UNIVERSITI PUTRA MALAYSIA

CLINICAL, MICROSCOPIC AND MECHANICAL EVALUATION OF CUTANEOUS TISSUE EXPANSION IN RATS AND DOGS USING IMMEDIATE AND GRADUAL TISSUE EXPANSION TECHNIQUES

AHMED KHALAF ALI

FPV 2018 19
CLINICAL, MICROSCOPIC AND MECHANICAL EVALUATION OF CUTANEOUS TISSUE EXPANSION IN RATS AND DOGS USING IMMEDIATE AND GRADUAL TISSUE EXPANSION TECHNIQUES

By

AHMED KHALAF ALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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 expression, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
DEDICATION

To my loving mother and memory of my late father for their kindness and love.

To my wife, my sons and my daughter; I greatly appreciate your support.
Tissue expansion is a technique used to stimulate the body to grow additional tissues, allowing coverage of a variety of complex wounds. In 1957, Neumann was the first to describe the clinical soft tissue expansion when an air filled rubber balloon was implanted subcutaneously to reconstruct an ear after traumatic ablation. This technique was improved by Radovan and Argenta in 1970 when they used saline solution to inflate a silicon balloon gradually via a filling port located subcutaneously. This device was highly standardized and different shapes and sizes are now available to fit various indications. A self-inflation model was described by Austad and Rose in 1982. The generation of clinically and physically viable expanded skin produced by Immediate or gradual expansion technique is possible in laboratory and small animals with reduced complications rate where careful planning of tissue expansion as well as attentive post-operative care are followed. The aim of this study was to examine the effects of immediate and gradual rate of mechanical expansion of limb skin tissue using an expansion technique in rats and dogs. The specific objectives were: (1) to evaluate clinically the expanded tissue following immediate or gradual skin tissue expansion. (2) to study the histological aspects of the cutaneous tissue following immediate or gradual skin tissue expansion. (3) to assess the mechanical properties of the expanded tissue caused by immediate or gradual skin tissue expansion. (4) to test the presence and evaluate the expression of the vascular endothelial growth factor (VEGF) in the immediately and gradually expanded skin tissue rats and dogs.
Immediate tissue expansion has been studied using different sizes of constant rectangle shaped tissue expanders made of polymethylmethacrylate (PMMA) which were surgically implanted under the subcutaneous tissue layer at the metacarpal area in dogs and metatarsal area in rats. Round and rectangle shaped self-inflating hydrogel tissue expanders were surgically implanted at the same site in dogs in different individual animals to evaluate gradual skin tissue expansion. Immediate skin tissue expansion of the metatarsal area was performed in four groups of rats (6 animals/group), using four different sizes of the PMMA expanders. In dogs, the immediate skin tissue expansion of the metacarpal area was induced using three different sizes of the PMMA expanders in three groups of dogs (6 animals/group). Immediate expansion procedure lasted for 14 days following implantation of the tissue expander in both rats and dogs. To induce gradual skin expansion of the metacarpal area, two groups of dogs (6 animals/group) were used using round and rectangle self-inflating tissue expanders. The skin expansion process lasted for 30 days following implantation of the tissue expander.

Clinical, histological and mechanical studies were undertaken on the immediately and gradually expanded skin samples collected at various time points. In general, immediate and gradual expansion processes in rats and dogs were well tolerated and associated with mild to moderate pain with low complication rates. Furthermore, there was no difference in terms of color, texture and hair bearance between normal and expanded skin. The thickness of the expanded skin was greater than that of the normal skin due to formation of a vascular fibrous capsule around the expander. Many histological changes were encountered as a result of the expansion process such as increased thickness of epiderm, decreased thickness of derm, increased fibroblast and collagen synthesis, increased mitotic activity, neovascularization, parallel realignment fashion of collagen fibers and the hair follicles, with sweat glands and sebaceous glands being farther apart. The evaluation of the VEGF of the immediately and gradually expanded skin in both rats and dogs revealed significant increase of VEGF expression. On the other hand, mechanical property evaluations of both normal and expanded skin of rats and dogs showed a significant decrease of the tensile strength of the immediately and gradually expanded skin in rats and dogs except the gradually expanded skin where rectangle shaped self-inflating tissue expander was used.

