Paul from the Department of Orthopaedics and Traumatology, Hospital Kuala Lumpur (HKL) in collaboration with IMR to assess the effectiveness of *L. cuprina* larval therapy for the treatment of diabetic foot ulcers, in comparison with conventional debridement (surgical debridement). In his study, 25 patients with infected diabetic foot wounds from the orthopaedics wards in HKL were treated with *L. cuprina* larval therapy whilst another 29 patients (control group) were treated by conventional debridement. The study outcome proved that MDT with *L. cuprina* larvae was as effective as conventional debridement in treating diabetic foot ulcers but in MDT-treated patients, the ward-stay time and amputation rate was reduced (Paul *et al*. 2009).

In 2007, in corporation with a local private company (Edaran Arif Medik Sdn Bhd), IMR has commercially produced and supplied sterile *L. cuprina* first-instar larvae to clinics and hospitals initially in the Klang Valley like Kuala Lumpur, Serdang and Sungai Buloh; and now throughout Malaysia to treat patients with intractable acute and chronic wounds. Figure 2.1.2 illustrates the application of medicinal *L. cuprina* larvae on the right leg of a 52-year-old diabetic woman and the progression of her intractable wound towards healing.

2.1.3 *Lucilia cuprina* Larvae - The Candidate for Maggot Therapy

In the western regions, the larvae of the green bottle, *L. sericata* (Meigen) (synonym: *Phaenicia sericata*) have been produced aseptically and commercially in large numbers for maggot debridement therapy and research purposes (Thomas *et al.* 1999; Nigam *et al.* 2006).

In contrast in Malaysia, *L. cuprina* (Wiedeman) larvae are employed as the medicinal maggots for maggot therapy. This local blowfly species is also known as the Australian sheep blowfly or green bottle fly. It is the sister species of *L. sericata* and therefore, it is virtually identical to *L. sericata* but occurs in drier areas. The identification between them requires microscopic examination of two main distinguishing characteristics. As opposed to *L.* sericata which has a blue-black femoral joint in the first pair of legs, *L. cuprina* is metallic green. Also, when looking at the occipital setae, *L. cuprina* has only one bristle on each side while *L. sericata* has six to eight (Bishop 1991). Besides, they each exhibit specific genetic variations which can be distinguished by using random amplified polymorphic DNA and/or mitochondrial DNA sequences (Stevens and Wall 1997).

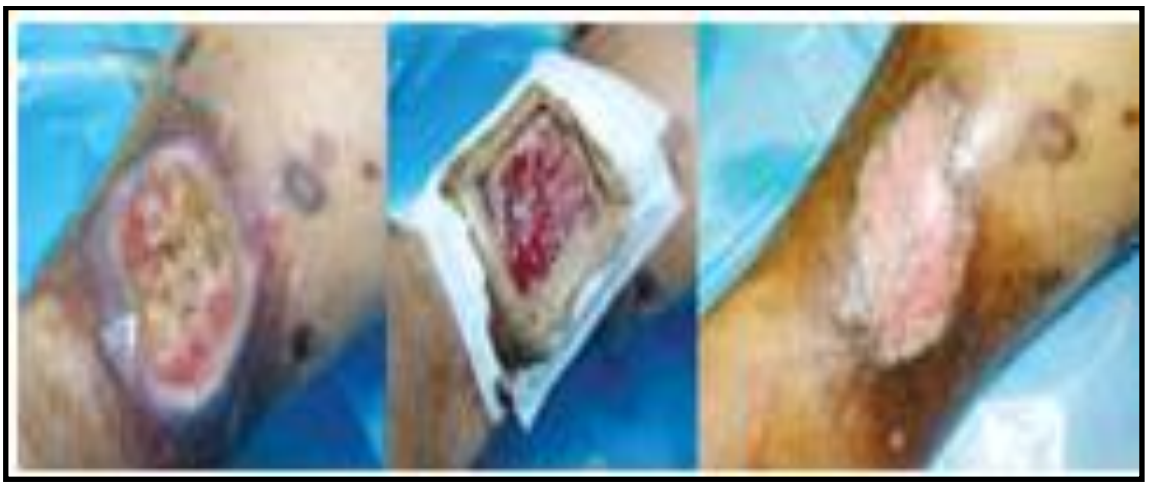

Figure 2.1.2: Condition of a 52-year-old diabetic woman's wound before, during and after undergoing maggot therapy

The larvae of *L. cuprina* are chosen as the therapeutic maggots for wound therapy in Malaysia because the temperate blowfly, *L. sericata* is not available in Malaysia. Besides, the selective debridement of *L. cuprina* larvae on necrotic tissue has made it a good alternative candidate for maggot therapy.

