Comparison of Moist Exposed Burn Ointment (MEBO) with Silver Sulfadiazine (Ag-S) for the Treatment of Deep Burn Injury

By

LAI Wing-Sze Vincy

Bachelor of Chinese Medicine
Bachelor of Science (Hons) in Biomedical Science

Principal Supervisor: Dr. Hong-Qi Zhang

Hong Kong Baptist University
School of Chinese Medicine

April 2004
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT (ENGLISH)</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT (CHINESE)</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 TYPES OF BURNS</td>
<td>1</td>
</tr>
<tr>
<td>1.2 CHARACTERISTIC FEATURES OF THE SKIN</td>
<td>1</td>
</tr>
<tr>
<td>1.3 STRUCTURE OF THE SKIN</td>
<td>2</td>
</tr>
<tr>
<td>1.4 ASSESSMENT OF BURNS SEVERITY</td>
<td>2</td>
</tr>
<tr>
<td>1.5 CLASSIFICATION OF BURNS BY DEPTH</td>
<td>3</td>
</tr>
<tr>
<td>1.5.1 Partial Thickness Burns</td>
<td>3</td>
</tr>
<tr>
<td>1.5.2 Full Thickness Burns</td>
<td>4</td>
</tr>
<tr>
<td>1.6 EXTENT OF BURNS IN TERMS OF TOTAL BODY SURFACE AREA (TBSA)</td>
<td>6</td>
</tr>
<tr>
<td>1.7 PATHOPHYSIOLOGY OF BURNS</td>
<td>7</td>
</tr>
<tr>
<td>1.7.1 Immunologic Response after a Burn Trauma</td>
<td>7</td>
</tr>
<tr>
<td>1.7.2 Vascular Response after a Burn Trauma</td>
<td>8</td>
</tr>
<tr>
<td>1.7.3 Edema Formation</td>
<td>9</td>
</tr>
<tr>
<td>1.7.4 Pulmonary Response</td>
<td>9</td>
</tr>
</tbody>
</table>
III. RESULTS................................................................................................................................. 37

3.1 WEIGHT VARIATIONS OF THE RATS OVER THE COURSE OF TREATMENT .......... 37

3.2 ABNORMAL SIGNS AND BEHAVIORS OBSERVED FROM THE RATS AFTER THE BURN INJURY ..................................................................................................................................... 37

3.3 GROSS AND MICROSCOPIC PATHOMORPHOLOGICAL FINDINGS ....................... 39

3.4 GROSS PHYSICAL APPEARANCE OF THE BURN WOUNDS .................................. 42

3.4.1 The Early Phase Appearance of the Burn Wounds .............................................. 42

3.4.2 The Physical Appearance of the MEBO Treated Wounds on Days 5 and 10 ......................................................................................................................................... 43

3.4.3 The Physical Appearance of the Ag-S Treated Wounds on Days 5 and 10 44

3.5 DEGREE OF WOUND HEALING .............................................................................. 45

3.6 HISTOLOGICAL ANALYSIS .................................................................................... 46

3.7 LIGHT ABSORBANCE (A) OF BACTERIAL CULTURE IN DIFFERENT GROUPS ...... 48

IV. DISCUSSION ......................................................................................................................... 51

4.1 THE ANTIBACTERIAL EFFECT OF MEBO AND AG-S ........................................ 51

4.2 THE ENLARGEMENT OF WOUND SIZE OBSERVED IN THE MEBO GROUP ....... 52

4.3 THE EFFECTIVENESS OF THE MOIST ENVIRONMENT CREATED BY MEBO ON HEALING ....................................................................................................................................... 53

4.4 THE ROLE OF TUMOR NECROSIS FACTOR (TNF) IN THERMAL INJURY COMPLICATIONS ......................................................................................................................... 54

4.5 PULMONARY COMPLICATIONS IN THERMAL INJURY ........................................ 54

4.6 GASTROINTESTINAL COMPLICATIONS IN THERMAL INJURY ............................. 55
4.7 RENAL COMPLICATIONS IN THERMAL INJURY .................................................. 56

4.8 EXPLANATIONS TO THE ABNORMAL SIGNS AND BEHAVIORS OF THE THERMALLY INJURED ANIMALS ........................................................................................................ 56

4.9 LIMITATIONS OF THIS STUDY ................................................................................ 57

4.10 FACTORS TO CONSIDER IN FURTHER STUDIES.................................................. 58

4.10.1 Monitor the Progression of Burn Wound Recovery ........................................... 58

4.10.2 Non-invasive Objective Parameters ................................................................................ 58

4.10.3 Non-invasive Methods of Burn Depth Assessment ................................................. 59

4.10.4 Monitor the Postburn Body Temperature and Identify the Strains of Bacterial Colonization .......................................................................................................................... 60

4.10.5 Comparison of Honey, MEBO and Ag-S .............................................................. 60

4.10.6 Benefit-cost Analysis ............................................................................................. 61

V. CONCLUSION ........................................................................................................ 62

VI. REFERENCES ......................................................................................................... 63

VII. APPENDIX .............................................................................................................. 74
LEGENDS OF TABLES AND FIGURES

TABLES

Table I : Classification of Burns
Table II : Definition of Different Types of Burns
Table III : Signs and Treatment of Different Degrees of Burns
Table IV : Rule of Nines
Table V : Classification of Burns Severity
Table VI : Clinical Signs of Burn Wounds Infection
Table VII : Commonly Used Topical Antimicrobial Agents for Burn Wound Care
Table IX : Different Stages of Burns Treatment with Chinese Medicines
Table X : The Wound Healing Percentage (%) of the Ag-S and MEBO Treated Groups
Table XI : The $P$-values of Different Groups on Days 5, 10, 15, 20 and 30

FIGURES

Figure 1: The structure of Ag-S: [4-amino-N-(2-pyrimidinyl-kappaN1)benzenesulfonamidato-kappaO]-silver
Figure 2: The back of the animal was shaved with an electric clipper (left). Plastic jelly cup collar with a hole at the bottom (right).
Figure 3: Two brass rods and heat-proof gloves (left). Thermostatic water bath and the immersed brass rods (right).
Figure 4: Identical burn wounds were created on the opposite side of the animals.
Figure 5: Ag-S and MEBO were applied on the left and right wounds respectively. -- 33

Figure 6: The procedures of hematoxylin and eosin staining. ----------------------------- 34

Figure 7: Bacterial samples were collected with a platinum loop closed to a Bunsen burner flame (left). The nutrition broth, the labeled culture tubes, the test tube rack and the platinum loop (right). ----------------------------- 35

Figure 8: The rat was lightly anesthetized with diethyl ether (left). The size of the wounds was outlined on an alcohol cleaned transparency (right). ---------- 36

Figure 9: The average weight of the rats over the 30 days postburn period. ---------- 37

Figure 10: Abnormal signs observed from the rats in the early postburn phase -- spiky hair (left) and nose bleed (right). ----------------------------- 38

Figure 11: Severe hair loss from the upper limbs, the lower abdomen and the lower limbs on day 15. ----------------------------- 38

Figure 12: Complete hair re-growth of the upper limbs, the lower abdomen and the lower limbs by day 30. ----------------------------- 39

Figure 13: Pulmonary and gastric wall hemorrhage (above); edematous enlargement of the large intestines (below) in the early dead rats. ----------------------------- 40

Figure 14: Dark bloody urine observed on the beddings (above); bloody urine that stained the hair of the groin region (below). ----------------------------- 41

Figure 15: Pulmonary alveolar hemorrhage and blood vessels congestion; 40x (left) and 100x (right). alveolar sacs (AS), pulmonary vessels (V). ----------------------------- 41

Figure 16: Erosion of the stomach (left) and the duodenum (right). Mucosa (M), muscularis mucosae (MM), submucosa (SM), circular muscle layer (CM), longitudinal muscle layer (LM). ----------------------------- 42

Figure 17: The appearance of the burn wounds in the early phase after the thermal injury. The locations of the wounds were indicated by the blue circles which were drawn on with a permanent marker. ----------------------------- 43
Figure 18: The appearance of the MEBO treated wound on day 5. 43

Figure 19: The appearance of the MEBO treated wound on day 10 (same wound as shown in figure 18). 44

Figure 20: The appearance of the Ag-S treated wound on day 5. 44

Figure 21: The appearance of the Ag-S treated wound on day 10 (same wound as shown in Figure 20). 44

Figure 22: The wound healing percentage of the Ag-S and MEBO treated groups on days 10, 15, 20 and 30. 45

Figure 23: The histology of the MEBO (left) and the Ag-S (right) treated wounds on day 5. Dermis (D), hair follicles (F), hypodermis (H), subcutaneous fat (SF) and blood vessels (V). 46

Figure 24: The Ag-S treated wound on day 10. 47

Figure 25: The histology of the MEBO (above) and Ag-S (below) wounds on day 30. Keratin (C), dermis (D), epidermis (E), hair follicles (F), hypodermis (H), scars (S) and fluid-filled vesicles (V). 48

Figure 26: The light absorbance of bacterial culture collected from the burn wounds of both the Ag-S and MEBO treated groups and the normal skins. 49
DECLARATION

I, Lai Wing-Sze Vincy, hereby declare that this thesis represents my own work, which has been done after registration for the degrees of BCM and BSc (Hons) Biomed. Sci. at the Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or other institution for a degree, diploma or other qualification.

Signature: ____________________

Date: April 5, 2004
Comparison of Moist Exposed Burn Ointment (MEBO) with Silver Sulfadiazine (Ag-S) for the Treatment of Deep Burn Injury

Abstract

Objective: To compare the wound healing property and the antibacterial ability of the Moist Exposed Burn Ointment (MEBO), a Chinese medicinal herbal product, with Silver Sulfadiazine cream (Ag-S), a first-line topical antibiotic for the treatment of deep burn injury (deep second to third degree burns).

Methods: Two identical deep burn wounds were created on each side of the back of fourteen SD rats by a heated cylindrical brass rod. The depth of burns and the degree of healing were verified by histological examination on days 5 and 30 respectively. All the wounds on the right side were treated with MEBO while all the wounds on the left were treated with Ag-S. Both topical agents were applied four times a day. The wound size and the amount of bacterial culture collected from both the wound surface and the normal skin were measured on days 5, 10, 15, 20 and 30 after the burn injury.

Results: The results did not show any significant difference \( (p=0.28) \) in the wound healing percentage between wounds treated with MEBO and Ag-S. MEBO had a poor antibacterial effect indicated by a high absorbance value compared to that of Ag-S.

Conclusion: MEBO is not desirable in managing deep burns.
摘要

目的: 比較美寶濕潤燒傷膏(MEBO), 一種含有中藥成份的燒傷膏，與磺胺嘧啶銀，一種常用處理燒傷的前線外用抗生素(Ag-S)，對深度(II-III°)燒傷癒合及抗感染的治療效果。

方法: 使用燙熱的鋼棒在十四隻 SD 大鼠背部造成左右相等的深度(II-III°)燙傷。燙傷的深度及其癒合程度分別在燙傷後的第五及第三十天在顯微鏡下作組織結構學分析。所有的右側傷口被塗上 MEBO，而所有的左側傷口則塗上 Ag-S，每日換藥四次。此外，分別在燙傷後的第五、第十、第十五、第二十及第三十天量度傷口的大小及測量其上的細菌量，並與沒有燙傷的正常皮膚作比較。

結果: MEBO 在傷口癒合率方面與 Ag-S 並無顯著的差異 \((p=0.28)\)，但是 MEBO 在抗菌方面則較 Ag-S 差。

結論: MEBO 不宜用於治療深度燒傷。
ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor, Dr. Hong-Qi Zhang, for his sincere guidance, patience and strict demands. Not only was the aim of the experiment clearly explained by Dr. Zhang at the very beginning, he also closely guided us along and was always available to give us insightful advice. However, he did not set boundaries to limit our thinking or forbid us from trying out new ideas. In contrast, he provided us plenty of rooms to explore and develop our logical thinking ability, organization power, communication skills and problem solving techniques. Despite the fact that he is a tough supervisor who sets high requirements, I enjoy working under his supervision. I honestly believe that I am very lucky to have the opportunity of becoming his student since the very first day of our pathology class dated back to my second year of university life.

I also wish to express my appreciation to our technician Ms. Nickie Chan. She is a strict yet helpful and kind “housekeeper” of our laboratory. Without her, our laboratory would not be neat and tidy with all the necessary tools and equipment in working order. She was there to oversee our progress and offered us invaluable technical advices on the overall flow of our experiments. She did not only skillfully assist us in overcoming our technical problems, but generously treated us with tea breaks to raise our morale on our hard days.

Miss Queenie Yip is another person I would like to acknowledge. She is a friendly research assistant who is experienced in conducting thermal injury experiment. I have to thank her for looking after our autoclaved equipments and operating the orbital shaker for us. I would not forget her outstanding tissue microtome slicing techniques. Moreover, she had always supported us and warned us ahead for possible unforeseen errors. Without her, our experiments would not have been completed on time.

I would also like to thank Mr. Wai-Shing Chung and Mrs. Yin-Sang Hui. Mr. Chung generously provided us with equipments and offered his assistance when some unexpected incidents came up. I am grateful to have Mrs. Hui to arrange and thoroughly clean the animal cages for us. We had indeed brought her a lot of work since the topical
burn agent (MEBO) that we used was particularly oily. Since we did not want cross contamination of the wounds, we changed the cages almost every two days.

Lastly, I have to thank my colleagues, Miss Irene Hui and Miss Ann Wong. They made me realize the importance of teamwork. There were many nights (10-11p.m.) when others were lying comfortably on the sofa watching television at home, my colleagues and I were working in the cold and isolated animal room. Miss Wong was unfortunately bitten by one of my rats when she was smearing topical burn agents for me during one of the many difficult nights. Early next morning (8:30a.m.), we appeared in the animal room again to perform the daily routine. The burn ointments application was carried out at 8:30a.m., 1p.m., 5:30p.m. and 10p.m. The four shifts per day schedule lasted for six weeks and in between there were four weeks that we had to participate in a sixteen hours per week clinical internship. This final year project had indeed brought us closer and allowed us to build a precious friendship that I really treasure.
I. INTRODUCTION

1.1 Types of Burns

Burns can be basically divided into six major categories based on the nature of causes. They are scalds (immersion in or splash by hot liquids, grease, and steam), contact burns (touching a hot object or substance), and burns caused by fire, chemicals, electricity and radiation (Table I). Among them, the most serious burns usually result from flames or scalds (Forrest et al. 1995). Generally, human skin can tolerate temperatures up to 40ºC. Any temperature above this will result in pain and tissue damage (Ashan 1997).