In conclusion, the immediate and gradual self-tissue expansion techniques in rats and dogs were able to provide a good and less invasive skin expansion to the animals, with minimal complications seen in the outcomes and producing viable and relatively normal additional skin tissues. It is potentially useful for surgical repair of relatively large skin wound defects. The gradual self-tissue expansion technique was significantly better than the immediate type in terms of complication rates, clinical toleration of the expansion process and the mechanical properties of the expanded skin. The histological and mechanical properties changes caused by immediate and gradual expansion
seemed to have no deleterious effects on the structural and functional skin features.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN KLINIKAL, MIKROSCOPIK DAN MEKANIKAL PENGEMBANGAN TISU KULIT DI DALAM TIKUS DAN ANJING MENGGUNAKAN TEKNIK PENGEMBANGAN SEGERA ATAU BERANSUR-ANSUR

Oleh

AHMED KHALAF ALI

Januari 2018

Pengerusi : Loqman Mohamad Yusof, PhD
Fakulti : Perubatan Veterinar


Kajian klinikal, histologi dan mekanikal telah dijalankan ke atas sampel-sampel kulit yang mengembang yang dikumpul di detik-detik masa yang berbeza. Secara umum, proses pengembangan secara segera dan beransur-ansur tertoleran dengan baik dan dikaikan dengan kesakitan yang sedikit atau sederhana dengan kadar komplikasi yang rendah. Tambahan lagi, tidak ada perbezaan dari segi warna, tekstur dan kelebatan rambut antara kulit yang normal dan mengembang. Ketebalan kulit mengembang adalah lebih daripada kulit biasa disebabkan pembentukan suatu kapsul vaskular berserabut di sekitar pengembang. Banyak perubahan histologi ditemui akibat daripada proses pengembangan seperti peningkatan ketebalan epiderma, penurunan ketebalan derma, peningkatan sintesis fibroblas dan kolagen, peningkatan aktiviti mitosis, neovascularisasi, penyajaran semula serat kolagen dan folikel rambut cara selari dengan kelenjar peluh dan kelenjar sebum menjadi lebih berjauhan. Pemeriksaan VEGF kulit yang dikembangkan secara segera atau beransur-ansur di kedua-dua tikus dan anjing menunjukkan peningkatan signifikan ekspresi VEGF. Sebaliknya, hasil penilaian sifat mekanikal di dalam kulit anjing dan tikus yang normal dan berkembang menunjukkan mengalami penurunan ketara kekuatan tegangan kulit yang diperkembangkan kecuali kulit yang dikembangkan secara beransur-ansur di mana pengembang tisu kendiri berbentuk empat segi tepat digunakan.

Kesimpulannya, teknik pengembangan tisu secara segera dan kendiri yang beransur-ansur bagi tikus dan anjing mampu memberikan pengembangan kulit yang baik dan kurang invasif terhadap haiwan berkenaan, dengan komplikasi minimum dan menghasilkan tisu kulit tambahan yang boleh hidup serta agak normal, berpotensi untuk digunakan untuk pembaikan secara
pembedahan bagi kecacatan luka kulit yang agak besar. Teknik pengembangan tisu-kendiri beransur-ansur adalah lebih baik daripada jenis segera dari segi kadar komplikasi dan toleransi klinikal terhadap proses pengembangan. Perubahan sifat histologi dan mekanikal disebabkan pengembangan segera dan beransur-ansur dilihat tidak memberi kesan mudarat kepada struktur dan fungsi kulit.
ACKNOWLEDGEMENTS

First of all, I thank Allah (SWT), by overcoming all the difficulties. I have experienced His guidance day by day through my study. I would like to express my special appreciation and thanks to my supervisor Senior Lecturer Dr. Loqman Bin Haji Mohamad Yusof, you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. I appreciate all your contributions of time, ideas, and funding to make my Ph.D. I would also like to thank my co-supervisors Associate Professor Dr. Zamri bin Radzi, Associate Professor Dr. Md Sabri Bin Mohd Yusoff and Senior Lecturer Dr. Nurul Hayah Binti Khairuddin for their valuable directions, guidance and encouraging during the different stages of my study, your support is so appreciated. My grateful goes to all the staff and members of our faculty at UPM especially the staff of the Department of Veterinary Pathology and Microbiology; all of you have been there to support me when I did my research.

Words cannot express how grateful I am to my late father, my loving mother, brothers and sisters for all of the sacrifices that you have made on my behalf; your prayers for me were what sustained me thus far. I would also like to thank to my faithful wife Mrs. Maysam Ibrahim, thank you for supporting me for everything, and especially I cannot thank you enough for encouraging me throughout this experience. My beloved sons Adam and Asef and my daughter Asia, I would like to express my thanks for being such a good family members always cheering me up.

