UNIVERSITI PUTRA MALAYSIA

EFFICACY OF EPIDERMAL GROWTH FACTOR TOCOTRIENOL RICH FRACTION CREAM FORMULATION IN DEEP-PARTIAL THICKNESS BURN IN Sprague-Dawley RATS

ASMA BINTI AHMAD ZAINI

IB 2018 11
EFFICACY OF EPIDERMAL GROWTH FACTOR TOCOTRIENOL RICH FRACTION CREAM FORMULATION IN DEEP-PARTIAL THICKNESS BURN IN Sprague-Dawley RATS

By

ASMA BINTI AHMAD ZAINI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

January 2018
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs, and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of University Putra Malaysia.

Copyright © Universiti Putra Malaysia
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFICACY OF EPIDERMAL GROWTH FACTOR TOCOTRIENOL RICH FRACTION CREAM FORMULATION IN DEEP-PARTIAL THICKNESS BURN IN Sprague-Dawley RATS

By

ASMA BINTI AHMAD ZAINI

January 2018

Chairman: Huzwha Khaza’ai, PhD
Faculty: Institute of Bioscience

Silver Sulfadiazine (SSD) is primarily used as a topical burn treatment. However, it has been reported that SSD cream can cause adverse reactions like allergy and toxicity. Thus, in this study the burn healing properties of epidermal growth factor (EGF) and tocotrienol rich fraction (TRF) formulation were evaluated. This study was conducted in four stages. The first and second stages were to evaluate the efficacy of TRF (3-5%) and EGF (A-C%) separately, meanwhile the third stage was to study the synergistic effect of both compounds in burn wound healing. Sprague-Dawley male rats were divided into 6 groups (n=7). The deep-partial thickness burn wounds were performed on the shaved skin with the exposure to 100°C heat for 10 second. Treatment was applied topically once daily to the burned areas for 21 days. The measurable outcomes involved rate of wound contraction, clinical evaluation, H&E staining and cellular population number. The results were analyzed for statistical significance using two-way ANOVA for microscopic study and two way repeated measure for macroscopic study. Bonferroni test was performed for the significant treatment means. The optimum dosage of both ingredients obtained was further used for the formulation of EGF-TRF cream and the synergistic effects were determined. TRF at 3% concentration showed most advanced healing indicated with better cosmetic and histopathological outcome, highest percentage of wound contraction rate at day 5, 9, 13, and 17 with 43.34±2.13, 62.87±1.74, 92.38±2.48 and 100.00±0.00 respectively, lowest count of neutrophils and macrophages. The highest dose (C%) of EGF increased the healing process indicated with better cosmetic and histopathological outcome and highest percentage of wound contraction rate at day 13 and 17 with 78.82±2.40 and 100.00±0.00 respectively. Hence, for the third stage study, A-C% EGF were mixed with 3% TRF. The best formulation was further used in the fourth stage. Microscopic changes of the collagen in the dermal layer for the optimum formulation (C% EGF + 3% TRF) was monitored. Current finding
demonstrated the C% EGF + 3% TRF treatment exhibited excellent gross appearance, highest percentage of wound contraction rate in all experimental period and full histological score as early as day 14. Microscopic evaluation demonstrated that there was a significant acceleration of the epidermal and dermal repair in C% EGF + 3% TRF. Collagen staining also showed increased fibroblast proliferation and collagen synthesis in C% EGF + 3% TRF. Combination of C% EGF + 3% TRF exhibited synergistic effects with better potential than the effects of these two compounds alone in accelerating burn wound healing. In addition, combination of EGF and TRF treatments showed better healing ability as compared to SSD. In conclusion EGF-TRF formulation is capable to accelerate the burn wound healing with better cosmetic outcome in the deep-partial thickness burn on various phase of burn wound healing.
Abstrak tesis yang dikenakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

EFIKASI FORMULASI EGF-TRF (FAKTOR PERTUMBUHAN EPIDERMIS-FRAKSI KAYA TOKOTRIENOL) TERHADAP TIKUS Sprague-Dawley YANG LUKA TERBAKAR PADA KETEBALAN SEPARA