Laboratory colonies of the adult fly of *L. cuprina* are successfully established in the insectarium of IMR using overnight tap water and granular sugar. The complete life cycle is approximately 24 days and the adult fly can live for approximately five to six weeks. Before egg-laying, the female fly needs to feed on protein (raw cow liver) for the maturation eggs. The female fly is ready for egg-laying after approximately four days of protein intake. Following this, the female blowfly has the capacity to lay several batches of eggs during her life-time at which up to 200 eggs can be laid in each single batch.

Eggs usually hatch into larvae in 4 - 24 hours. During the larval period (the main feeding stage of the fly), *L. cuprina* larvae are fed with raw cow liver and moistened mouse pellet (Figure 2.1.3 a). The front ends of larvae are armed with mouth hooks with which they rake in decaying or dead flesh. Their rears consist of a chamber, in which their anus and posterior spiracles are located. Spiracles are used for breathing and the possession of the posterior spiracles enables the larvae to breathe-feed vertically 24 hours a day (Figure 2.1.3 b). Between the anterior and posterior is a muscular, segmented body, a simple intestine and a pair of very large salivary glands. The larvae will normally take eight days to transform into pupae and subsequently emerge as adult blowflies after a pupal period of six to seven days.

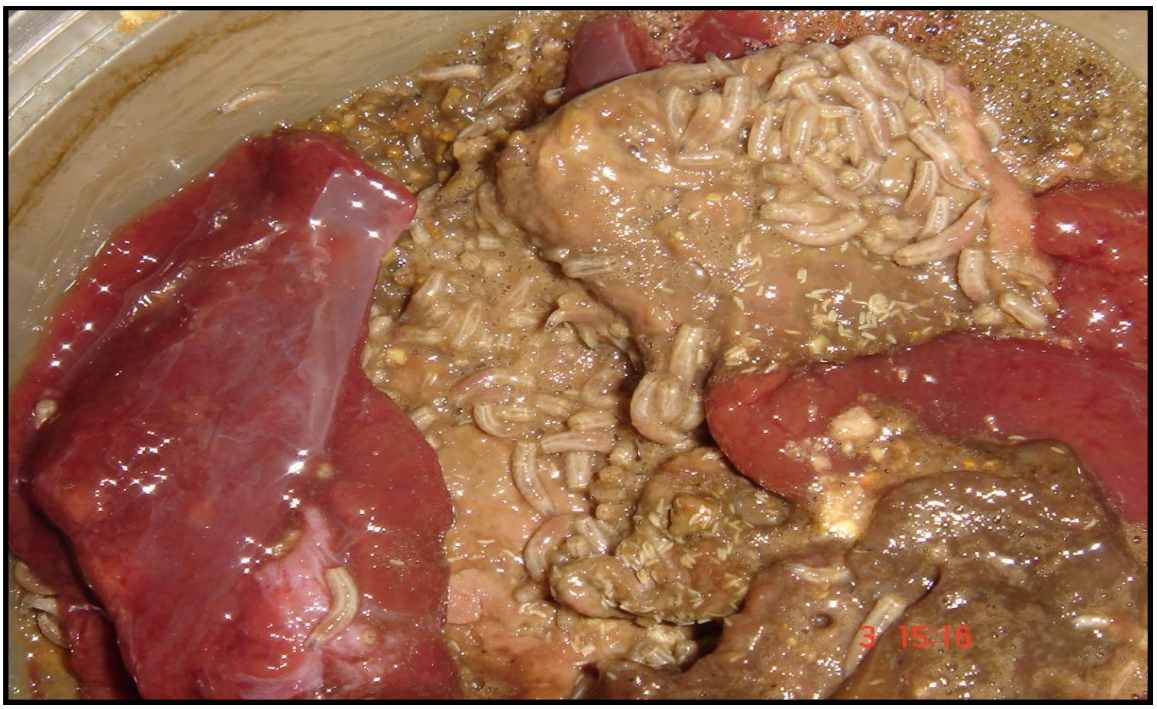

Figure 2.1.3a: Two-day old *Lucilia curpina* larvae fed on fresh, raw cow liver and mouse pellet