This work would not have been complete without the technical contributions and support of many individuals; among them are Dr. Faez Firdaus Jesse Abdullah, Dr Lau Fong, Mr Jeferi, Mr Jamil, Mrs Latifah, Mrs Jamila and many others that made technical assistance for accomplishment of this work to take shape. Many thanks also go to Dr. Abu Bakar adamu, Dr. Ubaid Kaka, Dr. Ali Saeed Al-chalaby, Dr. Wessam Monther Mohammed Salih, Dr. Kareem Obayes Handool, Dr. Mohammed Naji Auda and Mr. Mustafa Qaes. I would like to express my utmost appreciation and gratitude to Universiti Putra Malaysia, School of Graduate Studies and Ministry of Higher Education for giving me the opportunity to pursue this study. My thanks and appreciations also go to Faculty of Veterinary Medicine, UPM, Department of Veterinary Clinical Studies and Department of Pathology, Faculty of Veterinary Medicine. Last but not the least; my deepest thanks go to all people I did not mentioned their names. I greatly appreciate all the help and support that gave me positive energy whenever I needed it.

AHMED KHALAF ALI
August 2017
I certify that a Thesis Examination Committee has met on 19 January 2018 to conduct the final examination of Ahmed Khalaf Ali on his thesis entitled "Clinical, Microscopic and Mechanical Evaluation of Cutaneous Tissue Expansion in Rats and Dogs Using Immediate and Gradual Tissue Expansion Techniques" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Rasedee @ Mat bin Abdullah, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Zamri b Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Mohamad Aris bin Mohd Moklas, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Stephen Kiama Gitahi, PhD
Professor
University of Nairobi
Kenya
(External Examiner)

RUSLI HAJI ABDULLAH, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 27 September 2018
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

**Loqman Bin Haji Mohamad Yusof, PhD**  
Senior Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Zamri Bin Radzi, PhD**  
Associate Professor  
Faculty of Dentistry  
Universiti Malaya  
(Member)

**Md Sabri Bin Mohd Yusoff, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Nurul Hayah Binti Khairuddin, PhD**  
Senior Lecturer  
Faculty of Biotechnology and Biomolecular 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, popular writings, seminar paper, manuscripts, posters, reports, lecture notes, 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 the 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 No: Ahmed Khalaf Ali, GS37606 ___________________________
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 (Revision2012-2013) are adhered to.

Signature: 
Name of Chairman of Supervisory Committee: Dr. Loqman Bin Haji Mohamad Yusof

Signature: 
Name of Member of Supervisory Committee: Associated Professor Dr. Zamri bin Radzi

Signature: 
Name of Member of Supervisory Committee: Associated Professor Dr. Md Sabri Bin Mohd Yusoff

Signature: 
Name of Member of Supervisory Committee: Dr. Nurul Hayah Binti Khairuddin
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>APPROVAL</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xvii</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xviii</td>
</tr>
<tr>
<td></td>
<td>LIST OF APPENDICES</td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATIONS</td>
<td>xxx</td>
</tr>
</tbody>
</table>

## CHAPTER 1
1. INTRODUCTION
1.1 Background of the study 1
1.2 Statement of the problems 3
1.3 Research hypothesis 4
1.4 Objectives:- 4

## CHAPTER 2
2. LITERATURE REVIEW
2.1 Skin
2.1.1 Epidermis 5
2.1.2 Dermis 6
2.1.3 Hypodermis (subcutis) 7
2.2 Skin defects and closure 7
2.3 Tissue expansion 8
2.3.1 History of tissue expansion 8
2.3.2 Indications of tissue expansion 8
2.3.3 Advantages of tissue expansion 9
2.3.4 Disadvantages of tissue expansion 9
2.3.5 Limitations of tissue expansion 10
2.3.6 Contraindications of tissue expansion 10
2.3.7 Complications of tissue expansion 10
2.4 Expansion technique 11
2.4.1 Mechanism of tissue expansion 12
2.4.2 Development of tissue expander 12
2.4.3 Directions of tissue expansion 13
2.4.4 Differences between conventional and self-inflating skin tissue expansion 14
2.4.5 The choice of the appropriate expander geometry and size 15
2.4.6 Types of tissue expansions 15
2.4.6.1 Immediate or intraoperative tissue expansion 15
2.4.6.2 Rapid soft tissue expansion 16
2.4.6.3 Chronic or conventional tissue expansion 16
2.4.7 Polymethylmethacrylate (bone cement) 17
2.5 Microscopic changes of the expanded skin tissue 17
2.5.1 Roles of growth factors in tissue expansion 17
2.5.2 Histological changes of the expanded skin tissue 18
2.5.3 Normalization of the expanded skin tissue 19
2.6 Physiology of tissue expansion 19
2.7 Mechanical properties of the expanded skin tissue 20
2.8 Maintenance period 20
2.9 Methods and substances used to improve tissue expansion 20