Oleh

ASMA BINTI AHMAD ZAINI

Januari 2018

Pengerusi : Huzwah Khaza’ai, PhD
Fakulti : Institut Biosains

Silver Sulfadiazine (SSD) digunakan sebagai ubat sapuan utama untuk luka akibat terbakar. Walau bagaimanapun, krim SSD dilaporkan boleh menyebabkan kesan buruk seperti alergi dan toksik. Oleh itu, dalam kajian ini, sifat penyembuhan luka akibat terbakar dengan formulasi faktor pertumbuhan epidermis (EGF) dan fraksi kaya tocotrienol (TRF) dinilai. Kajian ini dijalankan dalam empat peringkat. Peringkat pertama dan kedua adalah untuk menilai kesan tindak balas dos TRF dan EGF secara berasingan, manakala tahap ketiga adalah untuk mengkaji kesan sinergistik kedua-dua sebatian dalam penyembuhan luka terbakar. tikus jantan Sprague-Dawley dibahagikan kepada 6 kumpulan (n = 7). Luka terbakar ketebalan separa dilakukan ke atas kulit yang dicukur dengan pendedahan kepada suhu panas 100°C selama 10 saat. Rawatan diberikan secara sapuan sekali sehari pada kawasan yang terbakar selama 21 hari. Keputusan untuk ketiga-tiga peringkat ini ditentukan mengikut gabungan kadar pengecutan luka, penilaian klinikal, pewarnaan H & E dan bilangan populasi sel kulit. Data dianalisis menggunakan dua cara ANOVA untuk kajian mikroskopik dan langkah berulang ANOVA untuk data makroskopik. Ujian Bonferonni dilakukan untuk penentuan data yang signifikan. Dos yang optimum daripada kedua-dua ramuan ini telah digunakan untuk membuat rumusan krim EGF-TRF dan kesan sinergi telah ditentukan. 3% TRF menunjukkan kadar pengecutan luka yang paling tinggi pada hari 5, 9, 13 and 17 dengan nilai 43.34±2.13, 62.87±1.74, 92.38±2.48 dan 100.00±0.00, bilangan neutrophil dan makrofaj yang terendah. Peningkatan dos yang responsif dapat diperhatikan dalam dos C% EGF ditunjukkan oleh hasil kosmetik yang baik, hasil histopatologi yang baik dan peratusan tertinggi kadar penguncupan luka pada hari 13 dan 17 dengan 78.82±2.40 dan 100.00±0.00 masing-masing. Oleh itu, untuk kajian peringkat ketiga, A-C% EGF telah dicampur dengan TRF 3%. Formulasi yang terbaik disiasat di peringkat keempat. Perubahan mikroskopik kolagen dalam lapisan dermal untuk formulasi terbaik telah (C% EGF + 3% TRF) dipantau. Penemuan terkini
menunjukkan rawatan C% EGF + 3% TRF menghasilkan rupa luaran luka yang baik, peratusan tertinggi untuk kadar penguncupan luka dalam semua tempoh eksperimen dan skor histologi yang penuh seawal hari 14. Penilaian mikroskopik menunjukkan bahawa terdapat akselerasi ketara perbaikan epidermis dan dermis dengan penggunaan C% EGF + 3%.TRF. Pewarnaan kolagen juga menunjukkan peningkatan proses percambahan fibroblast dan sintesis kolagen dalam kumpulan C% EGF + 3% TRF. Kombinasi C% EGF + 3% TRF menunjukkan kesan sinergi dengan potensi yang lebih baik daripada kesan kedua-dua rawatan itu secara sendirian dan rawatan lain dalam mempercepatkan penyembuhan luka terbakar ketebalan separa. Gabungan rawatan EGF dan TRF menunjukkan keupayaan penyembuhan yang lebih baik berbanding dengan SSD. Kesimpulannya, formulasi EGF-TRF mampu mempercepatkan penyembuhan luka terbakar tahap kedua di pelbagai proses tahap penyembuhan luka dan mempercepat proses penyembuhan secara langsung.
ACKNOWLEDGEMENTS

In the name of Allah, The most Gracious and Merciful.

First and foremost, I am greatly indebted to my supervisor, Dr Huzwah Khaza’ai for her endless guidance throughout the development of this study. Her comments, patience and examples have inspired me in so many ways. A highly gratitude also goes to my committee members Dr Razana Md Ali for serving as my supervisor with various suggestions and also for the help and encouragement during the research work.