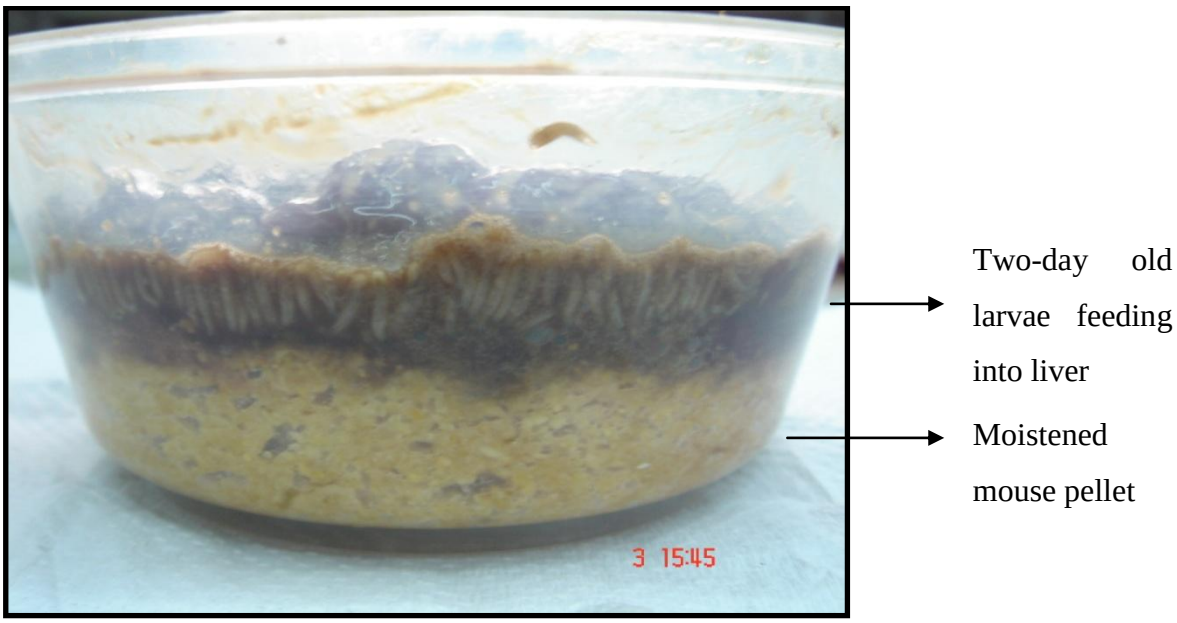

Figure 2.1.3b: Cross-sectional view of the vertically feeding *L. cuprina* larvae

2.1.4 Synergistic Therapeutic Mechanisms of Maggot Therapy

The functions of the therapeutic larvae or maggots are identical to the conventional wound debridement techniques (surgical debridement, mechanical debridement, enzymatic debridement and autolytic debridement), which involve debridement, disinfection and promotion of wound healing (Beasley and Hirst 2004; Bexfield *et al.* 2004; Sherman *et al.* 2007; Jones and Wall 2008).

Debridement is the removal of cellular debris, non-viable necrotic tissue and damaged ECM components which act as a microbial substrate in the wound bed. It is the first, critical step of wound care as the presence of devitalized tissue can impede the wound healing process. Debridement occurs as the larvae selectively ingest necrotic tissue and purulent materials by secreting proteolytic enzymes including collagenase (Ziffren *et al.* 1953), carboxypeptidases A and B, leucine aminopeptidase (Vistnes *et al.* 1981), trypsin-like and chymotrypsin-like enzymes (Chambers *et al.* 2003) to catalyze the breakdown of dead tissue on the wound which is then digested, leaving healthy, viable tissue exposed, and thus making the wound environment less suitable for bacterial growth (Robinson and Norwood 1933). The ability of the larvae to fully debride wounds appears superior to that of the surgeons as they cause minimal disruption of normal tissue planes and are able to selectively remove necrotic tissue on a microscopic scale (Beasley and Hirst 2004).