3 CLINICAL EVALUATION OF IMMEDIATE AND GRADUAL SKIN EXPANSION 22
3.1 Introduction 22
3.2 Specific objectives 23
3.3 Materials and Methods 23
3.3.1 Study Approval 23
3.3.2 Skin tissue expanders 23
   3.3.2.1 Preparation of the bone cement tissue expander (PMMA) for rats 23
   3.3.2.2 Preparation of the bone cement tissue expander (PMMA) for dogs 24
   3.3.2.3 Self-inflating tissue expander used in dogs 25
3.4 Animal model and housing 26
   3.4.1 Animal model and housing for rats 26
   3.4.2 Animal model and housing for dogs 26
3.5 Experimental design and management 26
   3.5.1 Experimental design and management of immediate skin expansion in rats 26
   3.5.2 Experimental design and management of immediate skin expansion in dogs 27
   3.5.3 Experimental design and management for gradual skin expansion in dogs 28
3.6 Anesthesia and patient preparation 29
   3.6.1 Anesthesia and patient preparation (rats) 29
   3.6.2 Anesthesia and patient preparation (dogs) 29
3.7 Surgical procedure 30
   3.7.1 Surgical procedure (rats) 30
   3.7.2 Surgical procedure (dogs) 31
3.8 Postoperative care 33
   3.8.1 Postoperative care of rats 33
   3.8.2 Postoperative care of dogs 33
3.9 Evaluation of tissue expansion process
   3.9.1 Clinical evaluation of tissue expansion process
   3.9.2 Measurement of the size of the expansion site
   3.9.3 Pain assessment of immediate and gradual skin tissue expansion in dogs
3.10 Statistical analysis
3.11 Results
   3.11.1 Immediate skin expansion in rats
      3.11.1.1 Clinical evaluation of tissue expansion
      3.11.1.2 Skin expansion measurement
      3.11.1.3 Macroscopic features of the expanded skin tissue
   3.11.2 Immediate skin expansion in dogs
      3.11.2.1 Clinical evaluation of tissue expansion
      3.11.2.2 Skin expansion measurement
      3.11.2.3 Macroscopic features of the expanded skin tissue
   3.11.3 Gradual skin expansion in dogs
      3.11.3.1 Clinical evaluation of tissue expansion
      3.11.3.2 Skin expansion measurement
      3.11.3.3 Macroscopic features of the expanded skin tissue
3.12 Discussion
3.13 Conclusion

4 HISTOLOGICAL EVALUATION OF THE EXPANDED SKIN
   4.1 Introduction
   4.2 Specific objectives
   4.3 Materials and methods
      4.3.1 Animals
         4.3.1.1 Rats (immediate skin tissue expansion)
         4.3.1.2 Dogs (immediate and gradual skin tissue expansion)
      4.3.2 Tissue processing for histological evaluation
   4.4 Statistical Analysis
   4.5 Results
      4.5.1 Changes in skin structure following immediate skin expansion in rats.
      4.5.2 Changes in skin structure following immediate skin expansion in dogs
      4.5.3 Changes in skin structure following gradual skin expansion in dogs
   4.6 Discussion
   4.7 Conclusions

xiv
5 EXPRESSION OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EXPANDED SKIN TISSUE

5.1 Introduction 89
5.2 Specific objectives 90
5.3 Material and Methods 90
  5.3.1 Animals 90
    5.3.1.1 Rats (immediate skin tissue expansion) 90
    5.3.1.2 Dogs (immediate and gradual skin tissue expansion) 90
  5.3.2 Biochemicals and Solutions 90
  5.3.3 Immunohistochemistry 90
  5.3.4 Scoring system for histomorphometry 91
5.4 Statistical Analysis 91
5.5 Results 91
  5.5.1 VEGF expression in the immediately expanded skin in rats 91
  5.5.2 VEGF expression in the immediately expanded skin in dogs 94
  5.5.3 VEGF expression in the gradually expanded skin in dogs 96
5.6 Discussion 98
5.7 Conclusions 99

6 MECHANICAL PROPERTIES OF THE EXPANDED SKIN

6.1 Introduction 100
6.2 Specific objectives 101
6.3 Material and methods 101
  6.3.1 Animals 101
    6.3.1.1 Rats (immediate skin tissue expansion) 101
    6.3.1.2 Dogs (immediate and gradual skin tissue expansion) 102
  6.3.2 Preparation of the skin samples 102
    6.3.2.1 Preparation of rat skin samples 102
    6.3.2.2 Preparation of dog skin sample 102
  6.3.3 Biomechanical test procedures 102
6.4 Statistical Analysis 104
6.5 Results 104
  6.5.1 Immediate skin expansion in rats 104
  6.5.2 Immediate skin expansion in dogs 106
  6.5.3 Gradual skin expansion in dogs 107
6.6 Discussion 109
6.7 Conclusion 111
7 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 General Discussion</td>
<td>112</td>
</tr>
<tr>
<td>7.2 General Conclusions</td>
<td>116</td>
</tr>
<tr>
<td>7.3 Recommendations for future studies</td>
<td>117</td>
</tr>
</tbody>
</table>