I would also like to acknowledge with much appreciation to Ms. Mu’mina, Mr. Ha’iz Sokhini, and Ms. Gayathri for helping me tremendously with the animal work. Their assistances were vital for my experiment. Many thanks are also due to all staff in Histopathology laboratory, Faculty of Medicine and Health Sciences especially to Ms. Normah and Ms. Juita Chupri for their kind assistance during research. Many more persons participated in various ways to ensure my research succeed, but special mentions are due to my labmates especially to Miss Guo Hui Fang for valuable exchange of ideas and support, Miss Najwa, Miss Afifah, Miss Hazirah and Miss Sarah. I am honoured to have the opportunity to work with such a dedicated postgraduate student who taught me the meaning of teamwork and camaraderie.

My deepest gratitude to my father and mother, Mr. Ahmad Zaini bin Hussin and Mrs. Rozita Abd. Samad for their endless support. Thanks also to my siblings (Umair, Ammar and Muadz). Last but not least I am immensely thankful for the loved, advised and support of my husband Mr. Ahmad Khaliq in my pursuit for higher education and expressed understanding and consideration towards me. This thesis is dedicated to my son Faheem bin Ahmad Khaliq whose inspired me to work harder.
I certify that a Thesis Examination Committee has met on 22 January 2018 to conduct the final examination of Asma binti Ahmad Zaini on her thesis entitled "Efficacy of Epidermal Growth Factor Tocotrienol Rich Fraction Cream Formulation in Deep-Partial Thickness Burn in Sprague-Dawley Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

**Suhaibah binti Abu Bakar @ Jamaludin, PhD**
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Norhaizan binti Mohd Esa, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Mahanem Mat Noor, PhD**
Associate Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

---

**NOR AINI AB. SHUKOR, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 March 2018
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Huzwah Khaza’ai, PhD**  
Senior Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Razana Md Ali, PhD**  
Senior Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**ROBIAH BINTI YUNUS, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:
● this thesis is my original work;
● quotations, illustrations and citations have been duly referenced;
● this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
● intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
● written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
● there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________ Date: ______________________

Name and Matric Number: Asma binti Ahmad Zaini, GS36125
Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: 
Name of Chairman of Supervisory Committee: Dr. Huzwah Khaza’ai

Signature: 
Name of Member of Supervisory Committee: Dr. Razana Md Ali
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background of study</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Problem statement</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Research objective</td>
<td>3</td>
</tr>
<tr>
<td>1.3.1 General objective</td>
<td>3</td>
</tr>
<tr>
<td>1.3.2 Specific objectives</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Hypothesis</td>
<td>4</td>
</tr>
<tr>
<td>1.5 Significance of the study</td>
<td>4</td>
</tr>
<tr>
<td>2 LITERATURE REVIEWS</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Integumentary system</td>
<td>5</td>
</tr>
<tr>
<td>2.2 Permeability of the skin</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Burns</td>
<td>7</td>
</tr>
<tr>
<td>2.3.1 Burn wound healing</td>
<td>8</td>
</tr>
<tr>
<td>2.3.2 Stages and mechanism of wound healing</td>
<td>8</td>
</tr>
<tr>
<td>2.3.2.1 Inflammatory phase</td>
<td>8</td>
</tr>
<tr>
<td>2.3.2.2 Proliferative phase</td>
<td>9</td>
</tr>
<tr>
<td>2.3.2.3 Maturation and remodeling</td>
<td>10</td>
</tr>
<tr>
<td>2.4 Epidermal growth factor (EGF)</td>
<td>11</td>
</tr>
<tr>
<td>2.4.1 Chemical structure of EGF</td>
<td>11</td>
</tr>
<tr>
<td>2.4.2 Sources, distribution, absorption and metabolism EGF</td>
<td>11</td>
</tr>
<tr>
<td>2.4.3 Properties of EGF</td>
<td>11</td>
</tr>
<tr>
<td>2.4.3.1 Function</td>
<td>11</td>
</tr>
<tr>
<td>2.4.3.2 Mechanism of action of EGF</td>
<td>12</td>
</tr>
<tr>
<td>2.4.3.3 EGF and wound healing</td>
<td>13</td>
</tr>
<tr>
<td>2.4.3.4 Pharmacological study of EGF</td>
<td>14</td>
</tr>
<tr>
<td>2.5 Vitamin E</td>
<td>15</td>
</tr>
<tr>
<td>2.5.1 Chemical structure of vitamin E</td>
<td>15</td>
</tr>
</tbody>
</table>
2.5.2 Absorption, transportation and metabolism of vitamin E
2.5.3 Properties of Vitamin E
  2.5.3.1 Function and mechanism of action
  2.5.3.2 Wound healing properties
  2.5.3.3 Antioxidative agent
2.6 Redox reaction
  2.6.1 Introduction to redox reaction
  2.6.2 Redox reaction in wound healing
2.7 Antioxidants
  2.7.1 Antioxidant enzyme
  2.7.2 Lipid peroxidation