On the other hand, disinfection takes place as the medicinal larvae (1) move on the wound surface and as a result, the irritation promotes the formation of a serous exudate by the wound and eventually causes continuous wound lavage and dilution of bacterial concentration (Mumcuoglu *et al.* 1999) as well as (2) unselectively ingest bacteria on the wound bed and destruct the ingested bacteria in the alimentary tract

(Robinson and Norwood 1934; Mumcuoglu *et al.* 2001) as part of the natural feeding process (Bowler *et al.* 2001). A study conducted by Lerch *et al.* (2003) has demonstrated that the ingestion and digestion of bacteria were accomplished within minutes. Additionally, (3) the secretion of antibacterial compounds such as phenylacetic acid and phenylacetaldehyde by the larval gut commensal *Proteus mirabilis* (Erdmann and Khalil 1986) also helps in the rapid elimination of pathogenic microorganisms. Together, these broad-spectrum antibacterial actions result in the rapid elimination of a wide range of pathogenic bacteria from the infected wounds and therefore accelerate healing.

Furthermore, it is believed that the movement of the larvae on the wound irritates healthy tissue and thus stimulates the formation of granulation tissue to enhance healing. Besides, substances in the larval excretions/secretions (ES), such as allantoin and urea (Pelle *et al.* 2001) aid healing process by providing an optimal environment for the growth of cells. Allantoin is the principal terminal product of purine metabolism which resulted from the oxidation of uric acid by uricase and it is also a constituent of the urinary secretions of plants and animals below man, including the larvae of *L. sericata* (Robinson 1935). These alkaline substances have been reported to involve in the promotion of wound healing (Rayner 1999).

In addition, *in vitro* studies have shown that haemolymph and alimentary secretion of maggots can stimulate human fibroblasts and hence promote the wound healing process (Prete 1997). Moreover, another *in vitro* study performed by van der Plas *et al.* (2007) has shown that larval ES potently inhibited the pro-inflammatory responses of phagocytes including chemotaxis, degranulation, respiratory burst and integrin expression that may accelerate progression towards on-going inflammation and

tissue damage in chronic wounds by excess production of bioactive substances like proteinases and reactive oxygen species. Besides, Wollina *et al.* (2002) concluded that the positive effect of substances within larval ES on wound healing is due to the enhanced development of granulation tissue and increased tissue oxygenation.

Recently, Bexfield *et al.* (2010) have identified three amino acid-like compounds from *L. sericata* larval ES as histidine, valinol and 3-guanidinopropionic acid and demonstrated the proliferative effect of these substances on the growth of human endothelial cells. This observation suggesting that the amino acid-like compounds present in larval ES may contribute to the therapeutic effects of maggot therapy via increased angiogenesis.

As a whole, it is the collective beneficial effect of the medicinal larvae that attributed to the rapid healing of intractable wounds in maggot therapy. However, the number of larvae applied for maggot therapy varies with type and size of wound as well as the bacterial inoculums on the infected wound.

2.2 BACTERIA

Bacteria are a large group of single-celled prokaryotes which have neither a cell nucleus nor other membrane-bound organelles such as mitochondrion and chloroplast. Unlike multicellular organisms, they grow to a fixed size and then reproduce asexually into identical clone daughter cells through binary fission (Black 2004). Bacteria are one of the microorganisms that can be found almost anywhere in the taxanomic organization of life on the earth. There are approximately $4.0 – 6.0 \times 10^{30}$ bacteria on earth, forming much of the world's biomass (Whitman *et al*. 1998).

Bacteria (formerly known as *eubacteria*), *archaea* and eukaryotes constitute the three-domain system, which is a taxonomic level above the traditional five-kingdom system of classification (Campbell and Reece 2002) and currently it is the most widely used classification system in microbiology. Bacteria and *archaea* have evolved independently from an ancient common ancestor but the *archaea* and eukaryotes are more closely related to each other than to the bacteria.