REFERENCES 118
APPENDICES 127
BIODATA OF STUDENT 133
LIST OF PUBLICATIONS 134
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The pros and cons of the conventional and self-inflating tissue expansion (Ronert et al., 2004)</td>
<td>15</td>
</tr>
<tr>
<td>2.2</td>
<td>Different types of tissue expansion (Johnson et al., 1993)</td>
<td>17</td>
</tr>
<tr>
<td>3.1</td>
<td>Median score (range) for Glasgow composite pain score of the immediate tissue expansion in dogs</td>
<td>41</td>
</tr>
<tr>
<td>3.2</td>
<td>Median score (range) for Glasgow composite pain score of the gradual tissue expansion in dogs</td>
<td>49</td>
</tr>
<tr>
<td>3.3</td>
<td>The differences of the gradual expansion process caused by round and rectangle self-inflating tissue expanders in dogs</td>
<td>52</td>
</tr>
<tr>
<td>5.1</td>
<td>Scoring system for the evaluation of VEGF expression in the expanded tissue</td>
<td>91</td>
</tr>
<tr>
<td>5.2</td>
<td>Median score (range) for VEGF expression score of the immediately expanded skin in rats</td>
<td>93</td>
</tr>
<tr>
<td>5.3</td>
<td>Median score (range) for VEGF expression score of the immediately expanded skin in dogs</td>
<td>96</td>
</tr>
<tr>
<td>5.4</td>
<td>Median score (range) for VEGF expression score of the gradually expanded skin in dogs</td>
<td>98</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>Skin anatomy and main cells of the epidermis layer</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td>Skin anatomy and main cells of the dermis layer and subcutaneous tissue</td>
<td>7</td>
</tr>
<tr>
<td>2.3</td>
<td>Description of the skin expansion process achieved by subcutaneous implantation of tissue expander</td>
<td>11</td>
</tr>
<tr>
<td>2.4</td>
<td>Different shapes of the tissue expanders used for the induction of the skin expansion process</td>
<td>13</td>
</tr>
<tr>
<td>2.5</td>
<td>Normalization of the expanded skin tissue after expander removal at the end of the skin expansion process</td>
<td>19</td>
</tr>
<tr>
<td>3.1</td>
<td>Different sizes of PMMA expanders, big (A), medium (B) and small (C) size used for immediate skin expansion in rats</td>
<td>24</td>
</tr>
<tr>
<td>3.2</td>
<td>Different sizes of PMMA expanders: big (A), medium (B) and small (C) sizes used for immediate skin expansion in dogs</td>
<td>25</td>
</tr>
<tr>
<td>3.3</td>
<td>Rectangle (A) and round (B) shaped self-inflating expanders used for gradual skin expansion in dogs</td>
<td>25</td>
</tr>
<tr>
<td>3.4</td>
<td>The animal groupings of immediate limb skin expansion experiment in rats</td>
<td>27</td>
</tr>
<tr>
<td>3.5</td>
<td>The animal groupings of immediate limb skin expansion experiment in dogs</td>
<td>28</td>
</tr>
<tr>
<td>3.6</td>
<td>The animal groupings of gradual limb skin expansion experiment in dogs</td>
<td>29</td>
</tr>
<tr>
<td>3.7</td>
<td>Creation of surgical subcutaneous pocket (black arrow) for implantation of PMMA expander at the metatarsal area in a rat</td>
<td>30</td>
</tr>
<tr>
<td>3.8</td>
<td>Surgical insertion of PMMA expander (black arrow) at the right metatarsal area in a rat</td>
<td>31</td>
</tr>
<tr>
<td>3.9</td>
<td>Surgical insertion of PMMA expander (A) through skin incision (B) at the right metacarpal area in a dog</td>
<td>32</td>
</tr>
</tbody>
</table>
3.10 Surgical implantation of round shaped self-inflating expander (A) through surgical skin incision (B) at the metacarpal area in a dog

3.11 Borders for expanded and normal skin flaps elevation at the end of skin expansion process of the metacarpal area in a dog

3.12 Circumferential elevation of both expanded and normal skin flaps (black arrow) at the end of skin expansion process of the metacarpal area in a dog

3.13 Rats body weight change during the first 7th post-operative days for all groups using 24 rats. Data were expressed as a mean. No significant difference in between days in group 2 and 3 at $P < 0.05$. Significant difference between days in group. Means with the same letter are not significantly different at $P < 0.05$