<table>
<thead>
<tr>
<th>Type of Burns</th>
<th>Tissue Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Injury</td>
<td></td>
</tr>
<tr>
<td>1. Scalds</td>
<td>Partial thickness/ deep dermal loss</td>
</tr>
<tr>
<td>2. Fat burns</td>
<td>Usually full thickness skin loss</td>
</tr>
<tr>
<td>3. Flame burns</td>
<td>Patches of full and partial thickness loss</td>
</tr>
<tr>
<td>4. Electrical burns</td>
<td>Full thickness loss with deep extensions</td>
</tr>
<tr>
<td>Friction Burns</td>
<td>Heat plus abrasion</td>
</tr>
<tr>
<td>Ionizing Radiation</td>
<td>Early tissue necrosis, later tissue dysplastic changes</td>
</tr>
<tr>
<td>Chemical Burns (Acid or Alkali)</td>
<td>Inflammation, tissue necrosis and allergic response</td>
</tr>
</tbody>
</table>

1.2 Characteristic Features of the Skin

The skin area of human beings ranges from 0.25m² at birth to 1.5-1.9 m² in adults. The skin accounts for about 15% of lean body mass and is one of the largest organs in the body. It is the first important barrier against entry of microorganisms. It also regulates heat loss by means of hair and sweat glands, receives stimuli, excretes waste products, protects from injury, ultraviolet light and desiccation (Ratcliffe 1983).
1.3 Structure of the Skin

The skin is composed of two main layers known as the epidermis and the dermis. The epidermis is a layer of keratinized, stratified squamous epithelium which delivers the three appendages (hair follicles, sweat glands and sebaceous glands) into the underlying dermis. These appendages are usually the source of new cells for reconstitution of the epidermis due to their deep location (Forrest et al. 1995). The dermis is mainly connective tissues forming the majority of the skin. In rats, the dermis consists of fibrous connective tissue, hair follicles, hair roots and arrector pili, sebaceous glands, panniculus carnosus, lymphatic and blood vessels, and two types of nerve endings (Meissner’s and Pacinian corpuscles). Beneath the dermis lies a layer of connective tissue with subcutaneous fat which is known as the hypodermis (Ratcliffe 1983).

1.4 Assessment of Burns Severity

When evaluating burn severity, the following factors need to be considered:

1. The age of the patient.
2. The actual site involved.
3. The depth of the burn.
4. The extent of the burn.
5. The presence of coexisting diseases.
6. The occurrence of inhalation burn.

The age of the patient is an important factor for burn severity evaluation. Burn patients below three and over sixty years of age have more serious consequences than patients at other ages. The coexisting diseases including cardiovascular, respiratory and renal disorders can complicate the treatment and increases mortality (Ahsan 1997). Moreover, burns of the face, neck, hands, feet and perineum pose special concerns in their reconstruction and rehabilitation. If the burn patients also have inhalation injury producing extensive damage to the airways with pending obstruction, they are particularly vulnerable and require special medical attention (Mozingo 2001).
I. Introduction

1.5 Classification of Burns by Depth

Coagulative necrosis of the epidermis and the underlying tissues are the consequence of thermal injury. The depth of thermal damage depends on both the temperature to which the skin is exposed to and the duration of exposure. Furthermore, the specific heat of the causative agent also affects the depth. Grease, for instance, causes a deeper damage than a hot water scald burn at the same temperature and exposure time since the specific heat of fat is higher than that of water (Wolf and Herndon 2001).

The depth of burns is classified into partial thickness and full thickness injury with respect to the extent of dermal destruction. The first and second degree burns are considered as partial thickness injury while the third degree burns are known as full thickness injury (Table II).

<table>
<thead>
<tr>
<th>Type of Burns</th>
<th>First Degree</th>
<th>Superficial Second Degree</th>
<th>Deep Second Degree</th>
<th>Third Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition</td>
<td>Injury localized to the epidermis.</td>
<td>Injury to the epidermis and superficial dermis.</td>
<td>Injury through the epidermis and deep into the dermis.</td>
<td>Full thickness injury through the epidermis and dermis into the subcutaneous fat.</td>
</tr>
</tbody>
</table>

1.5.1 Partial Thickness Burns

Partial thickness burns are often caused by brief contact with hot surfaces, flames, chemicals or hot liquids. They are limited to the epidermal and superficial dermal layers of the skin. The skin often appears red, warm, edematous and blistered. With the denuded epithelium, the burned surface often appears moist with mottled white or pink. The injured tissue is very painful especially when exposed to air. However, deep second degree burns can be insensitive to pinprick if the neural receptors are damaged.

This kind of burns heals in time spontaneously by epithelial migration from the survived skin appendages. The time of skin healing is proportional to the depth of dermal destruction (Mozingo 2001). Healing will be completed within a few days with the outer
I. Introduction

Injured cells peel off leaving no scar (Ahsan 1997). If early fluid resuscitation is inadequate, areas of partial thickness burn can convert to full thickness injury over time (Doherty 1999).

1.5.2 Full Thickness Burns

Full thickness burns cause damage to all layers of the skin and some subcutaneous tissues. It lacks viable epithelial elements, thus skin grafting for wound closure is often required if the burned area is large. Since the dermal hair follicles, sweat glands, sebaceous glands and sensory fibers for touch, pain, temperature and pressure are destroyed, the burned skin is painless and appears dry, white and leathery or charred and cracked. The underlying fat may also be exposed. Additional information of the signs and treatment of different degrees of burns are listed in Table III (Wolf and Herndon 2001).

<p>| Table III: Signs and Treatment of Different Degrees of Burns (Wolf and Herndon 2001) |
|-----------------------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Type of Burns</th>
<th>Signs</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Degree</td>
<td>• Red</td>
<td>1. Apply cool, wet compresses, or immerse in cool, fresh water. Continue until pain subsides.</td>
</tr>
<tr>
<td></td>
<td>• Erythematous</td>
<td>2. Cover the burn with a sterile, non-adhesive bandage or clean cloth.</td>
</tr>
<tr>
<td></td>
<td>• Painful to touch</td>
<td>3. Do not apply ointments or butter to burn; these may cause infection.</td>
</tr>
<tr>
<td></td>
<td>• Usually moist</td>
<td>4. Over-the-counter pain medications may be used to help relieve pain and reduce inflammation.</td>
</tr>
<tr>
<td></td>
<td>• No blisters</td>
<td>5. First degree burns usually heal without further treatment. However, if a first degree burn covers a large area of the body or the victim is an infant or elderly, seek emergency medical attention.</td>
</tr>
</tbody>
</table>
## I. Introduction

### Table III: Signs and Treatment of Different Degrees of Burns (Wolf and Herndon 2001)

<table>
<thead>
<tr>
<th>Type of Burns</th>
<th>Signs</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Second Degree</strong></td>
<td></td>
<td>1. Immerse in fresh, cool water, or apply cool compresses. Continue for 10 to 15 minutes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Dry with clean cloth and cover with sterile gauze.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Do not break blisters.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Do not apply ointments or butter to burns; these may cause infection.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Elevate burned arms or legs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Take steps to prevent shock: lay the victim flat, elevate the feet about 12 inches, and cover the victim with a coat or blanket. Do not place the victim in the shock position if a head, neck, back, or leg injury is suspected, or if it makes the victim uncomfortable.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. Further medical treatment is required.</td>
</tr>
<tr>
<td><strong>Third Degree</strong></td>
<td></td>
<td>1. Cover burn lightly with sterile gauze or clean cloth. (Don’t use material that can leave lint on the burn).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Do not apply ointments or butter to burns; these may cause infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Take steps to prevent shock: lay the victim flat, elevate the feet about 12 inches.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Have the victim sit up if face is burned. Watch closely for possible breathing problems.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Elevate burned area higher than the victim’s head when possible. Keep person warm and comfortable, and watch for signs of shock.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Do not place a pillow under the victim’s head if the person is lying down and there is an airway burn. This can close the airway.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. Immediate medical attention is required.</td>
</tr>
</tbody>
</table>
1.6 Extent of Burns in terms of Total Body Surface Area (TBSA)

The determination of burn size estimates the extent of injury. Since all currently used resuscitation formulas are based upon the percentage of the TBSA burned and the weight of patient, it is important to obtain them early in the course of treatment. The extent of body surface area burned can be estimated using the “Rule of Nines” (Table IV) which assigns 9 or 18% of the TBSA to specific anatomic regions. In adults, each upper extremity and the head and neck are 9% of the TBSA. While for the lower extremities and the premium and genitalia are assumed to be 1% TBSA (Wolf and Herndon 2001). If the burned area is small or irregularly shaped, it can be estimated by a simpler method by equating the area of the palm of the patient, including the fingers, to be approximately 1% of his or her TBSA. The measurement is then transposed visually onto the wound for its size determination. The physiological impact of the severity of burns can be classified according to the final TBSA% obtained (Table V).

<table>
<thead>
<tr>
<th>Table IV: Rule of Nines (Tompkins 1994)</th>
<th>Body Surface Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and Neck</td>
<td>9</td>
</tr>
<tr>
<td>Face and Neck</td>
<td>4.5</td>
</tr>
<tr>
<td>Scalp</td>
<td>4.5</td>
</tr>
<tr>
<td>Arm, Forearm and Hand</td>
<td>9</td>
</tr>
<tr>
<td>Anterior</td>
<td>4.5</td>
</tr>
<tr>
<td>Posterior</td>
<td>4.5</td>
</tr>
<tr>
<td>Leg and Foot</td>
<td>18</td>
</tr>
<tr>
<td>Anterior</td>
<td>9</td>
</tr>
<tr>
<td>Posterior</td>
<td>9</td>
</tr>
<tr>
<td>Anterior Trunk</td>
<td>18</td>
</tr>
<tr>
<td>Chest</td>
<td>9</td>
</tr>
<tr>
<td>Abdomen</td>
<td>9</td>
</tr>
<tr>
<td>Posterior Trunk</td>
<td>18</td>
</tr>
<tr>
<td>Upper Back</td>
<td>9</td>
</tr>
<tr>
<td>Lower Back (includes buttock)</td>
<td>9</td>
</tr>
<tr>
<td>Perineum</td>
<td>1</td>
</tr>
</tbody>
</table>
Table V: Classification of Burns Severity (Green and Wajed 2000)

<table>
<thead>
<tr>
<th></th>
<th>Major Burn</th>
<th>Moderate Burn</th>
<th>Minor Burn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partial Thickness</strong></td>
<td>&gt;25% Adults</td>
<td>15-25% Adults</td>
<td>&lt;15% Adults</td>
</tr>
<tr>
<td></td>
<td>&gt;20% Children</td>
<td>10-20% Children</td>
<td>&lt;10% Children</td>
</tr>
<tr>
<td><strong>Full Thickness</strong></td>
<td>&gt;10%</td>
<td>2-10%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td><strong>Primary areas involved (head, neck, hands, feet and perineum)</strong></td>
<td>Major Harm</td>
<td>Not Involved</td>
<td>Not Involved</td>
</tr>
</tbody>
</table>

1.7 Pathophysiology of Burns

The human skin can be injured by heat from two mechanisms: an immediate direct cellular injury that results in protein denaturation and a delayed injury caused by progressive dermal ischemia. Furthermore, tissue destruction is closely related to the time of exposure and the temperature involved (Mozingo 2001). Generally, a burn wound is divided into three zones: the zone of necrosis, the zone of stasis and the zone of hyperemia. In the zone of necrosis, coagulation and cell death has occurred. In the zone of stasis, which extends radially from the zone of necrosis is characterized by vascular damage and vessel leakage. This zone has the potential to completely regenerate if there is enough blood supply. However, immediately after burn injury, the vessels in the zone of stasis dilate and cause a reduction of perfusion. If there is desiccation, infection, or inadequate perfusion, this zone will eventually convert to necrosis (Noble, Robson and Kricke 1977). As for the zone of hyperemia, it sustains only minimal injury and lies peripheral to the zone of stasis. The cells in this zone usually recover over a period of seven to ten days (Mozingo 2001).

1.7.1 Immunologic Response after a Burn Trauma

Burns cause a systemic depression in immune function, including the activation and activity of neutrophils, macrophages, T and B lymphocytes, thus the patients are at
I. Introduction

A greater risk of a number of infectious complications. Basically, after a burn injury, the destruction of the normal skin barrier results in loss of mechanical protection from microbial organisms that allow them to invade into the normal tissues. In burns that are of more than 20% TBSA, the impairment of the cellular and humoral immune function is proportional to the extent of injury (Wolf and Herndon 2001). During the first week after a burn injury, the total white blood cell count is elevated while the peripheral blood lymphocyte counts are reduced. Moreover, the delayed hypersensitivity reactions and peripheral blood lymphocyte proliferation in the mixed lymphocyte reaction are both inhibited (Mozingo 2001).

1.7.2 Vascular Response after a Burn Trauma

Various substances such as the prostaglandins, leukotrienes, histamine and oxygen free radicals are released after a burn injury which causes an increase in vascular permeability and plasma extravasations resulting in hypovolemia and hemoconcentration (Martyn 1990). This systemic damage may affect many organs but more prominently in loose tissues such as the lung. Leukotrienes are active metabolites derived from arachidonic acid that intervene in the inflammatory processes. They cause bronchoconstriction, increase in vascular permeability and vasoconstriction (Lockhart and Jing-Xuan 1997). Histamine, on the other hand, elicits a characteristic response in venules by increasing intercellular junction space formation. They are released in large quantities immediately after injury by the mast cells in the burned skin (Wolf and Herndon 2001). Moreover, the increase in microvascular permeability is often due to injury of the inter-endothelial junctions and the vascular endothelial injury. It could also be a result of endothelial contraction caused by an increment in intra-endothelial calcium concentration (Wang et al. 1994). Another study suggested that endothelial apoptosis was one of the main factors causing the increase in vascular permeability. Factors recognized to be inducing cell apoptosis include bacterial infection, cytokines, stress response, ischemia and reperfusion injury (Zhang et al. 1994).
1.7.3 Edema Formation

Following a burn trauma, edema may be formed in the wound and deep in the unburned tissues as a result of superficial tissues destruction and inflammatory response of the tissues. The formation is greatest in the first six hours after injury and continues to a lesser extent for the first twenty-four hours (Dermling et al. 1981). After the initial changes in physical characteristics of burn tissues, subsequent edema formation is generally attributed to an increase in microvascular permeability which is caused by cytokines produced by the activated leukocytes and humoral factors liberated from the burned tissues. Because the postcapillary venular constriction markedly increases the capillary hydrostatic pressure and the production of interstitial edema in the early post injury phase, a strong negative interstitial fluid hydrostatic pressure takes place within thirty minutes of injury. Moreover, an early increase in the interstitial fluid colloid osmotic pressures following burn injury results in a reversal of the transcapillary osmotic pressure gradient. In addition, the plasma concentration of histamine which is a regulator of vascular permeability increases in proportion to the burn size immediately after injury (Mozingo 2001).