Basically, the classification of bacteria is based on cell structure, cellular metabolism, nutritional requirements, physiology, biochemistry or differences in cellular components such as DNA, fatty acids, pigments, antigens and quinines (Black 2004). There are three most common bacterial cell shapes or morphology being spheres (cocci), rods (bacilli) and helices (Campbell and Reece 2002). The microscopic examination of bacterial morphology is an important step in the identification of bacteria. Additionally, staining reaction, particularly the Gram staining is amongst the first properties other than morphology to be used in the classification of bacteria. In 1884, Hans Christian Gram developed the Gram staining technique to characterize and classify bacterial species based on the structure of bacterial cell walls. The Gram-positive bacteria with thick layers of peptidoglycan in their cell wall are able to withstand the alcohol-decolorization process and retain the purple dyes (crystal violet), whist alcohol-decolorization against the Gram-negative bacteria removes the purple dyes trapped within the thin peptidoglycan layer and subsequent counter-staining with phenol-red makes them appear pink (Black 2004).

By combining the Gram-staining and morphology, bacteria can be generally classified into Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci and Gram-negative bacilli. However, further biochemical tests that based on bacterial

metabolisms or serology tests that based on the presence of bacterial antigens are required for the identification of bacteria to a particular genus and species. From the clinical aspect, timely identification of causative pathogenic bacteria is imperative in facilitating early and appropriate treatment of a bacterial infection.

Like any other living organisms, bacteria need nutrients for energy generation and synthesis of enzymes, proteins, nucleic acids and cellular components. Nutrients needed by bacteria include carbon, nitrogen, sulphur, phosphorus, certain trace elements and vitamins. However, some bacteria often require a variety of vitamins and are thus able to grow and reproduce only when they can obtain these substances from the host organisms. Such bacteria often interact in groups with other bacterial species or eukaryotes like human, animals, plants and fungus via symbiosis.

2.2.1 Symbiosis of Bacteria with Other Organisms

Symbiosis implies complex ecological relationships between organisms of different species that are in direct contact. There are three categories of symbiotic relationships being mutualism, commensalism and parasitism (Campbell and Reece 2002).

In mutualism, both the symbiotic parties benefit from each other. For instance, the mutualistic symbionts (*Lactobacillus*) that live in the human small intestine and vagina produce lactic acids of pH 4.0 to 4.5 and thus inhibit the colonization and growth of yeast and other potentially pathogenic bacteria such as *Gardnerella vaginalis, E. coli* and *Streptococcus agalactiae* (Boris *et al.* 1998). Similarly, the gram-negative *E. coli* that live in the large intestine of human release useful products such as Vitamin K, which the human host uses to make certain blood-clotting factors. These bacteria, in turn, get a favourable environment in which to live and obtain nutrients (Black 2004).

On the other hand, commensalism is a symbiotic relationship where one organism benefits while neither harming nor benefiting the host. Bacteria like *S. epidermidis, S. aureus, Micrococcus, Streptococcus pneumonia, Neisseria sp., Enterococcus, E. coli* and *Lactobacillus* are the normal human commensal of the skin, oral cavity, upper respiratory tract, lower gastrointestinal tract and urogenital tract (Black 2004). However, the presence of these commensal bacteria does out-compete other potentially harmful pathogens through competitive exclusion and thus preventing them from colonizing the host. Hence, these bacteria have indirectly conferred benefit to the host and are said to be mutualistic. Despite these indirect mutualisms, many of these commensals have potential parasitic relationship with the human host as well.

Some commensal bacteria may become pathogenic when the host's immune systems are weakened by factors such as primary infection by other microbial species, poor nutrition or aging or when they gain entry into a part of the body where they could not normally exist and these bacteria are called opportunistic commensals, parasite or pathogen. In a parasitic relationship, one symbiont, called the parasite or pathogen benefits at the expense of the host (Campbell and Reece 2002). Bacterial pathogenicity manifests when they invade and multiply on or within human cells and tissue, and finally, establish an infection within the body (Atlas 1994).

2.2.2 Pathogenic Bacteria

Pathogenic bacteria cause diseases when the growth of the pathogen or the virulence factors (toxins) produced in the host have adversely disrupted normal body physiological functions. The production of toxins either the exotoxins or endotoxins by these disease-causing microorganisms further enhances their ability to disrupt normal body functions (Atlas 1994).