3.14 Swelling and redness of the right metatarsal region (black arrow) on the 1st post-surgical implantation of PMMA expander in a rat

3.15 Skin necrosis and exposure of the expander (black arrow) of expanded skin on the 9th day-surgical implantation of big PMMA expander at the metatarsal area in a rat

3.16 Skin tissue expansion of the right metatarsal region (black arrow) on the 14th day following surgical implantation of PMMA expander in a rat

3.17 The difference in height of the skin between expanded and normal skin in group 1 (small size), group 2 (medium size) and group 3 (big size of PMMA expander) using 6 rats. * denotes significant difference within groups at $P < 0.05$. Mean with different letters denotes significant difference of the expanded skin at $P < 0.05$ among groups 1, 2 and 3

3.18 PMMA expander (A), Expanded skin (B), fibrous capsule (C) on the 14th day following surgical implantation of PMMA expander in a rat

3.19 Swelling and redness of the right metacarpal area on the 1st day post-surgical implantation of medium PMMA expander in a dog of group 2

3.20 Skin tissue expansion of the right metacarpal region on the 14th day post-surgical implantation of medium PMMA expander in a dog of group 2
3.21 Skin necrosis and exposure of the expander (black arrow) at expanded skin area on the 10th day post-surgical implantation of big PMMA expander in a dog of group 3

3.22 The difference in height of the skin between expanded and normal skin in group 1 (small size), group 2 (medium size) and group 3 (big size of PMMA expander) using 6 dogs. * denotes significant difference within groups at $P < 0.05$. Mean with different letters denotes significant difference of the expanded skin at $P < 0.05$ between groups 3 and 1

3.23 The differences in the length of the explanted expanded and normal skin flaps in group 1 (small size), group 2 (medium size) and group 3 (big size of PMMA expander) using 6 dogs. * denotes significant difference within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

3.24 PMMA expander (A), expanded skin (B), fibrous capsule (C) following euthanasia on the 14th day post-surgical implantation of medium PMMA expander in a dog of group 2

3.25 The gross appearance of immediately expanded skin (a highly vascularized area) (A) on the 14th day post-surgical implantation of medium PMMA expander and normal skin (control) (B) of metacarpal area in a dog

3.26 Swelling and redness of the right metacarpal area (black arrow) on the 1st day post-surgical implantation of rectangle shaped self-inflating expander in a dog

3.27 Swelling and redness of the right metacarpal area (black arrow) on the 4th day post-surgical implantation of round shaped self-inflating expander in a dog

3.28 Swelling and redness of the right metacarpal area (black arrow) on the 4th day post-surgical implantation of rectangle shaped self-inflating expander in a dog

3.29 Swelling and redness of the right metacarpal area (black arrow) on the 7th day post-surgical implantation of round shaped self-inflating expander in a dog

3.30 Skin expansion process of the right metacarpal area (black arrow) on the 30th day post-surgical implantation of round shaped self-inflating expander in a dog

xx
3.31 Skin expansion process of the right metacarpal area (black arrow) on the 30th day post-surgical implantation of rectangle shaped self-inflating expander in a dog

3.32 Skin necrosis and exposure of the expander (black arrow) at expanded skin area on the 20th day post-surgical implantation of round shaped self-inflating expander in a dog

3.33 Healed hemorrhagic spots (black arrows) at the right metacarpal area on the 7th day post-surgical implantation of round shaped self-inflating expander in a dog

3.34 Hair loss at the expanded area (black arrow) of the right metacarpal area on the 30th day post-surgical implantation of round shaped self-inflating expander in a dog

3.35 Gradual skin expansion process using round and rectangle shaped self-inflating tissue expanders implanted at the right metacarpal area using 6 dogs. * denotes significant difference in the height of the expanding site for both shapes at 30th day from 1st day following expander implantation at \( P < 0.05 \). There was no significant difference of the expanded skin between groups at \( P < 0.05 \)

3.36 The length difference between normal (A) and gradually expanded skin (B) metacarpal area on the 30th day post-surgical implantation of rectangle shaped self-inflating expander (C) in a dog

3.37 The differences in the length of the explanted expanded and normal skin flaps in group 1 (round-shaped expander) and group 2 (rectangle-shaped expander) using 6 dogs. * denotes significant difference of flap length of the expanded tissue from the normal (control) within groups at \( P < 0.05 \). There was no significant difference between expanded skin of different groups at \( P < 0.05 \)

4.1 Photomicrograph of normal skin tissue of the metatarsal area of a rat showing normal thickness of the epidermis (A) and dermis layer (B) (H&E stain 200X)