Many other inflammatory mediators such as activated proteases, prostaglandins, leukotrienes and substance P rise after burn injury and enhance microvascular permeability (Mozingo 2001). In short, the formation of burn wound edema can be due to several factors including vasodilatation with increased effective transcapillary filtration pressure, increased extravascular osmotic activity created in damaged tissue, increased microvascular permeability to micromolecules, and generalized impairment in cell membrane function resulting in cell swelling (Arturson 2000).

1.7.4 Pulmonary Response

Immediately after a thermal injury, the minute ventilation is unchanged or slightly increased as a result of anxiety and pain that induces hyperventilation. However, the respiratory rate and tidal volume progressively increase that result in a two to two and a half times increase of the minute ventilation than that of the normal. The degree of
increase is proportional to the extent of burns which is related to the postburn hypermetabolism. Furthermore, the increase in pulmonary vascular resistance immediately after a burn is more prolong than the increase in peripheral vascular resistance (Mozingo 2001). The adult respiratory distress syndrome is frequently found in thermally injured patients. It is, nonetheless, difficult to distinguish from inhalation injury which bronchogenic infections or pneumonia often occurs because of organism colonization in the burn wounds. In addition, pulmonary edema may take place following inhalation injury or fluid overload. In that case, close attention should be given to monitor the arterial oxygen tension, the respiratory mechanics and the administered fluid load (Tompkins 1994).

1.7.5 Gastrointestinal and Biliary Response
As discovered by Curling in 1842, duodenal ulcers frequently occur in association with burns on the body surface. The pathophysiology of the initial mucosal injury appears to be related to mucosal hypoxia that is more prone to gastric acids damage even at their normal concentration. This hypoxia may be due to submucosal arteriovenous shunting or diminished organ blood flow (Mozingo 2001). Moreover, deep skin burns always trigger a pronounced inflammatory reaction involving the synthesis and release of inflammatory mediators that directly or indirectly leads to vasoconstriction and progressive ischemia (Denzlinger et al. 1985). Among these mediators, thromboxane (TBX) A₂, which increases dramatically in the plasma and wounds of burned patients, likely play a role in the changes in permeability and fluid shifts. On one hand, TBX is a potent vasoconstrictor that causes vasoconstriction and platelet aggregation in the wound and contribute to the expansion of the zone of stasis locally. On the other hand, TBX also leads to prominent mesenteric vasoconstriction and decreases the gut blood flow that compromises its mucosal integrity and weakens its immune function (Wolf and Herndon 2001).

Another important gastrointestinal complication is the motility impairment of the gastrointestinal tract and the hepatobiliary system. Intestinal paralytic ileus and acute
gastric dilation are commonly seen as a result of sepsis, fluid overload and electrolyte imbalances. In addition, acute acalculous cholecystitis, manifested as sepsis, right upper quadrant pain and mass, and abnormalities in liver function are often seen in severe cases (Tompkins 1994).

1.7.6 Renal and Cardiovascular Response

Acute renal failure may be secondary to hypofusion and hypoxia occurring before plasma volume replacement in resuscitation which results in oliguric or non-oliguric acute tubular necrosis (Tompkins 1994). Acute tubular necrosis is one of the complications of extensive burns injury. It is especially common in the elderly and those with pre-existing renal disease. Hemoglobinaemia or myoglobinuria are seen as a result of massive red cell destruction or extensive muscle destruction which damages the tubules and obstructs urine flow by cast formation (Forrest et al. 1995). Recently, it has become clinically accepted that early resuscitation and the prevention of sepsis may reduce the incidence of acute renal dysfunction in burned adults (Chrysopoulo et al. 1999). Apart from renal failure, congestive heart failure may also occur either in the acute phase of the burn injury or during the mobilization of the peripheral edema (Tompkins 1994).

1.8 Prevention and Treatment of Burn Related Infections

1.8.1 Local Infections

Local infections and burn wound sepsis are detrimental problems in the treatment of thermally injured patients. The burn wound bacterial cultures are negative during the first few hours after injury. The wound becomes rapidly colonized by gram-positive cocci that are present within twenty-four hours, but gram-negative aerobes usually take over within three to seven days, and fungi usually appear later in the postburn course (Ahsan 1997). Conversely, in the untreated wounds, the surface colonization often leads to the invasion of healthy tissues. Early surgical excision and the use of topical antimicrobials were reported to decrease the infection rate and improve the survival rate of burn patients in
the last twenty-five years (Fujii 1990). Likewise, the presence of avascular nonviable eschar provides an excellent culture medium for microbial proliferation. The bacteria colonize the nonviable eschar while invading beneath the eschar into the viable tissues which leads to burn wound infection (Wolf and Herndon 2001).

1.8.2 Systemic Infections

Systemic infection is one of the principal causes of death in burned patients. It had been repeatedly reported by many investigators that severe thermal injury was usually accompanied by immune suppression that mediated an increased susceptibility to infections (Alexander and Moncrief 1966). Previous study had demonstrated that the increased release of prostaglandin E$_2$ (PGE$_2$) postburn suppressed granulocytes and macrophage growth in the burn wound thereby increasing the risk of infections (Gamelli et al. 1998). PGE$_2$ was also shown to play a crucial role in the suppression of the B-cell function and the inhibition of T-lymphocytes postburn (Yamamoto et al. 1996; Grbic et al. 1991). Another study indicated that severe impairment of both B and T lymphocytes, either circulating or in the lymphoid organs, took place shortly after thermal injury and lasted for several days. The severe damage of the lymphocytes is considered to be closely related to the immune suppression after thermal injury (Maekawa et al. 2002).

Wound infection by exogenous means is a major cause of systemic infections. Careful and efficacious treatment of wounds is perhaps the most important measure to prevent burn infection in addition to early extensive eschar excision. Moreover, the use of antibiotics which were frequently administered in combination through the venous route is still an effective measure for preventing and treating burn infections. A clinical study investigating the fourteen cases of full thickness burns of more than 70% TBSA demonstrated that every effort should be made to have no more than 5% of the wound infected in the entire therapeutic course. A patient’s condition would progress uneventfully if their infected wound remained under 5%. Nonetheless, the incidence of systemic infection and internal organ complication was higher as the area of wound infected increased (Zhou et al. 1998).
1.8.3 Indications of Invasive Infections

The clinical signs of invasive burn wound infection that are commonly seen include tachycardia, tachypnea, hyperthermia or hypothermia, disorientation, glucose intolerance and ileus. These systemic signs are often indistinguishable from those observed in the uninfected hypermetabolic burn patients or burn patients who have other sources of infection (Mozingo 2001). Thus, the physical changes in the burn wound are more reliable signs of invasive infection (Table VI).

<table>
<thead>
<tr>
<th>Table VI: Clinical Signs of Burn Wounds Infection (Mozingo 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Focal dark-brown or black discoloration of wound</td>
</tr>
<tr>
<td>- Degeneration of wound with neoeschar formation</td>
</tr>
<tr>
<td>- Hemorrhagic discoloration of subeschar fat</td>
</tr>
<tr>
<td>- Conversion of partial thickness to full thickness necrosis</td>
</tr>
<tr>
<td>- Unexpectedly rapid eschar separation</td>
</tr>
<tr>
<td>- Erythematous edematous wound margin</td>
</tr>
<tr>
<td>- Metastatic septic lesions in unburned skin or distant organs</td>
</tr>
</tbody>
</table>

1.9 Definitive Burn Wound Care

Burn sepsis is a leading cause of mortality and morbidity in patients with major burns. In order to minimize the chance of infections, special care should be paid to wound care. After initial wound assessment, thorough cleansing and debridement, topical antimicrobial agents should be applied on the burn wound to limit bacteria proliferation and invasion. The three most extensively used topical agents that are efficacious in controlling bacterial proliferation are silver sulfadiazine (Ag-S) cream, mafenide acetate cream and 0.5% silver nitrate soaks (Mozingo 2001). The clinical use of effective topical antimicrobial agents have markedly decreased the incidence of invasive burn wound infections and subsequent sepsis. Nevertheless, each agent has its own specific advantages and limitations, thus special attention should be paid when selecting the appropriate agent to ensure optimal benefit and safety (Table VII).
Table VII: Commonly Used Topical Antimicrobial Agents for Burn Wound Care (Mozingo 2001)

<table>
<thead>
<tr>
<th>Topical Agent</th>
<th>Silver Sulfadiazine</th>
<th>Silver Nitrate</th>
<th>Mafenide Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active Component</strong></td>
<td>1.0% in water-miscible base</td>
<td>0.5% in aqueous solution</td>
<td>11.1% in water-miscible base</td>
</tr>
<tr>
<td><strong>Spectrum of Antibacterial Activity</strong></td>
<td>Gram(-), selectively good; Yeasts, good</td>
<td>Gram(-), good; Gram(+), good; Yeasts, minimal</td>
<td>Gram(-), good; Gram(+), good; Yeasts, good</td>
</tr>
<tr>
<td><strong>Method of Wound Care</strong></td>
<td>Exposure or single-layer dressings</td>
<td>Occlusive dressings</td>
<td>Exposure</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Painless; greater effectiveness against yeasts</td>
<td>Painless</td>
<td>Penetrates eschar; no Gram(-) resistance</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Neutropenia; hypersensitivity is uncommon; limited eschar penetration; resistance of certain Gram(-) bacteria and Clostridia</td>
<td>Deficits of sodium, potassium, calcium and chloride; no eschar penetration; staining of environment and equipment</td>
<td>Painful on partial thickness burns; acidosis as a result of inhibition of carbonic anhydrase; hypersensitivity reactions in 7% of patients</td>
</tr>
</tbody>
</table>

Apart from the above mentioned topical agents, sulfamylon is another antimicrobial agent which is used less frequently than Ag-S. Since sulfamylon has broad spectrum activity but requires frequent administration due to its strong wound penetration ability, it may easily result in toxic complications because of systemic absorption. Other antimicrobials such as povidine iodine and nitrofurazone are less commonly used. Povidine iodine has broad spectrum in vitro activity but may be absorbed systemically and can be inactivated by wound exudates (Monafo and West 1990). As for nitrofurazone, it has limited bactericidal activity and has the potential to cause nephrotoxicity and ototoxicity as it is again readily absorbed from the wounds. Nitrofurazone is thus not recommended for major burn injury (Monafo and Freedman 1987).

1.10 Silver Sulfadiazine (Ag-S)

Silver sulfadiazine (Ag-S) cream, with commercial names of Silvadene, SSD, SSD AF and Thermazene in the United States and Flamazine in Canada, is the standard topical treatment for burn wounds. It is a 1% suspension of Ag-S with nonmedicinal ingredients
including isopropyl myristate, methylparaben, polyoxyxyl 40 stearate, propylene glycol, purified water, sodium hydroxide, sorbitan monooleate, stearyl alcohol and white petrolatum. It is used as a topical antiseptic for thermal and chemical burns and is applied after prompt emergency treatment and debridement of burns. It does not require to be covered by dressings and therefore, can be used under less than ideal economic conditions (Noronha and Almeida 2000).

1.10.1 Antibacterial Properties of Ag-S
Ag-S is a sulfa medicine for the prevention and treatment of bacterial or fungal infections in second and third degree burns. It is a topical bactericidal agent effective against many gram-negative and gram-positive bacteria as well as yeasts (Tompkins 1994). In a study that compared the effects of Ag-S, silver nitrate solution, sulfamylon acetate and gentamicine, the sulfamylon and Ag-S treated groups had the least sepsis occurrence and the lowest frequency of positive *Pseudomonas* cultures (Hummel, MacMillan and Altemeier 1970). An experimental study on mice with burns contaminated with *P. aeruginosa* showed that *Pseudomonas* strains disappear under the influence of Ag-S (Fox 1968). However, more recent investigations illustrated that Ag-S became ineffective against certain gram-negative organisms such as some *Pseudomonas* strains and all *Enterobacter cloacae* species (Heggers and Robson 1978). In order to verify the antibacterial ability of Ag-S which is a conventional topical agent for burns treatment, further investigations are required.

1.10.2 Analgesic Properties and Wound Healing Ability of Ag-S
Ag-S offers particular advantages when used in small children because of its painless application and soothing effect. Moreover, it causes no acid-base abnormalities and restricts fluid and heat loss from the burn surface. Ag-S is also the adjunctive treatment of skin grafts, incisions, leg ulcers and other clean lesions, abrasions, minor cuts and wounds (Tompkins 1994). Nevertheless, the use of Ag-S is often associated with the formation of “pseudo-eschar” within two to four days. This is due to the interaction of
Ag-S with proteinaceous exudates in the wound (Noronha and Almeida 2000). Since Ag-S treatment is not associated with infections that caused the dead tissues to slough off quickly, formation of hypertrophic or atrophic scar were observed in partial thickness burns (Dickinson 1973). Apart from the possibility of forming hypertrophic scars due to dead tissue sloughing retardation, Ag-S was reported to slow down wound healing (Sawhney et al. 1989). Similar result was noted in another study which illustrated that despite Ag-S gave a dry scar that was not favorable for the growth of bacteria, it also hindered the regrowth of the epidermal tissues because of dryness which led to further downward damages (Fang et al. 1989).

1.10.3 The Mode of Action of Ag-S

The chemical name of Ag-S is [4-amino-N-(2-pyrimidinyl-kappaN1) benzenesulfonamidato-kappaO]-silver. Its chemical structure is shown in Figure 1. Ag-S is a combined formulation made from silver nitrate and sodium sulfadiazine by substituting a silver atom for a hydrogen atom in the sulfadiazine molecule. (Fox 1969). The mechanism of the antimicrobial action of Ag-S has not been fully elucidated. Nonetheless, it is thought to act via inhibition of DNA replication and modifications of the cell membrane and cell wall. In addition, silver ions in Ag-S interfere relatively non-specifically with a number of enzymes, including some involved in the synthesis of the bacterial cell wall (Robb and Nathan 1981). The dominant manifestation of the reaction of Ag-S with sensitive organisms may result from the displacement of hydrogen bonds within the bacterial DNA. Once these bonds that serve to connect the two strands of the DNA double helix are displaced, bacterial replication and cell viability are effectively reduced. Since the mammalian cells have approximately one hundred times more DNA than bacterial cells, the ratio of inhibitory concentrations of Ag-S to bacterial DNA is high enough to prevent
bacterial division. The ratio of Ag-S to epithelial DNA is sufficiently low that epithelial cell regeneration is not impaired (Noronha and Almeida 2000).