Exotoxins can produce disease symptoms even without the presence of the pathogens. *Clostridium botulinum, Vibrio cholerae* and some strains of *E. coli* are the examples of endotoxin-producing pathogens that produce exotoxins into the surroundings. On the contrary, endotoxins or lipopolysaccharides (LPS) are the components of the outer membrane of certain Gram-negative bacteria (Campbell and Reece 2002). Nearly all members of the genus *Salmonella* produce endotoxins, however the endotoxins are not toxic unless released from the cell membrane (Atlas 1994).

Nevertheless, despite the bacterial pathogenicity, the human body has a complex network of interactive defense mechanisms against the potentially pathogenic microorganisms which includes the non-specific and specific immune system. The first line physical defender of the non-specific immune system is the intact and impermeable skin that serves as a protective and formidable external barrier to pathogens (Atlas 1994). However, disruption of the intact skin exposes the body to a vast diversity of pathogenic microorganisms that can then establish infections. Wounds disrupt the protective barrier of the skin and render passage to the microorganisms into the circulatory system and deep tissue, causing bacterial species which are previously harmless commensals of the skin, such as *S. epidermidis, S. aureus, Lactobacillus* spp. and *Propionibacterium acnes* may now become pathogenic in a wound environment.

2.3 WOUNDS

A wound is a breach in the intact skin, which may allow the entry of microorganisms and provide a warm, moist and nutritious environment for the colonization and proliferation of bacteria (Bowler *et al.* 2001).

2.3.1 Wound Types

Types and causes of wounds are wide ranging, and health care professionals have several different ways of classifying them. Generally, wounds can be categorized as having either an acute or chronic etiology. Acute wounds are caused by external damage to the intact skin and are usually instigated by a sudden, solitary insult, such as animal bites, burns, minor cuts or abrasion, surgical wounds and more severe traumatic wounds such as lacerations or gun-shot injuries. Irrespective of the nature of the cutaneous injury, acute wound generally proceed through the healing process in an orderly manner within a predictable time frame (Bowler *et al* 2001).

In conspicuous contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition which is usually due to an underlying pathological process that ultimately compromises the integrity of dermal and epidermal tissue. Impaired arterial supply, impaired venous drainage and metabolic diseases such as diabetes mellitus as well as sustained external skin pressure are the pathophysiological abnormalities that may predispose to the formation of chronic wounds such as foot ulcers, leg ulcers and pressure sores (Bowler *et al* 2001). Chronic wounds heal slowly and normally do not progress through the healing process due to repeated and prolonged insult to the tissue and severe tissue damage, and often remain in the inflammatory and infected phase, causing much discomfort and distress to the patient (Nigam *et al.* 2006).

2.3.2 Wound Healing

Wound healing is a complex series of events that begin at the moment of injury**.** It is a natural body's process to regenerate dermal and epidermal tissue which comprises three phases: inflammation, proliferation and remodeling (van der Plas *et al.* 2007).

The inflammatory phase is marked by platelet accumulation, coagulation, and leukocyte migration. In the inflammatory phase, bacteria and debris are phagocytosized and removed, and factors are released to stimulate the migration and division of cells involved in the proliferative phase or reconstruction phase. About two or three days after the wound occurs, fibroblasts begin to enter the wound site, marking the onset of the proliferative phase even before the inflammatory phase has ended.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. In angiogenesis, new blood vessels are formed from pre-existing vessels by vascular endothelial cells. During granulation tissue or fibrous connective tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and migrate across the newly formed granulation tissue, covering the new tissue (Midwood *et al*. 2004). When fibroblasts have differentiated into myofibroblasts, contraction commences (Eichler and Carlson 2005).

In the remodeling or maturation phase, the originally disorganized collage fibers are rearranged, cross-linked and aligned along tension lines and cells that are no longer needed are removed by apoptosis. As this phase progresses, the tensile strength of the wound increases and ultimately becoming as strong as normal tissue. Since activity at the wound site is reduced, the scar loses its red appearance as blood vessels that are no longer needed are removed by apoptosis (Greenhalgh 1998).

The phases of wound healing normally progress in a predictable, timely manner, however, healing may progress inappropriately in chronic wounds. Chronic, non-

healing wounds are frequently hypoxic as a consequence of poor blood perfusion and this pathological condition will ultimately cause cell death and tissue necrotic that are likely to create ideal growth conditions for the wound microflora (Bowler *et al.* 2001).