4.2 Photomicrograph of immediately expanded skin tissue (medium PMMA expander) of the metatarsal area of a rat showing the increased thickness of epidermis (A), decreased thickness of dermis layer (B) and hyperkeratosis (C) (H&E stain 200X)
4.3 Photomicrograph of immediately expanded skin tissue (medium PMMA expander) of the metatarsal area of a rat showing the increased melanocytic activity (black arrows) (H&E stain 400X)

4.4 The difference in the thickness of the epidermis layer of the immediately expanded skin tissue of the metatarsal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in rats. * denotes significant difference of epidermis layer thickness of the expanded tissue compared with the normal skin tissue within groups at $P < 0.05$. There was no significant difference among the expanded skin of the different groups at $P < 0.05$

4.5 The difference in the thickness of the dermis layer of the immediately expanded skin tissue of the metatarsal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in rats using PMMA expanders. * denotes significant difference of dermis layer thickness of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among the expanded skin of the different groups at $P < 0.05$

4.6 The difference in the number of the hair follicle of the immediately expanded skin tissue of the metatarsal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in rats. * denotes significant difference of the hair follicle number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.7 The difference in the number of the sebaceous gland of the immediately expanded skin tissue of the metatarsal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in rats. * denotes significant difference of the sebaceous gland number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.8 Photomicrograph of immediately expanded skin tissue (small PMMA expander) of the metatarsal area of a rat showing the normal blood vessels (black arrows) (H&E stain 400X)
4.9 Photomicrograph of immediately expanded skin tissue (small PMMA expander) of the metatarsal area of a rat showing the increased in the number of blood vessels (black arrows) (H&E stain 400X)

4.10 Photomicrograph of normal skin tissue of the metatarsal area of a rat showing fibroblast (black arrows) (H&E stain 400X)

4.11 Photomicrograph of immediately expanded skin tissue (medium PMMA expander) of the metatarsal area of a rat showing the increased number of fibroblast (fibroplasia) (black arrows) (H&E stain 400X)

4.12 The difference in the number of the fibroblast cell of the immediately expanded skin tissue of the metatarsal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in rats. * denotes significant difference of the fibroblast number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.13 Photomicrograph of normal skin tissue of the metacarpal area of a dog showing normal thickness of the epidermis (A) and dermis layer (B) (H&E stain 200X)

4.14 Photomicrograph of immediately expanded skin tissue (big PMMA expander) of the metacarpal area of a dog showing the increased thickness of epidermis (A) and decreased thickness of dermis layer (B) (H&E stain 200X)

4.15 Photomicrograph of immediately expanded skin tissue (medium PMMA expander) of the metacarpal area of a dog showing normal keratosis (A) melanocytic activity (black arrows) (H&E stain 400X)

4.16 Photomicrograph of immediately expanded skin tissue (medium PMMA expander) of the metacarpal area of a dog showing hyperkeratosis (A) increased melanocytic activity (black arrows) (H&E stain 400X)

4.17 The difference in the thickness of the epidermis layer of the immediately expanded skin tissue of the metacarpal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in dogs. * denotes significant difference of epidermis layer thickness of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no
significant difference among expanded skin of different groups at $P < 0.05$

4.18 The difference in the thickness of the dermis layer of the immediately expanded skin tissue of the metacarpal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in dogs. * denotes significant difference of dermis layer thickness of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.19 The difference in the number of the hair follicle of the immediately expanded skin tissue of the metacarpal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in dogs. * denotes significant difference of the hair follicle number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.20 The difference in the number of the sebaceous gland of the immediately expanded skin tissue of the metacarpal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in dogs. * denotes significant difference of the sebaceous gland number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.21 Photomicrograph of immediately expanded skin tissue (big PMMA expander) of the metacarpal area of a dog showing the normal blood vessels (black arrows) (H&E stain 200X)

4.22 Photomicrograph of immediately expanded skin tissue (big PMMA expander) of the metacarpal area of a dog showing the increased in the number of blood vessels (black arrows) (H&E stain 200X)

4.23 Photomicrograph of normal skin tissue of the metacarpal area of a dog showing fibroblast (black arrows) (H&E stain 400X)

4.24 Photomicrograph of immediately expanded skin tissue (big PMMA expander) of the metacarpal area of a dog showing the increased number of fibroblast (fibroplasia) (black arrows) (H&E stain 400X)
4.25 The difference in the number of the fibroblast cell of the immediately expanded skin tissue of the metacarpal area of group 1 (small size), group 2 (medium size) and group 3 (big size PMMA expanders) in dogs. * denotes significant difference of the fibroblast number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference among expanded skin of different groups at $P < 0.05$