3 GENERAL MATERIALS AND METHODOLOGY
3.1 Materials
  3.1.1 Experimental animal preparation
  3.1.2 Preparation of formulation
  3.1.3 Burn wound creation and treatment protocol
  3.1.4 Macroscopic study
    3.1.4.1 Gross appearance
    3.1.4.2 Clinical evaluation
    3.1.4.3 Wound contraction
  3.1.5 Microscopic study
    3.1.5.1 Hematoxylin and eosin staining (H&E staining)
    3.1.5.2 Masson’s trichrome staining
3.2 Methodology
  3.2.1 Experimental design
  3.2.2 Experimental animal
    3.2.2.1 The effect of different concentration of TRF on burn healing
    3.2.2.2 The effect of different concentration of EGF on burn healing
    3.2.2.3 The effect of 3% TRF mixed with different concentration of EGF.
    3.2.2.4 The effect of C% EGF + 3% TRF on collagen changes
  3.2.3 Preparation of formulation
    3.2.3.1 Preparation of base cream
    3.2.3.2 Preparation of TRF cream
    3.2.3.3 Preparation of EGF cream
    3.2.3.4 Preparation of TRF-EGF cream
  3.2.4 Preparation of the skin
  3.2.5 The burn injury
    3.2.5.1 Thermal source
    3.2.5.2 Infliction of the burn wound
  3.2.6 Treatment protocol
  3.2.7 Macroscopic study
3.2.7.1 Gross appearance of the wound 33
3.2.7.2 Clinical evaluation 33
3.2.7.3 Rate of wound contraction 33
3.2.8 Microscopic study 34
3.2.8.1 Preparation of the slide 34
3.2.8.2 Light Microscope Evaluation 35
3.2.8.3 Haematoxylin and Eosin staining 36
3.2.9 Statistical analysis 37

4 THE EFFECT OF DIFFERENT CONCENTRATION OF TOCOTRIENOL RICH FRACTION UPON WOUND HEALING 38
4.1 Introduction 38
4.2 Materials 39
4.3 Methodology 39
4.4 Results 39
  4.4.1 Gross appearance of the wound 39
  4.4.2 Clinical evaluation 42
  4.4.3 Rate of wound contraction 44
  4.4.4 Haematoxylin and eosin staining 45
    4.4.4.1 H&E stained section on day 1 45
    4.4.4.2 H&E stained section on day 7 47
    4.4.4.3 H&E stained section on day 14 49
    4.4.4.4 H&E stained section on day 21 51
  4.4.5 Cell populations in burn wound 54
4.5 Discussion 56

5 THE EFFECT OF DIFFERENT CONCENTRATION OF EGF ON WOUND HEALING 60
5.1 Introduction 60
5.2 Materials 61
5.3 Methods 61
5.4 Result 61
  5.4.1 Gross appearance of the wound 61
  5.4.2 Clinical evaluation 64
  5.4.3 Rate of wound contraction 66
  5.4.4 Haematoxylin and eosin staining 67
    5.4.4.1 H&E stained section on day 1 67
    5.4.4.2 H&E stained on day 7 (×10) 69
    5.4.4.3 H&E stained section on day 14 71
    5.4.4.4 H&E stained section on day 21 74
  5.4.5 Cell populations in burn wound 76
5.5 Discussion 78
6 THE SYNERGISTIC EFFECT BETWEEN 3% TRF EACH MIXES WITH A%, B% AND C% EGF UPON WOUND HEALING ACTIVITY 82
6.1 Introduction 82
6.2 Materials 82
6.3 Methods 82
6.4 Result 83
6.4.1 Gross appearance of the wound 83
6.4.2 Clinical evaluation 85
6.4.3 Estimation of wound contraction rate 86
6.4.4 Haemaoxylin and eosin staining 87
6.4.4.1 H&E stained section on day 1 88
6.4.4.2 H&E stained section on day 7 89
6.4.4.3 H&E stained section on day 14 90
6.4.4.4 H&E stained section on day 21 93
6.5 Discussion 93