2.3.3 Wound Microflora

Wound microflora or contaminants are likely to originate from three main sources: the environment, involving the exogenous microorganisms in the air; the surrounding skin, involving the normal skin microflora; and the endogenous sources involving mucous membranes such as the oropharyngeal, gastrointestinal and genitouninary mucosae (Duerden 1994). The endogenous sources particularly the oral and gastrointestinal mucosae supply the vast majority of microorganisms that colonize wounds.

An acute wound that follow an orderly and reliable progression of healing may allow sufficient time for only a relatively small number of wound contaminants to take residence, whilst a slowly healing chronic wound is susceptible to the colonization of a vast variety of endogenous microorganisms. Nevertheless, both acute and chronic wounds are susceptible to colonization by mixed populations of aerobic and anaerobic wound bacteria.

The presence of aerobic or facultative pathogenic bacteria such as *S. aureus, P. aeruginosa* or beta-hemolytic streptococci in both acute and chronic wounds is the primary cause of delayed healing and progression to infection state (Halbert *et al.* 1992; Bowler *et al.* 2001). On the other hand, pathogenic anaerobes such as *Bacteriodes, Clostridium, Prevotella, Porphyromonas* and *Peptostreptococcus* are also the frequent wound colonizers (Bowler and Davies 1999).

The phases of bacterial activity in the wounds can be categorized into contamination, colonization, critical colonization and infection. Bacterial contamination and colonization are unlikely in hindering the wound healing process, whilst critical colonization occurs as the wound pathogens have adversely affected the healing process (Howell-Jones *et al*. 2005). Immunologically, infection occurs when the virulence factors expressed by the wound pathogens overwhelmed the host immune system and subsequent invasion in viable tissue incites a series of local and systemic host responses as characterized by purulent discharge and erythema (Bowler *et al*. 2001), while microbiologically, the indicators for infection would be a critical load of bacteria and synergistic interactions between the pathogenic bacteria (Howell-Jones *et al*. 2005) in the wounds.

The polymicrobial nature of wounds provides a ground for synergic microbial interaction between bacterial species and thus enhances the net pathogenic effect (Trengove *et al.* 1996) as well as worsens the infection at the wound in several ways (Bowler *et al.* 2001). Aerobic bacteria consume oxygen and induce tissue hypoxia which favors the growth of anaerobic bacteria. Besides, specific nutrients or essential growth factors produced by a particular bacterial species such as *S. aureus, E. coli* and *K. pneumoniae* may encourage the growth of some fastidious pathogenic cohabitants. Last but not least, some anaerobic bacteria are able to impair or compromise host cellular immune system and consequently, provide a competitive advantage to themselves and also other pathogenic cohabitants (Bowler *et al.* 2001).

In addition, the polymicrobial nature of wounds, particularly that of the intractable chronic wounds is likely to provide a suitable and appropriate environment for genetic exchange or transfer between the colonized bacteria (Howell-Jones *et al.*

2005) through transformation (assimilation of genetic materials from the surrounding environment) and conjugation (direct transfer of genes from one bacterium to another) and hence favors the emergence of mutated antibiotic-resistant bacteria as a result of the acquisition of resistance genes.

Furthermore, the misuse, overuse and abuse of antibiotics in the prophylaxis and healing of chronic wounds, indeed, foster the emergence of antibiotic-resistant bacteria. Though the inappropriate use of antibiotics does not induce bacterial chromosomal mutation, however, it creates environments or selection pressures that favor the survival of mutated resistant bacteria via Darwinian selection (Parnes and Lagan 2007). In 1999, Tentolouris *et al.* reported that there were significantly more methicillin-resistant *S. aureus* (MRSA) isolates from patients who had received prior antibiotic therapy, compared to those that had not. Hence, it is hardly surprising that chronic wound patients are a high risk group for the acquisition, carriage and dissemination of antibiotic-resistant bacteria (Howell-Jones *et al.* 2005).

2.4 ANTIBIOTIC-RESISTANT BACTERIA

Resistance of a microorganism to a particular antibiotic occurred when the microorganism which was formerly susceptible to the action of the antibiotic is no longer affected by it. Generally, microorganisms acquired antibiotic resistance by genetic changes (Black 2004).