1.10.4 Adverse Reactions of Ag-S

Adverse effects of topical silver therapy are limited; silver toxicity or argyrosis is uncommon and generally resolves with cessation of the therapy (Hollinger 1996). Although side effects of Ag-S are infrequent, they may include local skin reactions such as itching, skin rash and rarely, brownish-gray skin discoloration. Occasionally, cases of fungal infection of eschar have been seen (Sawhney et al. 1989). Thrombocytopenia and transient, self-limiting leucopenia are found in 5 to 15% of patients treated with Ag-S (Monafo and West 1990). Leukopenia that is associated with Ag-S administration is primarily characterized by a decreased neutrophil count. The maximal white blood cell depression occurs within two to four days after the initiation of the Ag-S therapy. Within two to three days following the onset of leukopenia, the leukocyte level rebounds to normal (Fuller and Engler 1988).

Previous study suggested that about 10% of the Ag-S applied to a burn wound was excreted via the kidneys (Grossman 1970). Furthermore, in early experimental animal studies, radioactive Ag-S was used to investigate whether the silver was resorbed. Only the skin was demonstrated to have silver presence but not in the blood or other organs (Fox 1968). In another silver resorption study, the serum silver level and the excretion of silver via the urine were determined in burn patients. No statistically significant relation was found between the size of the burn and the silver levels (Boosalis et al. 1987). A similar increase in silver concentration had also been found in an animal study without causing renal dysfunction or crystalluria (Robb and Nathan 1981). Although no renal dysfunction was observed in previous animal studies, it was suggested to take into account the theoretically possible toxicity of silver when applying Ag-S to extensive burns (Tsipouras, Rix and Brady 1995).
1.10.5 Contraindications of Ag-S

Ag-S itself had not been shown to cause birth defects or other problems in studies of rabbits treated with three to ten times the usual amount of Ag-S, yet sulfonamide derivatives were known to increase the possibility of kernicterus. Ag-S should be administered to pregnant women only when the potentially lifesaving benefits of Ag-S therapy in extensive burns (>20% total body surface area) outweighs the possible hazards to the fetus. It is not normally used in pregnant women approaching or at term, in premature infants, or in neonates less than two months of age. In addition, Ag-S should also be used with caution in patients with a history of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, as hemolysis may occur (Sawhney et al. 1989).

Despite the many attempts to find a better remedy for the topical treatment of burns than Ag-S, it is still the most frequently and widely used topical agent according to a recently published survey of the application of topical remedies in American burns centers because of its excellent spectrum of activity, low toxicity, and ease of application with minimal pain caused (Fakhry et al. 1995).

1.11 Chinese Medicine in Burn Treatment

Burn treatment in Chinese medicine has a long and remarkable history. In ancient times, burn injuries were classified as “external diseases”. Dated back to AD 281-341, Ge Hong wrote a book called “Zhou Hou Fang” which was the oldest preserved written Chinese scientific description of burn wound treatment. In his book, he suggested two topical applications of different prescriptions: old calcarea that was optionally blended with plant oil and the use of pig fat cooked willow bark. Either application would reduce the number of wound infections and was regarded as the oldest preserved Chinese description of anti-infectious astringent used in thermal injuries (Kopp et al. 2003).
According to the Chinese medicinal theory, burn injury is a disease brought about by noxious fire and heat attack. In mild cases, the damage is limited to the superficial skin layer where erythema, blisters and erosion are seen. However, in severe cases, the damage is extensive and deep to the muscles that seriously depleted the \textit{Yin}-fluid in the body leading to an imbalance of the \textit{Yin} and \textit{Yang}, together with the disturbance of the \textit{Zang Fu} (Hou and Geng 1996).

\subsection*{1.11.1 External Treatment of Chinese Medicine}

External application of selected Chinese herbal medicines can promote the eschar of deep second or third degree burns to dissolve with minimal scar remaining. Bandaging with gauze of Radix Arnebiae seu Lithospermi that includes Radix Arnebiae seu Lithospermi (10g), Radix Angelicae Dahuricae (30g), Caulis Lonicerae (30g), Radix Sanguisorbae (30g), Oleum Sesami (500g), Borneolum Syntheticum (1.5g) and wax (15g) add protection to the wound surface and enhance recovery (Hou and Geng 1996).

\subsection*{1.11.2 Internal Treatment of Chinese Medicine}

Internal treatment with Chinese medicine can be divided into three different stages: the early stage, the middle stage and the late stage. The recipes of Chinese herbal medicines and the therapeutic principles are different in respect to different clinical presentation of symptoms and signs in respective stages. In the early stage, the therapeutic principle is to nourish \textit{Yin}, clear away heat, recuperate the depleted \textit{Yang} and rescue the patient from collapse, while in the middle stage, clearing away heat and toxic materials, and cooling the blood are the main concerns. The therapeutic principles of the late stage are to vitalize \textit{qi} and blood, nourish \textit{yin} and replenish the stomach (Hou and Geng 1996). The details of the ingredients and proportions of the Chinese herbal recipes are listed in Table IX.
I. Introduction

Table IX: Different Stages of Burns Treatment with Chinese Medicines (Hou and Geng 1996)

<table>
<thead>
<tr>
<th>Early Stage of Treatment</th>
<th>Middle Stage of Treatment</th>
<th>Late Stage of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipe:</td>
<td>Recipe:</td>
<td>Recipe:</td>
</tr>
<tr>
<td>• Radix Rehmanniae 30g</td>
<td>• Radix Rehmanniae 30g</td>
<td>• Radix Codonopsis 15g</td>
</tr>
<tr>
<td>• Radix Seriphulariae 30g</td>
<td>• Radix Seriphulariae 30g</td>
<td>• Radix Astragal 15g</td>
</tr>
<tr>
<td>• Flos Lonicerae 30g</td>
<td>• Flos Lonicerae 30g</td>
<td>• Radix Andelicae Sinensis 15g</td>
</tr>
<tr>
<td>• Radix Ophiopogonis 15g</td>
<td>• Rhizoma Coptidis 10g</td>
<td>• Rhizoma Polygonati Odorati 15g</td>
</tr>
<tr>
<td>• Fructus Forsythiae 15g</td>
<td>• Radix Scutellariae 10g</td>
<td>• Radix Rehmanniae 10g</td>
</tr>
<tr>
<td>• Rhizoma Anemarrhenae 15g</td>
<td>• Cortex Phellodendri 10g</td>
<td>• Radix Rehmanniae Praeparata 10g</td>
</tr>
<tr>
<td>• Radix Salviae Miltiorrhizae 15g</td>
<td>• Fructus Gardeniae 10g</td>
<td>• Rhizoma Atractylodis Macrocephalae 10g</td>
</tr>
<tr>
<td>• Rhizoma Coptidis 10g</td>
<td>• Cortex Moutan 10g</td>
<td>• Radix Paeoniae Alba 10g</td>
</tr>
<tr>
<td>• Fructus Gardeniae 10g</td>
<td>• Radix Salviae Miltiorrhizae 15g</td>
<td>• Poria 10g</td>
</tr>
<tr>
<td>• Radix Ginseng 12g</td>
<td>• Fructus Forsythiae 15g</td>
<td>• Radix Ophiopogonis 10g</td>
</tr>
<tr>
<td>• Radix Aconiti Lateralis Praeparata 10g</td>
<td>• Cornu Rhinocerotis 3g</td>
<td>• Radix Glehniae 10g</td>
</tr>
<tr>
<td>• Fructus Schisandrae 10g</td>
<td>• Herba Lophatheri 6g</td>
<td>• Fructus Setariae Germinatus 10g</td>
</tr>
<tr>
<td></td>
<td>• Radix Glycyrrhizae 6g</td>
<td>• Fructus Hordei Germinatus 10g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Radix lycyrrhizae 6g</td>
</tr>
</tbody>
</table>

1.12 The Moist Exposed Burn Therapy (MEBT) and Moist Exposed Burn Ointment (MEBO)

MEBT and MEBO were invented two decades ago by Xu Rong-Xiang of the Beijing Chinese Burn Center. He is also the chief editor of the Chinese Journal of Burns, Wounds and Ulcer Society. As a researcher in the burn field, Xu put forward a moist therapy for burns which was contrary to the conventional dry therapy. His invention, MEBT, was a burn therapy that was designed to combine both the traditional Chinese medicine and modern medical science in treating patients suffering from minor to massive deep burns. MEBT was applied along with MEBO for achieving the desired therapeutic actions. With this invention, four major difficulties in burn treatment were claimed to be achieved: the control of infection and pain, the decrease of scar formation and the prevention of progressive necrosis of burn tissues (Xu 1989b).
I. Introduction

1.13 Moist Exposed Burn Therapy (MEBT)

Using MEBO, MEBT was a therapeutic procedure claimed to create an optimal physiological moist environment for the damaged burn tissues, thereby to promote necrotic tissue discharge and epithelial regeneration. The general theory behind MEBT was rather vague and broad that comprised of the followings (Xu 1989a):

1. Protection of the injured nerve endings and alleviation of the spasm of arrector pilorum of fine hair to relieve pain.
2. Absorption of the residual heat in burn wound using a frame-structured ointment to avoid secondary thermal injury.
3. Removal of necrotic skin through a non-damaging liquefaction process, to promote the regeneration of surviving tissue.
4. Provision of a physiological moist environment to protect the wound and the surviving tissue in order to promote wound repair.
5. Triggering skin regeneration with a mode in compliance with tissue regeneration.
6. Alteration of the bacterial ecology through promoting active drainage and interfering bacterial growth so that bacterial infection could be controlled and prevented.
7. Control of the physiological wound repair process by the comprehensive efficacy of the drug.

1.14 Moist Exposed Burn Ointment (MEBO)

MEBO was a topical agent particularly developed for MEBT in treating burns and scalds. It is light yellow-brown in color, consists of natural ingredients including beeswax, sesame oil, seventeen amino acids, fourteen fatty acids and four polysaccharides. The major active substance in the ointment is B-sitosterol in a concentration of 0.25%. MEBO also contains Chinese medicinal ingredients such as Radix Scutellaria, Cortex Phellodendri and Rhizoma Coptidis that were used to clear away heat and toxic materials in Chinese medicinal terms, to relieve pain, and to promote regeneration (Xu 1989b).
MEBO application was an open treatment that was supposed to provide a necessary moist environment for optimal healing and re-epithelialization without the need of additional covering dressing (Ioannovich et al. 2000). It was said to offer painless application and reduced the amount of fluids required for resuscitation (Dham et al. 1999).

1.15 Physiological Mechanisms of MEBT and MEBO

According to the inventor of MEBO, MEBO is a lipophilic ointment that adheres firmly to the burn wound and protects it by inhibiting evaporation. When the necrotic tissues start to liquefy and at the same time produce a hydrophilic liquefaction product, MEBO becomes non-lipophilic. This change is said to produce a dual effect that initially favors MEBO to react with the necrotic tissues when it is still lipophilic. Later, when MEBO loses its lipophilic property, it can be removed from the wound and creates an active drainage for the wound (Xu 1989a).

The frame structure and pharmacokinetic features of MEBO are said to account for its claimed moisture maintaining ability. MEBO has a beeswax base acting as a frame structure that embraces the Chinese medicinal ingredients in refined oil. The oil isolated in the frame is supposed to warm up at the body surface temperature when applies onto the wound and becomes in equilibrium of two phases, the liquid and semi-solid phase. It is then released from the frame and penetrates into the wound to ensure a continuous supply of indispensable nutritional substances to the surviving cells in the wounds. As the freshly applied MEBO continues to penetrate into the tissue, the liquefied necrotic skin layers are constantly removed from the superficial to the interior. With such active drainage effect, excessive water, liquefaction products and excretions are said to be removed through the drug layer. This liquefaction period lasts until all the necrotic tissues are discharged and removed (Du 1993). While the frame structure of MEBO base isolates the wound from external irritation and prevents excessive water evaporation from the wound surface, the claimed physiological moist environment is said to enhance wound recovery. In addition, since the MEBO base contains both hydrophilic and lipophilic groups and has high affinity to the wound tissue than water, it has been supposed to form
a strong adsorptive film on the wound to protect the wound from either maceration or dehydration (Xu 1998b).

1.16 The Claimed Therapeutic Effects of MEBO

MEBO has been claimed to pose the following therapeutic effects: antibacterial, analgesic, reduction of water evaporation from burn wound surface, promotion of epithelial repair and improved scar formation (Ioannovich et al. 2000).

1.16.1 Antibacterial Effects

Prevention of infection is the immediate goal of burn wound treatment so as to optimize regeneration. MEBO has been claimed to act mainly as a hyperosmolar medium which prevents bacterial growth. At the same time it changes the biological behavior of bacteria, decreases the bacterial toxicity and invasive capacity, increases the bacterial sensitivity to antibiotics and enhances both the local and systemic immunity (Ioannovich et al. 2000). In an experimental study, the antibacterial effect of MEBO was tested against anaerobic spore-bearing bacillus, anaerobic non-spore-bearing bacillus and fungi. The results revealed that there were variations of morphological structure and culturing characteristics of these bacteria. Their proliferation rate and invasive power were also significantly reduced (Qu et al. 1998). Furthermore, MEBO was said in one study to have similar antibacterial ability as Ag-S especially in the control of burn wound sepsis and systemic infection caused by *P. aeruginosa* (Xing 1989). However, another study demonstrated that MEBO had the worst antibacterial effect among other commonly used Chinese burn ointments (Wang et al. 1996). Similarly, MEBO showed no demonstrable bacteriostatic and bactericidal activity *in vitro* in another study conducted by Qu et al. It was believed that its oily composition hindered diffusion in a watery culture medium (Qu et al. 1996). In a literature review, MEBO was reported to have no antibacterial effects at all. On the contrary, it enhanced the growth of bacteria and sepsis in regions of high humidity by its excessive liquefactive necrosis (Cai and Wang 2001).
such conflicting results, it is thus worth conducting a thorough experiment that particularly investigates the antibacterial ability of MEBO.

1.16.2 Analgesic Effects

A recent study that evaluated the analgesic property of MEBO was conducted by randomly assigning one hundred fifty patients with partial thickness burns to either conventional or MEBO treatment. A verbal numerical rating score of pain was made in the morning, after burn dressing and some eight hours later. The patient pain profiles were analyzed statistically and was concluded that MEBO appeared to bring greater pain relief for the postdressing assessment during the first week after burns (Ang et al. 2003). However, due to the difference in the degree of pain tolerance among different patients that may include gender and age difference, it is difficult to provide objective evidence that truly reflects MEBO’s analgesic property. In a study that compared the efficacy of MEBO and Ag-S in treating perineal deep second degree burns, the degree of pain, infection, scar formation and the need for surgical operation in the MEBO group were significantly lower than that of the Ag-S group (Dai 2000). Nevertheless, bias might be involved in the pain level measurement as the experimenters relied on the crying response of the children such as quiet or restless and loud or soft crying noise as the rating indices. In addition, most of the experiments that claimed MEBO to have excellent efficacy were published in the “Chinese Journal of Burn Wounds and Surface Ulcers” whose chief editor is in fact Xu Rong-Xiang, the president of the MEBO Company. This can obviously lead to bias.