7 THE EFFECT OF THE OPTIMUM FORMULATON (3% TRF+C% EGF) ON THE DERMAL COLLAGEN CHANGES 97
7.1 Introduction 97
7.2 Materials 99
7.3 Methodology 99
7.4 Results 100
7.4.1 Effects of EGF-TRF on the dermal collagen changes 100
7.4.1.1 Masson’s Trichrome stained section on day 7 101
7.4.1.2 Masson’s Trichrome stained section on day 14 102
7.4.1.3 Masson’s Trichrome stained section on day 21 103
7.4.2 Effects of TRF-EGF on the total healing criteria 104
7.4.2.1 Masson’s trichrome stained section on day 7 107
7.4.2.2 Masson’s trichrome stained section on day 14 109
7.4.2.3 Masson’s trichrome stained section on day 21 111
7.4.3 Fibroblasts counts 113
7.5 Discussion 114

8 GENERAL RESULTS AND DISCUSSION 118

9 CONCLUSION AND RECOMMENDATION FOR FUTURE STUDIES 122

BIBLIOGRAPHY 123
APPENDICES 136
BIODATA OF STUDENT 144
LIST OF PUBLICATION 145
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Quantitative histological scoring of H&amp;E staining</td>
<td>37</td>
</tr>
<tr>
<td>4.1</td>
<td>Clinical evaluations of the wounds were observed for 21 days</td>
<td>43</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage of wound contraction in control groups, 3%, 4% and 5% TRF groups</td>
<td>44</td>
</tr>
<tr>
<td>4.3</td>
<td>The cell counts of control group as compared to different concentration of TRF at different time intervals</td>
<td>55</td>
</tr>
<tr>
<td>5.1</td>
<td>Clinical evaluations of the wounds were observed for 21 days</td>
<td>65</td>
</tr>
<tr>
<td>5.2</td>
<td>Percentage of wound contraction in control groups, A%, B% and C% EGF groups</td>
<td>66</td>
</tr>
<tr>
<td>5.3</td>
<td>The cell counts of control group and different concentration of EGF at different time intervals</td>
<td>77</td>
</tr>
<tr>
<td>6.1</td>
<td>Clinical evaluations of the wounds were recorded for 21 days</td>
<td>85</td>
</tr>
<tr>
<td>6.2</td>
<td>Percentage of wound contraction in EGF alone groups, 3% TRF and combination formulations (3% TRF + EGF)</td>
<td>86</td>
</tr>
<tr>
<td>6.3</td>
<td>Reference for quantitative histopathologic scoring of the wound healing parameter based on H&amp;E staining</td>
<td>87</td>
</tr>
<tr>
<td>6.4</td>
<td>H&amp;E score in EGF alone groups, 3% TRF and combination formulations (3% TRF + EGF)</td>
<td>88</td>
</tr>
<tr>
<td>7.1</td>
<td>Semi-quantitative histological scoring table of Masson’s trichrome staining</td>
<td>100</td>
</tr>
<tr>
<td>7.2</td>
<td>Quantitative histological scoring table of Masson’s trichrome staining</td>
<td>100</td>
</tr>
<tr>
<td>7.3</td>
<td>Semi quantitative histological findings in Masson’s trichrome</td>
<td>101</td>
</tr>
<tr>
<td>7.4</td>
<td>Quantitative histologic findings of total dermal healing in Masson’s trichrome staining</td>
<td>106</td>
</tr>
<tr>
<td>7.5</td>
<td>The cell counts of control group, 3% TRF and C% EGF + 3% TRF at different time intervals</td>
<td>113</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Human skin diagram shows three layers of the skin with different cells</td>
<td>5</td>
</tr>
<tr>
<td>2.2</td>
<td>Basic skin anatomy showing the depth of injury for first, second and third degree burn</td>
<td>7</td>
</tr>
<tr>
<td>2.3</td>
<td>Epidermal growth factor (EGF) signaling pathway</td>
<td>12</td>
</tr>
<tr>
<td>2.4</td>
<td>Roles of hEGF in various applications.</td>
<td>15</td>
</tr>
<tr>
<td>2.5</td>
<td>The relation between wound healing and ROS</td>
<td>20</td>
</tr>
<tr>
<td>3.1</td>
<td>Shaved dorsal part of the rat</td>
<td>28</td>
</tr>
<tr>
<td>3.2</td>
<td>Burn wound creation apparatus</td>
<td>29</td>
</tr>
<tr>
<td>3.3</td>
<td>Infliction of the burn lesion</td>
<td>30</td>
</tr>
<tr>
<td>3.4</td>
<td>A) Standard deep partial thickness burn 5 min after the burn; B) H&amp;E staining at 5 min post-burn (×10)</td>
<td>30</td>
</tr>
<tr>
<td>3.5</td>
<td>A) Standard deep partial thickness burn on day 7th after the burn; B) H&amp;E staining at day 7th post-burn (×10)</td>
<td>31</td>
</tr>
<tr>
<td>3.6</td>
<td>Summary of the experimental design</td>
<td>32</td>
</tr>
<tr>
<td>3.7</td>
<td>A photographic method for burn wound area measurement</td>
<td>34</td>
</tr>
<tr>
<td>3.8</td>
<td>Photomicrographs of wound section showing cells population in H&amp;E slides (×40)</td>
<td>36</td>
</tr>
<tr>
<td>4.1</td>
<td>Gross appearance of the control groups, 3%, 4% and 5% TRF</td>
<td>41</td>
</tr>
<tr>
<td>4.2</td>
<td>Microscopic view of the histological sections of control groups and different TRF groups at day 1 post-burn stained with H&amp;E (×10)</td>
<td>46</td>
</tr>
<tr>
<td>4.3</td>
<td>Microscopic view of the histological sections of control groups and different TRF groups at day 7th of post-burn stained with H&amp;E (×10)</td>
<td>48</td>
</tr>
<tr>
<td>4.4</td>
<td>Microscopic view of the histological sections of control groups and different TRF groups at day 14th of post-burn stained with H&amp;E (×10)</td>
<td>50</td>
</tr>
</tbody>
</table>
4.5 Microscopic view of the histological sections of TRF groups at day 14\textsuperscript{th} of post-burn for granulation tissue observation and alignment of endothelial cells (×40)  