Genetic changes or mutation in bacterial genome may be due to changes in the bacterial chromosome or acquisition of extrachromosomal DNA. Changes or mutation in chromosomal DNA leads to chromosomal resistance and the resulted resistance is

usually effective only against a single type of antibiotic. However, the acquisition of extrachromosomal DNA such as the resistance (R) plasmids or R factors that can carry more than one resistance genes had given rise to the emergence of multidrug-resistant bacteria as each of the R plasmids or R factors confers resistance to a different antibiotic. R plasmids can be transferred from one bacterial strain or species to another via transduction (bacteriophage as the mediator of gene transfer) or conjugation (gene transfer from a donor bacterium to a recipient bacterium) (Black 2004).

These multidrug-resistant bacteria are also known as multiple resistant bacteria or superbug and are defined as the bacteria that are resistant to more than two classes of antibiotics (Amorn 2005). The increasing levels of multiple resistance bacteria, such as MRSA, *E. coli* and *P. aeruginosa* has led to increased level of wound infections and difficulties in wound healing and wound management.

Chronic wounds are particularly susceptible to MRSA infection due to their acquisition of resistance to all commonly used antibiotics (Bexfield *et al.* 2008), including penicillin, methicillin and vancomycin (Sievert *et al.* 2002). The resistance to methicillin is conferred by the *mec*A gene which codes for penicillin-binding proteins that are insensitive to all penicillin-like antibiotics and hence obviate their clinical use in MRSA infections. On the other hand, the acquisition of vanA gene that codes for an alternative peptidoglycan to which the glycopeptides antibiotic vancomycin could not bind (Black 2004) has given rise to the emergence of vancomycin-resistant *S. aureus* (VRSA). The rapid spread of these dreadful strains of MRSA and VRSA has increased the number of infected patients and mortality globally.

Additionally, *P. aeruginosa* is an opportunistic pathogen characterized by an intrinsic resistance to multiple classes of antibiotics which is attributable to its chromosomal-encoded antibiotic resistance genes. In addition to this intrinsic resistance, *P. aeruginosa* possesses cellular envelopes of low permeability which obviate the actions of certain antibiotics by hindering their entry into the cytoplasm. Furthermore, *P. aeruginosa* easily develops acquired resistance via mutation in the chromosomally-encoded gene or horizontal transfer of antibiotic resistance genes. These genetic events have fostered the development of multidrug-resistant *P. aeruginosa* (Cornelis 2008). Multidrug-resistant *P. aeruginosa* has frequently been reported as the cause of nocosomial infection or as wound colonizers of burn patients (Richard *et al.* 1994).

Owing to the rapid emergence of multiple resistance strains of bacteria and increasing problems in the management of a wide spectrum of wounds, sores and ulcers, nowadays, maggot debridement therapy which employs sterile, live larvae of the green bottle blowfly has been deployed in the treatment of wound infections. Furthermore, the beneficial effects of the medicinal larvae have prompted massive research to reveal the underlying therapeutic mechanisms of maggot therapy.

Amongst the three core beneficial effects of maggot therapy, the antibacterial activity of larval ES has been studied most extensively. Many reports have proven that the larvae or maggots of *L. sericata* have great therapeutic effects on infected chronic wound via a series of mechanisms which encompasses debridement, disinfection and promotion of wound healing to rapidly eliminate infecting microbes, including the MRSA (Weil *et al.* 1933; Courtenay 1999; Thomas and Jones 2000), multidrug-resistant *P. aeruginosa* (Parnes and Lagan 2007) and *E. coli* (Mumcuoglu *et al.* 2001; Lerch *et al.* 2003).

Despite substantial research into the antibacterial effect of *L. sericata* larval ES, the antibacterial effect of the local blowfly *L. cuprina* against a wide range of potentially pathogenic wound bacteria has never been demonstrated. In view of this, the present study particularly aims to determine the antibacterial activity exhibited by the larvae of *L. cuprina* against seven species of wound pathogens including the MRSA via four established antibacterial bioassays, namely the turbidometric, colony-forming unit, agar well-diffusion and minimum inhibitory concentration assay.