1.16.3 Reduction of Wound Water Evaporation

In early postburn period, the increase in capillary permeability is one of the major pathophysiological changes. This leads to body fluid loss containing a large amount of plasma protein and electrolytes, and thus further decrease in the effective circulatory blood volume causing shock eventually. It had been suggested that wounds healed more readily under a moist, physiological environment (Field and Kerstein 1994). Therefore,
the maintenance of a moist wound environment might facilitate the wound healing process at least in theory. An experimental study on a rabbit model showed that MEBT and MEBO inhibited water evaporation from the wound and thus decreased body fluid loss. It claimed that MEBO was beneficial to prevent shock at the early stages of postburn and slowed down hypertonic dehydration at the liquefaction period (Pu et al. 1999). Another study demonstrated that wounds treated with MEBO had higher moisture values than those treated with Ag-S and povidine iodine (Ioannovich et al. 2000).

On top of preventing wound water evaporation, MEBO was also claimed to protect the tissues in the zone of stasis from progressive damage (Fang et al. 1989). Since burn injury destroyed body surface barrier and then accelerated the rate of water loss from the body through evaporation, burn wound would be deepened and enlarged due to dehydration and necrosis if no measure for preventing evaporation was adopted. Although the injured tissue in the zone of stasis could be recovered with enough fluid replacement, excessive dryness would lead to irreversible pathological changes of the tissues. MEBO was suggested to have a sebum-like ability for water retention to maintain the wound moisture without affecting drainage. Not only would the retention of biologic fluids over the wound prevent desiccation of the denuded dermis and deeper tissues, it also allows faster and unimpeded migration of keratinocytes over the wound surface (Kerstein 1997). In a study, the MEBO treated wounds were shown to have good permeability and adequate drainage and did not obstruct water evaporation from wound as Vaseline did. It was postulated that the maintenance of a physiological moist environment in the burn wound by MEBT and MEBO could promote the discharge and removal of necrotic tissues, and consequently enhanced the regeneration of the wound (Pu et al. 1999). In another experimental model, MEBO was found to provide a moisturizing environment that significantly accelerated the wound healing process in partial thickness burns compared with Ag-S and povidone iodine (Ioannovich et al. 2000). Nonetheless, it should be noted that there were possible adverse effects created by the moist environment of MEBO because excessive wetness of the burn wound might induce secondary tissue injury by maceration. It might also promote bacterial colonization which was the major cause of wound infection, including wound sepsis. Since the physiological
moist environment created by MEBO might lead to some hazardous effects, studies that focus on monitoring the histological wound healing process and particularly look for signs of infection and progressive wound deepening and enlargement should be conducted to verify the claimed advantages provided by the moist environment.

1.16.4 Wound Healing Ability of MEBO

In terms of wound healing ability, MEBO had been demonstrated experimentally to pose a statistically significant wound healing potential in the rabbit corneal epithelium when compared to saline, vitamin A, dexamethasone and homologous serum (Huang et al. 1995). Furthermore, it had been shown that the rabbit skin alkaline burns treated with MEBO healed much faster and with less scar than similar burns treated with Vaseline (Wang et al. 1989). Although there were Chinese literature studies suggested that MEBO had a better wound healing ability than Ag-S which was a proven conventional burn ointment, those studies were again published in the Chinese Journal of Burn Wounds and Surface Ulcers that might involve conflicts of interest (Cui and Yang 1999; Guo 1999; He and Qin 2002; Lin and Wu 1997; Wang 2000; Zhao 2000). Thereby, an experiment conducted with a neutral stance towards the wound healing effects of MEBO and Ag-S is needed to provide objective evidence on the efficacy of MEBO. In fact, MEBO is not as miraculous as the proponents proposed. The application of MEBO may also give rise to adverse effects such as tetanus, sepsis, gas gangrene and dermatitis (Zhang, Wang and Que 1998).

1.17 Rationales for the Importance of Burn Treatment Research

Burn injury is a worldwide problem that concerns both the developed and developing countries (Singh et al. 1998; Wilkinson 1998). The recognition of the advantage of debridement and the advances in burn care management continue to reduce mortality and improve quality of life for burn victims. Nevertheless, thermal injury is still recognized as among the most painful and devastating injuries a person can sustain and survive that
leave the victims with lifelong physical and psychological trauma. In particular, it is an important cause of mortality and morbidity in children, especially among those under five years old. Special attention to burn injury is required because it may cause deformities and have a lifelong sequel on the subsequent development of the injured child (Rivera 1995). Thus, it is meaningful and necessary to conduct research on the currently available burn ointments in the market by comparing their properties and efficacies in order to provide experimental evidence and reliable guidance on burn ointments selection.

1.18 Objective of This Study

MEBO had recently been praised to be an outstanding burn treatment topical agent that revolutionized the conventional dry topical therapy, yet its claimed effects were currently under furious debate because of the many contradictive results obtained from literature studies other than those published in the Chinese Journal of Burn Wounds and Surface Ulcers which was owned by the inventor of MEBO. In hope of clearing away the mist of the mythical therapeutic value of MEBO, this study focused on two particular therapeutic effects claimed by MEBO, the antibacterial effect and the wound healing ability.

This study was to be carried out based on the experimental model of last year’s pilot study in which MEBO was demonstrated to have little antibacterial or healing effect which was contradictory to what had been claimed. In this study, better control settings and expanded scale were employed to consolidate the preliminary result in a more systematic manner. In particular, the comparison between the efficacy of MEBO and Ag-S that was not included in last year’s research would be conducted using a rat model. In short, the aim of this study was to investigate the antibacterial effect of the Moist Exposed Burn Ointment (MEBO) in comparison with Silver Sulfadiazine (Ag-S) on deep burn wounds (second to third degree burns) and to assess their efficacy on wound healing. It was hypothesized that the use of MEBO in the treatment of deep burn injury was a viable and desirable alternative to the use of Ag-S.
II. METHODS AND MATERIALS

2.1 Equipments and Materials

2.1.1 Animal Model

- 14x Male Sprague-Dawley Rats
- 2x Brass Rods (1.96 kg each; 30cm long; contact surface of 2cm in diameter)
- 2x Heat-proof Gloves
- 1x Hot Plate
- 1x Stainless Steel Pot
- 1x Thermometer
- 1x Timer
- 1x Electric Shaver, size 40, 1/10mm, Oster
- 1x Weight Balance, Navigator™, OHAUS®
- 1x Water Soluble Marker
- 14x Jelly/Salad Plastic Cups (227mL, a hole with a diameter of 2.6cm is drilled at the bottom that serves as a neck collar), Good Life™
- 1x 1mL Syringe (0.5x16mm), Terumo®
- 3g Choral Hydrate Riedel-deHaën, RdH Laborchmikalien GmbH & Co., stored in 4°C
- 1Kg 5001 Rodent Diet, LabDiet®, PMI® Nutrition Internal Inc.

2.1.2 Postburn Treatment with Topical Burn Ointments

- Moist Exposed Burn Ointment (MEBO), 40g, Beijing Guangming Chinese Medicine Institute for Burns, 國藥準字：Z20000004, Expiry Date: January, 2005-March, 2006.
- Dermazin® Cream (Ag-S), 25g, Lek Pharmaceutical and Chemical Company, Expiry Date: November, 2005.
- Autoclaved Wooden Tongue Depressors, Clinicon Medical Ltd.
- Autoclave Machine, Autoclave ES-315, Kou Hing Hong Scientific Supplies Ltd.
2.1.3 Wound Size Measurement and Morphological Appearance Recording

- Diethyl Ether, \((C_2H_5)_2O\), *Farco Chemical Supplies*
- Transparent Plastic Box (18cm x18cm x18cm) with a lid
- Cotton Wools
- 75% Alcohol
- Transparency Papers
- Permanent Markers
- Graph Papers
- Sony Digital Still Camera (DSC-P7/P9)

2.1.4 Staining and Histological Analysis

- Forceps
- Petri Dish, Sterilin
- Sterilized Bottles and Beakers
- Filter Paper, Qualitative I, Whatman
- Thermo Shandon Cryotome
- 10% Formalin, Fisher Band
- Harris Hemotoxylin, Sigma Diagnostics, Expiry Date: May, 2004
- Eosin Y Solution Alcoholic, Sigma Diagnostics (Accustain)™, Expiry Date: May, 2004
- Absolute Ethanol, Fisher Band
- HCL, 37% BDH
- Xylene, \(C_6H_4(CH_3)_2\), UNI-CHEM Chemical Reagent
- Cover Slips
- Mounting Liquid, Sigma
- Microscope slides 72 pcs, Sail Brand
- Software for taking histology photo: SPOT version 3.4 for windows, *Diagnostic Instruments Inc.*
II. Methods and Materials

2.1.5 Wound Bacterial Culture and Spectrophotometry

- 1x Bunsen Burner
- 1x Pipette, 1mL, Pipetteman
- 210x 16mL Snap cap culture tube sterilized by gamma radiation
- 210x Curvettes (10 x 4 x 45mm), SARSTEDT, Aktiengesellschaft and Co.
- Nutrition Broth 3g/L, Qxid Ltd.
- Fume Cupboard
- Orbital Shaker, Kou Hing Hong Scientific Supplies Ltd.
- Hitachi U-2001 Spectrophotometer 600nm

2.2 Experimental Procedures

2.2.1 Animal Preparation

Fourteen adult male Sprague-Dawley rats weighing between 240g and 260g were obtained from the Laboratory Animal Center at the Chinese University of Hong Kong (Shatin, Hong Kong) were used. The rats were housed in an environmentally controlled breeding room (temperature: 20 ± 1°C, humidity: 55 ± 5%, 12 hours light-dark cycle). They were fed with standard laboratory chow with water ad libitum. The animals were kept in separate cages and acclimatized for one week before the experiment started. Consequently, the animals gained weight to between 280g and 310g at the beginning of the experiment.

This experiment was conducted in the School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong. The rats were first weighed and subsequently anaesthetized with an intraperitoneal injection of choral hydrate (2mL/kg) that was freshly dissolved in distilled water (2g/10mL). A supplementary dose was injected when necessary. The lower back portion of the animals was closely shaved using an electric clipper (Figure 2). Plastic jelly cups with a hole of 2.6cm in diameter cut at the bottom (Figure 2) were put around the neck of the rats as collars to prevent them from licking their wounds. Apart from the concern of cross-contaminations, ingestion of the topical
burn agents that might have systemic or gastrointestinal effects on the animals could thus be prevented by the collars.

2.2.2 Thermal Injury

Two deep burn wounds (deep II-III°) were first created by a cylindrical brass rod with a diameter of 2cm at the contact end (Figure 3). Two brass rods of the same weight and applied diameter were immersed in a thermostatic water bath where the water was boiled and was kept equilibrated for half an hour (Figure 3).
The rods were used alternatively and were briefly blotted before putting in contact with the rat’s skin to create two identical burn wounds 1 cm above the upper iliac crest of the rat. The heated rod was placed on the back for twelve seconds creating the deep burn injury. The twelve seconds duration was used based on last year’s pilot study where both twelve seconds and eighteen seconds created indistinguishable burn of deep second to third degrees. There was no additional pressure applied to the rod while in contact with the skin except the natural gravity. The depth of burns was later verified by histological examination. The used brass rod was re-equilibrated in the boiling water bath, while the other brass rod was used to create a second burn wound on the opposite side such that each animal received two identical burns, one on each side of the back (Figure 4).

![Figure 4: Identical burn wounds were created on the opposite side of the animals.](image)

### 2.2.3 Treatment

The Ag-S and MEBO treatments were applied on the burn wounds on the opposite sides, that is, all the left burn wounds were treated with Ag-S and all on the right were treated with MEBO (Figure 5). Both Ag-S and MEBO were smeared on the wounds to a thickness of around 1 mm using the autoclaved tongue depressors. The topical burn agents were applied four times a day at 8:30 a.m., 1:00 p.m., 5:30 p.m. and 10:00 p.m., with a ten and a half hours break at night. By following last year’s pilot study, daily debridement
II. Methods and Materials

was not performed prior to the application of topical agents so as to limit the amount of irritations, secondary injury and induced stress.

Figure 5: Ag-S and MEBO were applied on the left and right wounds respectively.

2.2.4 Daily Observations and Healing Progression Records

The general behavior of the rats, the gross appearance of the wounds, the evidence of infection and bleeding, the type of granulation tissues and the time taken for healing were noted daily until the wounds were healed. Apart from daily observation of the gross pathological changes, the wound tissues and tissues from important organs such as the lung, the stomach and part of the small intestines were sampled whenever there were unusual animal deaths. If the animals survived the whole course of experiment, they were euthanized for skin biopsies on day 30.

2.2.5 Histological Processing and Microscopic Analysis

The wound biopsies were fixed in 10% neutral formalin for at least a week before they were sectioned with a cryotome into 15μm sections. The sections were mounted on the Meyer affixative coated slides before passing through the hematoxylin staining procedures including alcohol dehydration and xylene clearing (Figure 6). The processed sections were protected by cover slips and were studied with a light microscope for
II. Methods and Materials

histopathologic analysis. The images were photographed at 40x, 100x, 200x and 400x magnifications using an Olympus CX-41 camera. These sections were then used to analyze the vascular and inflammatory changes that occurred during the early postburn period, confirm the depth of burns and examine the pathological changes and recovery.

![Figure 6: The procedures of hematoxylin and eosin staining.](image)

2.2.6 Bacterial Culture Absorbance of the Wounds

On days 5, 10, 15, 20 and 30, swab samples were collected from the surface of each burn wound and from the rat’s normal skin (the mid-point between the centers of the left and right burn wounds) for quantification of the bacterial concentration by means of spectrophotometry which was expressed in light absorbance (Figure 7). A standard platinum wire loop of 0.5mm in diameter was used to obtain specimen from the wound and skin surface. The loop was heated by a Bunsen burner flame until the entire wire was red hot. It was then cooled down in a sterile solution. After the loop was cooled down, it was used to obtain the bacterial culture by a single, light contact with the center of the wound surface. The loop was then quickly inoculated into a 3mL nutrient broth in a labeled culture tube, followed by a few gentle shakes (Figure 7). The loop was reheated and cooled down again. The above steps were repeated until all wound specimens were
collected. The whole process was carried out with extreme care and was performed close
to a Bunsen burner flame in a fume cupboard to ensure sterility.