4.6 Microscopic view of the histological sections of control groups and different TRF groups at day 21\textsuperscript{st} of post-burn stained with H&E (×10)  

5.1 Gross appearance of control groups, A\%, B\% and C\% EGF  

5.2 Microscopic view of the histological section of control groups and different EGF groups at day 1 post-burn stained with H&E (×10)  

5.3 Microscopic view of the histological sections of control groups and different EGF groups at day 7\textsuperscript{th} post-burn stained with H&E (×10)  

5.4 Microscopic view of the histological sections of control groups and different EGF groups at day 14\textsuperscript{th} post-burn stained with H&E (×10)  

5.5 Microscopic view of the histological sections of EGF groups at day 14\textsuperscript{th} of post-burn for granulation tissue observation and alignment of endothelial cells (×40)  

5.6 Microscopic view of the histological sections of control groups and different EGF groups at day 21\textsuperscript{st} post-burn stained with H&E (×10)  

6.1 Gross appearance of wound sites in EGF alone groups, 3\% TRF and combination formulations (3\% TRF + EGF)  

6.2 Photomicrographs of wound section of combination formulation; A\%, B\%, C\% EGF each mix with 3\% TRF at day 1 post burned stained with H&E (×10)  

6.3 Microscopic view of histological section of combination formulation; A\%, B\%, C\% EGF each mix with 3\% TRF at day 7 post burned stained with H&E (×10)  

6.4 Microscopic view of the granulation tissue of combination formulation; A\%, B\%, C\% EGF each mix with 3\% TRF at day 14 post burned stained with H&E (×10; ×40)  

6.5 Microscopic view of the histological sections of combination formulation; A\%, B\%, C\% EGF each mix with 3\% TRF at day 21 post burned stained with H&E (×10)  

7.1 Microscopic view of the histological sections of control groups, 3\% TRF and C\% EGF + 3\% TRF at day 7 post-burn in Masson’s trichrome staining (×10)
7.2 Microscopic view of the histological sections of control groups, 3% TRF and C% EGF + 3% TRF at day 14 post burned in Masson’s trichrome staining (×10) 103

7.3 Microscopic view of the histological sections of control groups, 3% TRF and C% EGF + 3% TRF at day 21 post burned in Masson’s trichrome staining (×10) 104

7.4 Microscopic view of histological section of total dermal healing in control groups, 3% TRF and C% EGF + 3% TRF at day 7 post burned in Masson’s trichrome staining (×10) 108