![Image](image1.png)

**Figure 7** Bacterial samples were collected with a platinum loop closed to a Bunsen burner flame (left). The nutrition broth, the labeled culture tubes, the test tube rack and the platinum loop (right).

The inoculated culture tubes were shook in an orbital shaker for 12 hours at 37ºC and
270rpm. After 12 hours, 1mL of each bacterial cultured sample was pipetted into a sterile cuvette. The bacterial concentration was measured by the Hitachi-U-2001 spectrophotometer at 600nm wavelength, calibrated with a 1mL sterile nutrient broth. The primary function of a spectrophotometer is to determine the concentration of a sample solution. It shines a set wavelength of light through a sample while the machine determines the percentage of light that is absorbed by the sample. The higher the concentration, the larger is the light absorbance. The reading thus reflects the concentration of bacteria in the nutrient broth in our scenario.

### 2.2.7 Wound Size Measurement and Healing Percentage Calculation

The wound sizes of the rats were also measured on days 5, 10, 15, 20 and 30. Each rat was transferred to a transparent, air tight plastic box with diethyl ether soaked cotton wools for general anesthetization by inhalation. The small volume of the box limited the movements of the rat and saved the drug; yet there was sufficient air space to prevent suffocation when the lid was closed (Figure 8). As soon as light anesthetization was
achieved (when the rat stopped moving), the rat was taken out for weighing, photographing and tracing of the wound size. A 75% alcohol cleaned transparency was laid on the wound surface while the wound size on the transparency was outlined with a permanent marker (Figure 8).

![Image showing a rat and a wound outline on a transparency.]

**Figure 8:** The rat was lightly anesthetized with diethyl ether (left). The size of the wounds was outlined on an alcohol cleaned transparency (right).

The wound size was later measured by counting the number of squares on a standard graph paper that were included by the wound outlines on the transparency. The healing percentage of the wounds was calculated by comparing the wound size on day 30 to that of day 5 for both treated groups.

### 2.2.8 Data Analysis

The results regarding the bacterial culture absorbance, wound size and healing percentage of both Ag-S and MEBO groups collected on days 5, 10, 15, 20 and 30 were compared and analyzed statistically using the paired $t$-test. The results were considered significant if $p<0.05$. The Microsoft® EXCEL 2002 program was employed in the data processing. The data were presented as mean value ± S.E (Standard Error).
III. RESULTS

3.1 Weight Variations of the Rats over the Course of Treatment

![Graph showing weight variations](image)

Figure 9: The average weight of the rats over the 30 days postburn period.

The average weight of the twelve rats (excluding the two rats that died on days 7 and 10 respectively) used was 300.4g initially after acclimatizing for one week before the start of the experiment. There was a sharp decrease of 34g, from 300.4g to 266g, four days after the induced burn injury (Figure 9). From day 5 onwards, the rats gradually gained weight to 273.1g, 286.2g, 294.6g and 305g on days 10, 15, 20 and 30 respectively.

3.2 Abnormal Signs and Behaviors Observed from the Rats after the Burn Injury

Soon after the burn injury, the animals immediately showed signs of pain. Almost all of the rats arched their backs after the thermal trauma until around day 5 postburn. Piloerection (erection of hair) without chills was another sign observed that gave the rats a spiky outlook (Figure 10). Also, nose bled was found in four of the rats on day 2
III. Results

postburn (Figure 10), which might be due to nasal mucosal capillary damage resulted from dehydration after the burn injury. Occasionally, diarrhea was observed in some of the rats which were also lighter in weight. Hair loss was another major sign noticed from the animals that was observed in half of the rats in which plenty of hair was collected from their beddings during the daily routine beddings change. Most of the hair loss was from the upper limbs, the lower abdomen and the lower limbs (Figure 11). Hair loss started to cease around day 15. Subsequently, new hair was seen around the bare regions that finally resumed the original hairy appearance by day 30 (Figure 12). Almost every time when MEBO was applied to the wound, the animal used its hind leg to scratch the MEBO wound. In contrast, it did not scratch when Ag-S was applied. The scratching reflex may be indications of pain or irritations.

Figure 10: Abnormal signs observed from the rats in the early postburn phase -- spiky hair (left) and nose bleed (right).

Figure 11: Severe hair loss from the upper limbs, the lower abdomen and the lower limbs on day 15.
III. Results

3.3 Gross and Microscopic Pathomorphological Findings

At the beginning of the experiment, fourteen rats were available for assessment. However, two of them died on days 7 and 10 respectively, possibly from pulmonary, gastrointestinal and/or renal complications. The dead animals were autopsied to look for possible causes of their unexpected death. Moreover, tissue samples were taken from the lung, the stomach and the small intestines for histopathological analysis.

Grossly from the autopsy, there were obvious pulmonary and gastric mural hemorrhage and edematous enlargement of the large intestines (Figure 13). In addition, hemouria was noticed from the dark urine stained beddings and the hair of the groin region (Figure 14). Microscopically, sections obtained from the lung revealed arterial congestions with hemorrhagic appearance (Figure 15). At higher magnification, the alveolar blood vessels were engorged with blood. Apart from alveolar damage, erosions were apparent in the gastroduodenal regions (Figure 16). Tissue necrosis, however, did not extend through the full thickness of the wall. The submucosa and the muscle layers were still intact. Moreover, the margins of the lesions were lined by necrotic tissues. Fibrous granulation tissues or fibrous scars were absent because the destructive process was too soon to have any apparent repair.
Figure 13: Pulmonary and gastric wall hemorrhage (top); edematous enlargement of the large intestines (bottom) in the early dead rats.
**III. Results**

Figure 14: Dark bloody urine observed on the beddings (top); bloody urine that stained the hair of the groin region (bottom).

Figure 15: Pulmonary alveolar hemorrhage and blood vessels congestion; 40x (left) and 100x (right). Alveolar sacs (AS), pulmonary vessels (V).
III. Results

3.4 Gross Physical Appearance of the Burn Wounds

3.4.1 The Early Phase Appearance of the Burn Wounds

In the early phase (usually 3 to 4 days postburn) after the thermal injury that created two identical round wounds on the back of the rats, wounds in both groups were round and clearly demarcated. They appeared pale with mild swelling over the level of the wound edges (Figure 17).
III. Results

3.4.2 The Physical Appearance of the MEBO Treated Wounds on Days 5 and 10

The wounds treated with MEBO were always brown (possibly stained by MEBO which is brown in color) and oily with zones of blister-like edema that began to subside around day 5 postburn. No slough was present over the wounds resulting in a clear assessment of the healing progression. The wounds remained round and were encircled by an obvious red outline which was not artificially drawn (Figure 18).

However, the wounds became macerated and deteriorated further from day 8 onwards. The necrotic tissues began to liquefy which gave the MEBO treated wounds a brownish and oily appearance. The hair grown from the surrounding uninjured skin was stained brown and appeared oily as well. On day 10, almost all of the MEBO treated wounds
were enlarged with an irregular shape. They were dominated by a yellowish color in the central area of the wounds together with a small proportion of red areas in the periphery (Figure 19).

3.4.3 The Physical Appearance of the Ag-S Treated Wounds on Days 5 and 10

The changes in physical appearance of the Ag-S treated wounds were quite different from those observed in the MEBO treated group. The Ag-S treated wounds did not appear oily, yet they had some Ag-S residues piling on top which appeared grayish-white (Figure 20). Moreover, the wounds treated with Ag-S were round in shape and did not show tissue swellings after day 5. On day 10, almost all of the Ag-S treated wounds remained round with no enlargement (Figure 21). They were generally grayish white in color encircled by red edges.
3.5 Degree of Wound Healing

The percentages of wound healing of the Ag-S and MEBO treated groups on days 10, 15, 20 and 30 were shown in Figure 22.

![Figure 22: The wound healing percentage of the Ag-S and MEBO treated groups on days 10, 15, 20 and 30.](image)

The wound healing percentage of day 10 which was obtained by comparing the wound size difference between days 5 and 10 was 6.4% for the Ag-S group and 4.8% for the MEBO group. The wound healing percentage increased further to 29.6% and 50.8% for the Ag-S group and 22.8% and 49.7% for the MEBO group on days 15 and 20 respectively. Finally, on day 30, the wound healing percentage of the Ag-S group reached 90.1% while the MEBO group attained 88.4%. Nevertheless, no significant difference was found in the wound healing percentage between the Ag-S and MEBO treated groups in all four days (days 10, 15, 20 and 30) with \( P > 0.05 \) (Table X).

| Table X: The Wound Healing Percentage (%) of the Ag-S and MEBO Treated Groups |
|-----------------|------|------|------|
| Day             | Ag-S | MEBO | P-value |
| Day 10          | 6.4% | 4.8% | 0.42   |
| Day 15          | 29.6%| 22.8%| 0.12   |
| Day 20          | 50.8%| 49.7%| 0.38   |
| Day 30          | 90.1%| 88.4%| 0.28   |
3.6 Histological Analysis

Histological analysis was used to determine the true burn depth. The state of viable adnexal structures (hair follicles) provided helpful information of the level of burn depth. Apart from the necrosis observed in the surrounding tissues, it could be inferred that thermal injury had reached at least that depth if such structure appeared damaged. On day 5 after the infliction of burn injury, histological samples taken from both the MEBO and Ag-S treated groups showed that the burn depth had reached the deep dermis (D) layer that was equivalent to deep second to third degree burn injury (Figure 23). The epidermis layers of both groups were completely destroyed leaving behind a few hair follicles (F) with the hair shafts gone. The deep dermal structures and the fibroadipose tissues (M) were viable, indicating deep second degree burn at least.

Figure 23: The histology of the MEBO (left) and the Ag-S (right) treated wounds on day 5. Dermis (D), hair follicles (F), hypodermis (H), subcutaneous fat (SF) and blood vessels (V).
On the day 10 histology of the Ag-S treated wound, severe hyperemia and granulation tissues that were close to the dermis were present (Figure 24). It was due to blood vessels dilatation, infiltration of white blood cells and proliferation of fibroblastic cells. Since the microcirculation was still in a dilated and congested state, the infiltrated inflammatory cells were found spreading around the blood vessels. Hyalinization that gave a glass-like appearance could be seen, as when the skin was thermally damaged, the fibers lost their linearity and fused with their neighbors forming dense aggregative degeneration of collagens. The appearance of hyalinization also suggested that thermal injury took place to that particular depth.

Figure 24: The Ag-S treated wound on day 10.

By day 30, almost all wounds showed complete healing. Microscopic examinations revealed complete regeneration of the epidermis (E) and dermis (D) with few scars present in both groups. The healing of skin involved epithelialization with epithelium proliferation at the edges of the lesions and granulation tissue formation. Furthermore, fibrous scar could be seen clearly with some condensed nuclei of inactive fibroblasts and capillaries persisted, accounting for the red appearance of recent scars. The immature collagenous tissue formed a pale scar (S) that was distinctive from the normal pink collagen of the dermis. In addition, there were no skin appendages in the scar. A region with lots of vacuoles (V) remaining was noticed in the MEBO wound that was left behind from previous inflammatory response (Figure 25). Concerning the current experiment, it was important to point out that there was no histological difference between the MEBO and Ag-S wounds in terms of wound healing.
III. Results

Figure 25: The histology of the MEBO (top) and Ag-S (bottom) wounds on day 30. Keratin (C), dermis (D), epidermis (E), hair follicles (F), hypodermis (H), scars (S) and fluid-filled vesicles (V).

3.7 Light Absorbance (A) of Bacterial Culture in Different Groups

The light absorbance of bacterial culture collected from the MEBO and Ag-S treated burn wounds and from the normal skins in five sample collection days (days 5, 10, 15, 20 and 30) were represented by a bar chart in Figure 26.
### III. Results

The Light Absorbance (A) of Bacterial Culture Collected from the Burn Wounds of Both the Ag-S and MEBO Treated Groups and the Normal Skins at 600nm Wavelength (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag-S</td>
<td>0.01</td>
<td>0.22</td>
<td>0.88</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>MEBO</td>
<td>0.15</td>
<td>1.16</td>
<td>1.67</td>
<td>1.17</td>
<td>0.35</td>
</tr>
<tr>
<td>Norm</td>
<td>0.05</td>
<td>0.19</td>
<td>0.77</td>
<td>0.22</td>
<td>0.03</td>
</tr>
</tbody>
</table>

![Graph showing light absorbance over time](image)

Figure 26: The light absorbance of bacterial culture collected from the burn wounds of both the Ag-S and MEBO treated groups and the normal skins.

On day 5, moderate amount of bacteria with the absorbance of 0.15A was found on the MEBO treated wounds as compared to the normal skin control group of 0.05A and Ag-S treated group of 0.01A (Figure 26). The absorbance of the MEBO group continued to increase to 1.16A and 1.67A on days 10 and 15 respectively. Similarly, the absorbance peaked at day 15 in all the groups. Nonetheless, the maximum absorbance of the MEBO group was 1.67A while the maximum absorbance of the Ag-S group was 0.88 which was almost half the value of the MEBO group. After day 15, the absorbance of the MEBO group decreased to 1.17A and 0.35A on days 20 and 30 respectively which were still much higher than that of the normal skin control group (0.22A and 0.03A) and the Ag-S group (0.25A and 0.05A).

The comparison of the antibacterial ability of the MEBO and Ag-S was very significant with the $P$-values less than 0.01 ($P<0.01$) except for day 5 ($P=0.012$) which was also
III. Results

significant \((P<0.05)\) (Table XI). The results suggested that MEBO had a poor antibacterial ability because a large amount of bacteria was growing on the wound as represented by the high absorbance values.

Conversely, the results revealed that Ag-S had an excellent antibacterial ability as shown by the consistently lower light absorbance values for all five sample measurement days that had no significant difference \((P>0.05)\) from that of the normal skin control group (Table XI). On day 5, the light absorbance of Ag-S (Figure 26) was extremely small \((0.01A)\) which was even smaller than the normal skin control group \((0.05A)\) \((P=0.031)\). However, the absorbance increased to 0.22A on day 10. Eventually, it peaked at 0.88A on day 15 and dropped to 0.25A and 0.05A on days 20 and 30 respectively. It should also be noted that the absorbance of the normal skin control group also peaked at day 15 and varied closely with the Ag-S treated group on days 20 and 30. The reason behind the peaked absorbance of the normal skin control group on day 15 was that there may be bacteria spreading from the nearby MEBO wounds whose absorbance also peaked at day 15 \((1.67A)\) which was more than twice the absorbance value of the normal skin control group.

Concluding from both the gross and histological observations, our results did not show any significant difference in the ultimate wound healing of the MEBO and Ag-S treated wounds. Nevertheless, wounds treated with MEBO consistently showed a larger amount of bacterial colonization than the Ag-S treated wounds.
IV. DISCUSSION

Several findings of the present investigation are noteworthy. Firstly, MEBO does not have the claimed antibacterial effect, as there was a high concentration of bacterial colonization and signs of wound infection in the MEBO treated wounds. Moreover, progressive wound enlargement was observed in the MEBO treated group possibly due to bacterial invasion and infection of healthy tissues. Secondly, MEBO does not have better wound healing ability than Ag-S, and this raises questions on the effectiveness of the claimed physiological moist environment that MEBO is supposed to create for the overall healing enhancement. Thirdly, complications of multiple organ failure may occur after thermal injury. Lastly, some abnormal behaviors and signs that are observed from the animals after thermal injury requires further consideration.

4.1 The Antibacterial Effect of MEBO and Ag-S

Following a burn injury, the wound is a site with serious bacterial contamination and infection. Both the type of wound and the microbial factors affect the rate of microbial proliferation in and penetration of the eschar. The burned tissue which is rich in degenerated protein, and moisture from the transeschar movement of fluid and serum, are excellent microbial culture medium (Wolf and Herndon 2001). The avascularity of the burned tissue, resulting from thermal thrombosis, limits both the delivery of endogenous phagocytic cells and the effectiveness of systemically administered antibiotics. Without an effective antibacterial topical therapy, the bacteria penetrate the eschar down to the viable-nonviable tissue interface. At this area, further microbial proliferation continues to take place and promotes the denatured collagen lysis and sloughing of the eschar. In the later course of burn injury, the exposure and desiccation of granulation tissues may result in the formation of neochars that further support bacterial growth and proliferation. When the host defense is weakened which is often the case after thermal injury or when the topical therapy is ineffective, the subeschar organisms will invade the underlying uninjured tissues and spread systemically (Ioannovich et al. 2000). Moreover, the toxins that are released concurrently by the bacteria will lead to lesions to the whole wound and
IV. Discussion

beyond causing systemic problems (Xu 1989a). Hence, prevention and control of bacterial colonization and infection of burn wounds should be the primary aim of treatment so that there is optimal cell regeneration. Otherwise, the viable cells with the capacity of skin regeneration will be rapidly destroyed. An agent that can prevent the tissues and cells from bacterial harm is therefore needed.

From our result, even though MEBO has been claimed to have the ability to promote and alter the change in the morphology of bacteria and thus limiting bacterial toxicity, it did not seem to occur in our experiment. Conversely, the antibacterial property of Ag-S is significantly stronger than MEBO. Ag-S has long been recognized to have an outstanding antibacterial effect and in this experiment, it was successfully demonstrated to limit the amount of bacterial growth on the wound surface.

In the future, a modified study that involves the exact bacterial count, bacteriology to quantify the bacterial amount and identification of the strain of bacteria infecting the wounds could be carried out. Generally speaking, if the bacterial density in the burn wound equals or exceeds $10^5$ per gram, there is likelihood to have an invasion that may lead to infections or systemic sepsis. However, the strains of bacteria are also an influential factor (Ioannovich et al. 2000). These additional assessments will provide a more complete picture on the bacterial inhibiting specificity of the topical agents. In addition to the lack of antibacterial ability of MEBO, it is feasible that the moist environment provided by MEBO also ironically creates a marvelous niche for the growth of fungi and other skin disease related organisms. Close observations on other skin diseases such as eczema would be beneficial.

4.2 The Enlargement of Wound Size Observed in the MEBO Group

Various factors such as the conserved heat energy and the actions of the inflammatory mediators may influence the postburn progression of necrosis of the burn wounds (Decamara, Raine and Robson 1981; Robb 1967). Vasoconstriction leads to a temporary reduction of skin diffusion can further increases burn necrosis significantly (Knabl et al.
IV. Discussion

1999). Apart from hemodynamic influences, infections may also result in a progression of burn wound necrosis (Williams and Phillips 1995). According to a study that examined the dermal influence of vasoconstrictors with reference to the development of burn necrosis in white rabbits, vasoconstriction caused a temporary reduction of skin diffusion that led to a significant increase of burn necrosis (Knabl 1999). In the early state of severe burn injuries, hypovolemia and decreased cardiac output leads to systemic vasoconstriction caused by the release of the body’s own stress hormones including catecholamines, angiotensin, vasopressin and aldosterone. These hormones bring about a hypermetabolic condition that further decreases vascular perfusion (Chance et al. 1989). Moreover, the increased vessel permeability leads to massive plasma excretion into the interstitium in both the burned and uninjured areas that worsens intravasal hypovolumia. Hence, under such critical perfusion situation, the ischemic changes of the zone of stasis may increase and enhance the progression of burn necrosis. Nevertheless, it was interesting that in our experiment, only the MEBO treated wounds demonstrated wound size progression. It would be worth looking into the possible vasoconstriction property of MEBO and determines whether the sesame oil base of MEBO has any negative influence on the progression of burn necrosis.

4.3 The Effectiveness of the Moist Environment Created by MEBO on Healing

Is it possible that MEBO on one hand builds a moist environment by preventing evaporation while on the other hand creates a “blanket effect” by its sesame oil base that allows further wound damage? In the rabbit model experiment designed to investigate the effect of MEBO on burn wound water evaporation showed that MEBO effectively inhibited wound water evaporation and decreased body fluid loss (Pu et al. 1999). It may be beneficial to shock resuscitation especially when the burned wound was large, yet it undoubtedly prevented adequate heat loss from the wound at the same time. Consequently, the application of MEBO which contains sesame oil may hinder heat dissipation from the burn wound. Ironically, progressive thermal damage may continue even when the heat source was removed, thereby creating more tissue damages as seen in this study, represented by the enlarged wound size of the MEBO treated wounds.
compared to the Ag-S treated wounds. In contrast, Ag-S presumably does not have such “blanket effect” because it has a 1.0% water-miscible base instead of an oil base. Not surprisingly, Ag-S allows more water loss from the wounds than MEBO (Ioannovich et al. 2000). In order to confirm our speculation that MEBO actually enhances progressive wound enlargement by limiting wound water evaporation, an experiment that compares the wound water evaporation and the wound size of the MEBO and Ag-S treated wounds should be conducted in the future.

4.4 The Role of Tumor Necrosis Factor (TNF) in Thermal Injury Complications

Burn injury itself is a stimulus for the production of monocytes, macrophages, TNF-α and inflammatory cytokines IL-6 and IL-8 as a result of the marked inflammatory reaction. Marano et al. were the first to report the close relation of TNF to the severity of the morbid condition in patients of burn injury (Marano et al. 1990). TNF-α was reported to be related to the prognosis of burn injury (Endo et al. 1993). TNF is believed to activate neutrophils which release lysozomic enzymes such as elastase, to produce active oxygen and to damage cells and vascular endothelial cells of important organs which results in functional organ failure (Gamble et al. 1985). The death of the two rats in this study was suspected to be due to multiple organ failure including pulmonary, gastrointestinal and renal complications. As for the causes of these complications, TNF is thought to be the major culprit.

4.5 Pulmonary Complications in Thermal Injury

TNF (also called Cachectin in early days) is a protein produced by the activated macrophages. It is found to be an important mediator of the lethal effect of endotoxin which causes hypotension, metabolic acidosis, hemoconcentration and even death (Beutler et al. 1986). In this study, diffuse pulmonary hemorrhage was apparent on both the gross and histopathologic examinations, along with hemorrhagic and ulcerative lesions of the gastrointestinal tract and acute renal tubular necrosis with the presentation
of hematuria. These complications can be explained by the systemic release of TNF by macrophages that initiate shock and tissue injury after a burn injury.

### 4.6 Gastrointestinal Complications in Thermal Injury

Apart from pulmonary hemorrhage, complications of Curling’s ulcer and ileus were identified by the histopathological examination and autopsy of the two rats that died in the current study. The possible gastric retention increases gastric secretion and the chance of ulceration. Gastrointestinal ulceration (Curling’s ulcer) if complicated by hemorrhage will be potentially lethal after a major burn injury. Clinically, gastrointestinal bleeding in burn patients can be explained by initial hypotension, hypoxia and resultant mucosal ischemia (Fadaak 2000). Furthermore, adynamic ileus, gastrointestinal hemorrhage and the redistribution of blood flow with a decrease of blood flow in the splanchnic bed are the detrimental changes commonly observed in the gastrointestinal system after thermal injury (Czaja et al. 1976; Jones et al. 1992).

Ischemic lesions in the small and large intestines are also a common gastrointestinal complication after a thermal injury. Many investigators have reported that the early phase appearance of activated inflammatory cells such as polymorphonuclear leukocytes (PMNs) and macrophages are the major cause for the postburn intestinal vascular barrier failure (Sir et al. 2000). Moreover, the translocation of the enteric pathogens through the compromised mucosal barrier may cause primary peritonitis, and it is also the main factor for septicemia and multiple organ failure in the thermally injured patients (Fadaak 2000; Gentillini et al. 1999). In addition, the intestinal and colonic motility rate was shown to decrease together with a delay in gastric emptying following burn injury (Chen et al. 1982). The above mentioned experimental results correlate well with our autopsy observation of the two rats that died in this study and thereby provide explanations to their cause of death.
IV. Discussion

4.7 Renal Complications in Thermal Injury

Acute renal failure (ARF) is a well known complication of severe burns and is an important factor causing an increase in mortality (Holm et al. 1999). When there is extensive cutaneous burns, local changes may cause general effects such as changes in blood volume, fluid exchanges and drug interactions that may result in renal damage (Sevitt 1965). In general, clinical complications of ARF usually occur after severe burn injury when there is hypovolemia, massive presence of necrotic tissues, septic shock and prolonged hypercatabolic state of the body. Firstly, hypovolemia during a burn shock is responsible for nearly all the cases of pre-renal ARF. Secondly, massive necrotic tissues are released from the wounds with the presence of myoglobinuria that may jam the glomerulotubules of the kidneys and cause hemoglobinuria and renal failure. Thirdly, the presence of endotoxemia from septic shock also results in pre-renal or renal ARF with a clinical picture of persistent hypotension due to circulating toxins. Lastly, the hypercatabolic state of the body is manifested by dystrophy of organs and the kidneys which have an increased demand after injury and are particularly vulnerable. (Belba and Belba 2000). In future studies, it is meaningful to dynamically and continuously detect the progressive increase of creatinemia together with oligoanuria on top of progressive uremia, acidosis, hyperkalemia and hyponatremia which are typical presentations of ARF.

4.8 Explanations to the Abnormal Signs and Behaviors of the Thermally Injured Animals

The sharp drop in weight at the beginning of the postburn period could be explained by an increase in catabolism. After a burn injury, there is a long term hypermetabolism requiring additional nutrition. Furthermore, traumatic burn injury causes frailty and loss of appetite of the animals. The loss of appetite and poor nutrition intake often lead to a state of negative nitrogen balance until the wound is healed (Wolf and Herndon 2001). In this study, when the overall body conditions became stable again, the animals began to regain weight. Similarly, hair loss of the four limbs and the abdominal regions could be explained by frailty and stress induced by the thermal trauma. As the body began to recover, hair loss ceased and was replenished by newly grown hair. Though formal pain
IV. Discussion

evaluation was not carried out in the current study, pain responses were observed such as the arched back and piloerection of the rats in the early postburn period. Moreover, by observation, almost every time when MEBO was applied to the wound, the rats used their hind leg to scratch the MEBO wound. On the contrary, they rarely scratched the Ag-S treated wounds. This suggests that MEBO may cause irritations. Similarly, no quantitative analysis of heat, redness, swelling, and moisture of the wounds are available but the clinical effects of the different treatments are borne out empirically by the overall healing rate and the decreasing wound size.

4.9 Limitations of This Study

The present study, being time-limited, has several limitations which merit further discussion. First, it is difficult to assess the pain level of the rats when both topical agents were applied to the same animal. Nonetheless, when evaluating the clinical applicability of a burn agent, its analgesic ability is an important criterion to consider. In this study, pain level could only be speculated through observations of the rats’ behavior. Almost during every MEBO application, the rats produced a reflexive action of hind leg scratching that was seldom observed during Ag-S application. This might be accounted for pain or irritations induced by MEBO. Second, while the rat model was used to simulate the human model, it was unclear whether our results would mimic the clinical scenario. Apart from using rats as animal model, domestic pigs could be selected to predict wound healing in humans because of the morphological and functional similarities of pig skin with human skin. Third, this study examined relatively small areas of deep second to third degrees burn wounds at the lower portion of the animal’s back. It is unclear whether the results would differ when more extensive wound, superficial burn wounds or wounds at different body locations, for example, the abdomen or the neck regions were used instead. Fourth, it is doubtful whether there are any systemic adverse effects caused by either tested agent alone since both agents were applied simultaneously to the same animal. Fifth, since the scrubbing action associated with ointment removal was painful, in order to limit the amount of irritations administered to the rats, previous ointment removal was not performed every time when a new application was smeared as
was customary under clinical setting. However, remnants of the previously applied ointments may form an overlying slough on the wounds that renders wound assessment difficult and may affect the diffusion of the topical agents. Finally, microscopic analysis of the kidneys was not included in the autopsy of the dead animals, although renal problems were highly suspected. It could be anticipated to see epithelial cloudy swelling of the proximal convoluted tubules with intracapillary cavity collapse of the renal glomeruli and hyaline cast formation within renal tubules. Thrombosis of the vascular lumen may also be seen.

4.10 Factors to Consider in Further Studies

4.10.1 Monitor the Progression of Burn Wound Recovery

The best indicator of invasive infection of a burn wound is the histological evidence of bacterial invasion of viable tissues at the base of the wound. Biopsy of the wound could be performed to include the viable wound base, and the specimen could be subjected to grinding and bacterial quantization, as well as to routine morphology, including proper stains for bacteria and yeasts (Tompkins 1994). In this study, punch biopsy of burned tissues was not sampled on a regular basis because when obtaining biopsy, additional scar and damage to the already injured tissues may be induced together with added stress to the animals. Furthermore, the application of punch biopsy may be impractical under clinical setting, thus, non-invasive methods for burn depth assessment should be considered in future studies.

4.10.2 Non-invasive Objective Parameters

In future studies, apart from monitoring and comparing the burn wound size, the antibacterial ability and the healing progress of the wounds treated with different topical agents, it may be useful to monitor the pH of wound surface, the transepidermal water loss and the moisture level of the wounds as well. As for the measurement of transepidermal water loss, it is an important non-invasive method for assessing the
efficacy of skin integrity since the stratum corneum provides a barrier against diffusion of water through the epidermis (Ioannovich et al. 2000).