7.5 Microscopic view of histological section of total dermal healing in control groups, 3% TRF and C% EGF + 3% TRF at day 14 post burned in Masson’s trichrome staining (×40) 110

7.6 Microscopic view of histological section of total dermal healing in control groups, 3% TRF and C% EGF + 3% TRF at day 21 post burned in Masson’s trichrome staining (×40) 112
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>GPX</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>GRB 2</td>
<td>Growth factor receptor-bound protein two</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine-5’-triphosphate</td>
</tr>
<tr>
<td>GTPase</td>
<td>Guanosine-5’-triphosphatase</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HDLs</td>
<td>High-density lipoproteins</td>
</tr>
<tr>
<td>hEGF</td>
<td>Human epidermal growth factor</td>
</tr>
<tr>
<td>MAP</td>
<td>Mitogen-activated protein</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>O$_2$</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SSD</td>
<td>Silver sulfadiazine</td>
</tr>
<tr>
<td>VLDLs</td>
<td>Very low density lipoprotein</td>
</tr>
</tbody>
</table>
α-TTP  Alpha tocopherol transport protein
CHAPTER 1

INTRODUCTION

1.1 Background of study

Burns are the fourth most common type of trauma worldwide which results in limb deformity, large amount of expenditure in health care, and trauma in both physical and psychological status of an individual (Lazarus et al., 1994). According to the World Health Organization (WHO), 238,800 individuals died due to fire-related burn in the year 2000 (Orgill & Ogawa, 2013)( Orgill and Ogawa, 2013; Afify et al., 2012). Annually, about 2 million people are wounded, 80 000 warded, and 6,500 fatalities due to burn wound in the United States. As in Malaysia, incidence rates of burn is 31, 176 cases per year. The remodeling of damages skin initiated by edema, skin inflammation and scar formation. Poor wound management and delayed progress of wound healing will lead to keloid, hypertrophic, non-raised and contracture scar (Tavares Pereira et al., 2012). Incidence rates of hypertrophic scarring is up to 91% for burn injury (Gauglitz et al., 2011). These consequences have an impact on the psychological and social behaviour of an individual (Clouatre et al., 2013). Skin-related complications also significantly reduced the ability of an individual to move by causing joint contracture, pain from inflammatory mediators and the worst case is that it could leads to deformation from severe scarring (Orgill & Ogawa, 2013).

The damage of epithelial part of the skin exposed the wound area unprotected (Enoch & Leaper, 2005). Generally, pathophysiology and management of burn and normal wound healing is similar and divided into four different but intersecting stages: hemostasis, inflammation, proliferation and remodeling. Physiologically, wound can heal by regeneration and reparative process. Successful tissue regeneration is the ideal form of healing process giving good cosmetic and functional results. On the other hand, in reparative process specialized tissue is replaced with collagen and result in a loss of functional and cosmetic outcome. The reparative events are generally preceded by hemostatic and inflammatory phenomena which may in turn influence the final result of wound healing (Orgill and Ogawa, 2013). It was suggested by Hsu and Mustoe (2010) that a proper technique to optimize the healing process is by reducing the inflammation, increasing tissue regeneration, minimizing tissue destruction and providing a moist environment on the wound area. There are four different classes of burn namely; superficial, superficial partial thickness, deep partial thickness and full thickness burn. However, the main concern in burn unit is infection that will trigger the other complications in healing burn wound. In deep partial-thickness burn, the patients have a high risk to develop into a full-thickness burn (Chan., et al 2002). Hence, it is a critical requirement to create a better treatment for deep-partial thickness burn.
The goal of wound care is to heal wounds in the shortest time with minor pain and scarring. It is concluded that antibacterial formulation can prevent infection from bacteria, nevertheless, they can hinder healing cells from proliferating during wound healing followed by delayed in wound closure. Silver sulfadiazine (SSD) is a standard topical treatment for burn but could cause adverse side effects upon long term usage. In addition, most current therapeutic approaches for burn healing treatment can also cause scar formation and disfigurement (Aarabi et al., 2007). They are different functions of wound healing formulation in a market. The active ingredients and properties were depending on their main function; whether to clean, to protect, to keep in good condition or to change the appearance.