4.10.3 Non-invasive Methods of Burn Depth Assessment

Since burn depth determination is one of the crucial factors in deciding the treatment of thermal injury, an accurate means of detecting the depth of wound in a non-invasive manner is necessary. A report has illustrated that a technique called the polarization sensitive optical coherence tomography (PS-OCT) is an alternative to histological analysis. Since the collagens in the skin is denatured by heat, the PS-OCT is particularly designed to measure the resulting reduction in collagen birefringence using depth resolved changes in the polarization of light propagated and reflected from the skin sample (Droog, Steenbergen and Sjöberg 2000). With the application of the PS-OCT, samples can be taken more frequently without anesthetizing the animals.

In addition to the PS-OCT, there are other methods for assessing burn depth such as injection of radioisotopes and dyes, ultrasound and thermography. Nonetheless, Laser Doppler Flowmetry (LDF) has advantage over other methods because it is more accurate and non-invasive. In the LDF, laser light is quasielastically scattered into the tissue and the back-scattered light is spectrally broadened as a result of the moving blood cells. The back-scattered signal is then transferred into electrical signals by a photodiode. After the signal has been processed, the output voltage correlates linearly with perfusion (Stern 1975).

Laser Doppler Perfusion Imaging (LDPI), a more advance development of LDF, has a collimated laser beam that scans a certain area of the tissue to form an image of its perfusion (Wårdell, Jakobsson and Nilsson 1993). The main advantage of LDPI is that it provides spatial information of the microvascular blood flow. Microvascular damage often results in stasis and subsequent vessel blockage which is one of the most obvious signs of damage in burns (Chvapil et al. 1984). Moreover, unlike LDF, the probe of LDPI does not come into contact with the tissue because it is a scanning method and it enables
the assessment of microvascular blood flow in a predefined skin area rather than just one place (Droog, Steenbergen and Sjöberg 2000). Together with the adjunction of digital photography, additional information can be provided for the analysis of the LDPI images.

4.10.4 Monitor the Postburn Body Temperature and Identify the Strains of Bacterial Colonization

Detection of postburn fever of the rats is a useful indicator of infections and metabolic status. Postburn fever is not uncommon clinically since extensive epidermal loss and the presence of necrotic tissue place the patient at particular risk of infections and multiple organ failure. On top of monitoring the postburn body temperature, it may be beneficial to precisely identify the strains of bacterial colonization of the wounds. Generally, there are relatively few organisms detected from the burned surface immediately after the injury. For those that are present are predominantly gram-positive bacteria. In contrast, the density and type of organisms in the untreated burn wound change with time so that by the fifth day after injury, gram-negative bacteria can be recovered from 60% of patients. By the middle of the second postburn week, gram-negative bacteria are the predominant organisms found in the burn wound and the density increased to the level of $10^2$ to $10^4$ per cm$^2$ of wound surface (Ioannovich et al. 2000). By identifying the strains of bacteria infecting the wounds, not only will the antibacterial effect of the topical agents be tested but their specificity could also be known as well.

4.10.5 Comparison of Honey, MEBO and Ag-S

Topical application of honey to burn wounds and other wounds has been found effective in controlling infection and producing a clean granulating bed (Subrahmanyam 1996; Subrahmanyam 1991). Further studies that compare the wound healing ability of honey, MEBO and Ag-S could be considered. Honey is non-irritant, non-toxic, convenient and inexpensive. It is a mixture of sugars produced by bees through nectar collection from flowers. The medicinal properties of honey have been known since ancient times. Back in 1937, Voigtlander used honey to treat scalds and highlighted the pain relieving property
of honey (Voigtlander 1937). In recent years, a number of articles concerning honey’s ability to treat burn injury appeared in the literature. Honey has been found to control wound infection, particularly against gram-positive and gram-negative bacteria. The antibacterial activity of honey is mainly due to inhibines which are hydrogen peroxide, flavinoids and phenolic acids, plus many other unidentified inhibines in honey. The possible explanation for the antibacterial activity of honey is that it causes shrinkage disruption of the bacterial cell wall due to the osmotic effect of its sugar content (Subrahmanyam 2001b). By this virtue, however, it should be noted that honey works in a totally different direction of MEBO that moisturizes the wound and its contents. Furthermore, honey also acts as a hyperosmolar medium preventing bacterial penetration and colonization of the wound surface (Subrahmanyam 2001a). The anti-oxidant effect of honey presumably assists to limit lipid peroxidation and contributes to the rapid healing of wounds apart from its other mentioned beneficial effects (Subrahmanyam 2001b).

In a study carried out in India, honey was shown to enhance wound healing through its antibacterial and analgesic effects (Subrahmanyam 2001a). In another study, honey was proved to be more effective in the sterilization of wounds than Ag-S (Philips 1993). From the above mentioned antibacterial properties of honey, it would be worthy to include honey in the choices of topical agents in comparison with MEBO and Ag-S in future studies.

4.10.6 Benefit-cost Analysis

Lastly, it is practical to carry out a benefit-cost analysis of MEBO and Ag-S (both treatments use the exposed method for burn wound care without the need for secondary covering dressing) in the future under clinical settings to compare their benefit-cost value apart from merely comparing their efficacy. It is especially important under the current economic downfall that raises the concerns of cutting hospital expenditures and tightening management costs. Hence, reducing expenditures without adversely affecting the quality of care has become important. When analyzing the benefit-cost value of the topical agents, comparisons should include the length of hospital stay, the cost of topical
agents used, the amount of analgesics and concomitant antibiotics used, and other pharmaceutical agents required.

V. CONCLUSION

The data and observations collected from this study have not lent considerable support to the hypothesis that the use of MEBO in the treatment of deep burn injury is a viable and desirable alternative to the use of Ag-S. Although MEBO had been claimed to accelerate wound healing, reduce bacterial colonization of the wounds and at the same time results in scarless wound healing under the moist environment created, this study demonstrated that MEBO had a similar re-epithelialization rate of the second to third degree burn wounds as Ag-S and had a poor antibacterial ability. In both groups, all wounds were completely re-epithelialized by day 30 and there was no difference in the characteristic of scars. In short, the application of MEBO in managing deep burn injury is not a desirable alternative to the use of Ag-S.

Even though the results of this animal study may not be readily applicable to burned patients, they provide valuable insights into the selection of a proper and appropriate topical treatment for deep burn injury. We understand that the results of this study are just a small step to a better understanding of the applicability of MEBO and Ag-S. Hence, we recommend that in future studies, identification of the type of bacterial colonization, histological progression of wound healing by non-invasive measures and comparison of MEBO with honey should be included. This will hopefully provide additional information and a more complete view on the efficacy of MEBO whose clinical effects on burn injury is still under serious debate.
VI. REFERENCES


VI. References


VI. References


VI. References


Fraser, J. F., Bodman, J., Sturgess, R. et al. (2003) An in vitro study of the antimicrobial efficacy of a 1% silver sulphadiazine and 0.3% chlorhexidine digluconate cream, 1% silver sulphadiazine cream and a silver coated dressing. *Burns* 30:35-41.


VI. References


VI. References


VI. References


VI. References


## VII. APPENDIX

### The Manufacturing Details of Each MEBO Used in This Study

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>20020406</th>
<th>20020107</th>
<th>20020801</th>
<th>20020703</th>
<th>20030303</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production Date</td>
<td>20020424</td>
<td>20020130</td>
<td>20020801</td>
<td>20020720</td>
<td>20030322</td>
</tr>
<tr>
<td>Total Number Used</td>
<td>6</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Anti-fake Number</td>
<td>53895290-81738150</td>
<td>51375794-18518565</td>
<td>53894105-43202811</td>
<td>59773483-01624465</td>
<td>50844415-08305833</td>
</tr>
<tr>
<td></td>
<td>5714997-69519062</td>
<td>58375795-16518568</td>
<td>51521075-49686777</td>
<td>59773465-01624060</td>
<td>5554985-6983891</td>
</tr>
<tr>
<td></td>
<td>52854935-34901159</td>
<td>59763243-74958834</td>
<td>52560341-71261449</td>
<td>5726994-73514872</td>
<td>57788879-26473841</td>
</tr>
<tr>
<td></td>
<td>5365291-81738170</td>
<td>53782246-80533369</td>
<td>5556926-29886851</td>
<td>53757547-21224598</td>
<td>5376284-52138574</td>
</tr>
<tr>
<td></td>
<td>5254341-51763418</td>
<td>59174295-43095711</td>
<td>53757547-21224598</td>
<td>5376284-52138574</td>
<td>53757592-71224090</td>
</tr>
<tr>
<td></td>
<td>55045373-87699558</td>
<td>53711354-41527177</td>
<td>57805390-20548358</td>
<td>59649095-14553064</td>
<td>54547205-99992350</td>
</tr>
</tbody>
</table>

### The Individual Wound Size of the Twelve Rats on Days 5, 10, 15, 20 and 30

<table>
<thead>
<tr>
<th>Wound Size</th>
<th>D5/cm²</th>
<th>D10/cm²</th>
<th>D15/cm²</th>
<th>D20/cm²</th>
<th>D30/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Ag-S MEBO</td>
<td>Ag-S MEBO</td>
<td>Ag-S MEBO</td>
<td>Ag-S MEBO</td>
<td>Ag-S MEBO</td>
</tr>
<tr>
<td>V1</td>
<td>2.37 2.97</td>
<td>1.87 2.52</td>
<td>1.65 1.80</td>
<td>1.39 1.44</td>
<td>0.63 0.67</td>
</tr>
<tr>
<td>V3</td>
<td>3.19 3.17</td>
<td>2.03 2.87</td>
<td>1.27 2.12</td>
<td>1.22 1.56</td>
<td>0.31 0.28</td>
</tr>
<tr>
<td>V4</td>
<td>2.33 2.47</td>
<td>2.08 2.21</td>
<td>1.60 2.14</td>
<td>1.12 1.40</td>
<td>0.60 0.82</td>
</tr>
<tr>
<td>V5</td>
<td>2.39 2.86</td>
<td>1.75 2.49</td>
<td>1.93 2.04</td>
<td>1.43 1.39</td>
<td>0.00 0.23</td>
</tr>
<tr>
<td>V6</td>
<td>3.11 2.91</td>
<td>1.88 2.28</td>
<td>1.99 1.84</td>
<td>1.31 1.04</td>
<td>0.88 1.01</td>
</tr>
<tr>
<td>V7</td>
<td>2.36 2.64</td>
<td>2.17 2.50</td>
<td>2.35 1.94</td>
<td>1.09 1.01</td>
<td>0.00 0.00</td>
</tr>
<tr>
<td>V8</td>
<td>2.58 2.81</td>
<td>2.11 2.86</td>
<td>1.12 1.63</td>
<td>0.72 1.10</td>
<td>0.00 0.26</td>
</tr>
<tr>
<td>V9</td>
<td>3.01 2.76</td>
<td>2.29 2.44</td>
<td>1.94 1.63</td>
<td>1.47 1.60</td>
<td>0.00 0.00</td>
</tr>
<tr>
<td>V10</td>
<td>2.08 2.74</td>
<td>2.60 2.38</td>
<td>1.85 2.22</td>
<td>1.60 1.69</td>
<td>0.00 0.00</td>
</tr>
<tr>
<td>V11</td>
<td>1.13 1.39</td>
<td>2.60 2.26</td>
<td>1.70 2.32</td>
<td>1.02 1.12</td>
<td>0.00 0.25</td>
</tr>
<tr>
<td>V12</td>
<td>1.81 2.42</td>
<td>1.46 2.13</td>
<td>0.21 1.23</td>
<td>0.16 0.75</td>
<td>0.07 0.12</td>
</tr>
<tr>
<td>V13</td>
<td>2.60 3.01</td>
<td>1.86 2.72</td>
<td>1.65 2.66</td>
<td>1.17 1.70</td>
<td>0.63 0.00</td>
</tr>
<tr>
<td>Ave</td>
<td>2.41 2.68</td>
<td>2.06 2.47</td>
<td>1.60 1.96</td>
<td>1.14 1.31</td>
<td>0.26 0.30</td>
</tr>
<tr>
<td>SE</td>
<td>0.17 0.13</td>
<td>0.10 0.07</td>
<td>0.07 0.11</td>
<td>0.11 0.09</td>
<td>0.10 0.10</td>
</tr>
</tbody>
</table>
### VII. Appendix

#### The Individual Absorbance of Different Groups of the Twelve Rats on Days 5, 10, 15, 20 and 30

<table>
<thead>
<tr>
<th>Rat</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ag-S</td>
<td>MEBO</td>
<td>Norm</td>
<td>Ag-S</td>
<td>MEBO</td>
</tr>
<tr>
<td>V1</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
<td>0.01</td>
<td>1.75</td>
</tr>
<tr>
<td>V3</td>
<td>0.00</td>
<td>0.40</td>
<td>0.10</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>V4</td>
<td>0.01</td>
<td>0.00</td>
<td>0.23</td>
<td>0.74</td>
<td>1.89</td>
</tr>
<tr>
<td>V5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>1.47</td>
</tr>
<tr>
<td>V6</td>
<td>0.02</td>
<td>0.19</td>
<td>0.00</td>
<td>0.42</td>
<td>0.97</td>
</tr>
<tr>
<td>V7</td>
<td>0.01</td>
<td>0.03</td>
<td>0.00</td>
<td>0.14</td>
<td>1.65</td>
</tr>
<tr>
<td>V8</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>V9</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>1.10</td>
<td>1.78</td>
</tr>
<tr>
<td>V10</td>
<td>0.01</td>
<td>0.13</td>
<td>0.08</td>
<td>0.04</td>
<td>0.48</td>
</tr>
<tr>
<td>V11</td>
<td>0.00</td>
<td>0.49</td>
<td>0.08</td>
<td>0.01</td>
<td>0.34</td>
</tr>
<tr>
<td>V12</td>
<td>0.00</td>
<td>0.07</td>
<td>0.06</td>
<td>0.01</td>
<td>1.65</td>
</tr>
<tr>
<td>V13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>1.70</td>
</tr>
<tr>
<td>Ave</td>
<td>0.01</td>
<td>0.15</td>
<td>0.05</td>
<td>0.22</td>
<td>1.16</td>
</tr>
<tr>
<td>SE</td>
<td>0.00</td>
<td>0.05</td>
<td>0.02</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>Median</td>
<td>0.00</td>
<td>0.05</td>
<td>0.00</td>
<td>0.03</td>
<td>1.56</td>
</tr>
</tbody>
</table>