Vitamin E composed of eight different isoforms, four tocopherol and four tocotrienol. The difference is that tocotrienol has an unsaturated phytol tail at carbon number 3,7 and 11 whereas tocopherol possess saturated isoprenoid side chain. These eight forms of vitamins E have different biological activity. It is well establish that vitamin E has the ability to prevent the lipid oxidation and can act as antioxidant molecules to scavenge reactive oxygen species (ROS). The action of vitamin E in accelerating wound repair is increasingly welcomed. However, much of the present work on wound healing has focused on α-tocopherol. It has been proven that tocotrienols have higher antioxidant activity than the tocopherols and it possess a few medicinal properties that are not present in tocopherol (Zingg, 2007). These characteristic are due to the presence of three double bonds on the hydrophobic side chain of tocotrienol (Sen et al., 2010). Therefore, vitamin E in the form of tocotrienol is highly welcomed and should lead to the development of strategies aimed specifically in reducing ROS produced upon burn injury.

Due to the importance of cellular proliferation during burn wound healing, any treatment that can increase the mitogenic effect of healing cells is highly desirable. Each of the phases in wound healing is controlled and regulated by cytokines called growth factors. Primary function of growth factors in wound healing is as mitogen, angiogenic and chemoattractant to command the progression of healing stages. Currently, cytokines have a limited role in clinical practice. The only growth factor currently available commercially is platelet-derived growth factor (PDGF). Wound healing managed with exogenous growth factors has been beneficial on the healing process. However, all of these studies were focused on the healing ability of vitamin E and growth factor as individual compound. By far, there are no experimental reports on the study of the healing ability of the combination of tocotrienol-rich fraction (TRF) and epidermal growth factor (EGF). Therefore it is of current interest to study on the interaction of TRF and EGF on deep partial thickness burn wound. In concert with combining antioxidant properties of TRF and mitogenic properties of EGF, increase ability of wound healing is expected. In this regards, reduced inflammation and increase cellular proliferation in wound healing could be achieved and eventually offered an alternative in topical based therapy.
1.2 Problem statement

One of the major culprits in burn injury is the presence of oxygen radicals that can form chain reaction of lipid peroxidation. Increase free radicals will result in longer inflammation which may cause the wound to lock into chronic state and result in delayed wound healing. Fortunately, protection against oxidative stress can be provided by radical scavenger compounds such as vitamin E which can help to arrest the chain propagation. Vitamin E usually used in topical formulation, such as in wound healing and cosmetic products. Nevertheless, most vitamin E skin care products contain only alpha-tocopherol and synthetic alpha-tocopheryl acetate, which involve hydrolyzation during absorption to demonstrate its activity (Henegouwen et al., 1995). Other factor that will delay wound healing include minimal re-epithelialization and minimal cellular proliferation. Increased cellular proliferation can be provided by cytokines and growth factor. EGF can exert a powerful mitogenic effect particularly on epithelial cells and fibroblasts. These two cells are responsible for regeneration and collagen production of the skin. Therefore in this study, the synergistic effect of both TRF and EGF in burn wound healing were evaluated.

1.3 Research objective

1.3.1 General objective

To elucidate the burn wound healing efficacy of EGF-TRF formulation in Sprague-Dawley rats.

1.3.2 Specific objectives

1. To evaluate the macroscopic and histopathological changes of burn wound healing treated with different concentration of TRF and EGF separately upon wound healing.
2. To investigate the synergistic effect between the optimum concentration of TRF mix with three different concentration of EGF separately upon wound healing activity via macroscopic and histopathological changes.
3. To compare and identify the best combination formulation for burn wounds.
4. To monitor the dermal collagen changes of the best treatment formulation at specific time of healing.
1.4 Hypothesis

EGF-TRF formulation is efficient in treating deep-partial thickness burn.

1.5 Significance of the study

Silver sulfadiazine (SSD) is a standard topical treatment for burn but could cause adverse side effects upon long term usage. SSD can cause allergy and toxicity. It is concluded that antibacterial formulation can prevent infection from bacteria, nevertheless, they can hinder healing cells from proliferating during wound healing followed by delayed in wound closure. So, it is important to find safer and effective treatment without any toxicity effect and aimed at promoting the stage of wound healing with better cosmeceutical outcome.


fraction improves wound healing by modulating collagen and decreasing reactive oxygen Species, (6), 43-300.


Xu, C., Bentinger, M., Savu, O., Moshfegh, A., Sunkari, V., Dallner, G., Tekle, M.