4.26 Photomicrograph of normal skin tissue of the metacarpal area of a dog showing normal thickness of the epidermis (A) and dermis layer (B) (H&E stain 200X)

4.27 Photomicrograph of gradually expanded skin tissue (rectangle-shaped-expander) of the metacarpal area of a dog showing the increased thickness of epidermis (A) and decreased thickness of dermis layer (B) (H&E stain 200X)

4.28 Photomicrograph of gradually expanded skin tissue (rectangle-shaped-expander) of the metacarpal area of a dog showing hyperkeratosis (A) increased melanocytic activity (black arrows) (H&E stain 400X)

4.29 The difference in the thickness of the epidermis layer of the gradually expanded skin tissue of the metacarpal area of group 1 (round) and group 2 (rectangle-shaped self-inflating expander) in dogs. * denotes significant difference of epidermis layer thickness of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference between expanded skin of different groups at $P < 0.05$

4.30 The difference in the thickness of the dermis layer of the gradually expanded skin tissue of the metacarpal area of group 1 (round) and group 2 (rectangle-shaped self-inflating expander) in dogs. * denotes significant difference of dermis layer thickness of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference between expanded skin of different groups at $P < 0.05$

4.31 The difference in the number of the hair follicle of the gradually expanded skin tissue of the metacarpal area of group 1 (round) and group 2 (rectangle-shaped self-inflating expander) in dogs.* denotes significant difference of the hair follicle number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference between expanded skin of different groups at $P < 0.05$
4.32 The difference in the number of the sebaceous gland of the gradually expanded skin tissue of the metacarpal area of group 1 (round) and group 2 (rectangle-shaped self-inflating expander) in dogs.* denotes significant difference of the sebaceous gland number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference between expanded skin of different groups at $P < 0.05$.

4.33 Photomicrograph of gradually expanded skin tissue (rectangle-shaped expander) of the metacarpal area of a dog showing the increased in the number of blood vessels (black arrows) (H&E stain 200X)

4.34 Photomicrograph of normal skin tissue of the metacarpal area of a dog showing fibroblast (black arrows) (H&E stain 400X)

4.35 Photomicrograph of gradually expanded skin tissue (round-shaped expander) of the metacarpal area of a dog showing the increased number of fibroblast (fibroplasia) (black arrows) (H&E stain 400X)

4.36 The difference in the number of the fibroblast cell of the gradually expanded skin tissue of the metacarpal area in dogs using round and rectangle shaped self-inflating expanders. * denotes significant difference of the fibroblast number of the expanded tissue from the normal skin tissue within groups at $P < 0.05$. There was no significant difference between expanded skin of different groups at $P < 0.05$.

4.37 Photomicrograph of gradually expanded skin tissue (rectangle-shaped expander) of the metacarpal area of a dog showing the infiltration of the inflammatory cells (black arrows) (H&E stain 400X)

5.1 Micrograph of immunoperoxidase staining showing no detection of VEGF in normal skin specimen of a rat (200 X)

5.2 Micrograph of immunoperoxidase staining showing weak expression of VEGF in the epidermis (black arrow) of the normal skin specimen of a rat (black arrow) (200 X)

5.3 Micrograph of immunoperoxidase staining showing increased expression of VEGF in the epidermis (black arrow) of the immediately expanded skin specimen of a rat (black arrow) (200 X)
5.4 Micrograph of immunoperoxidase staining showing no detection of VEGF in normal skin specimen of a dog (200 X) 94

5.5 Micrograph of immunoperoxidase staining showing weak expression of VEGF in the epidermis (black arrow) of the normal skin specimen of a dog (black arrow) (200 X) 95

5.6 Micrograph of immunoperoxidase staining showing increased expression of VEGF in the epidermis (A) and hair follicle (B) of the immediately expanded skin specimen of a dog (200 X) 95

5.7 Micrograph of immunoperoxidase staining showing no detection of VEGF in normal skin specimen of a dog (200 X) 96

5.8 Micrograph of immunoperoxidase staining showing weak expression of VEGF of the normal skin specimen of a dog (black arrow) (200 X) 97

5.9 Micrograph of immunoperoxidase staining showing increased expression of VEGF in the epidermis (A) and hair follicle (B) of the gradually expanded skin specimen of a dog (black arrow) (200 X) 97

6.1 Instron mechanical testing machine (3365) used for tensile strength measurement of expanded and normal skin in rats and dog 103

6.2 Tensile strength measurement of expanded skin sample (black arrow) of metatarsal area in rats 103

6.3 Tensile strength measurement of expanded skin flap (black arrow) of metacarpal area in dogs 104

6.4 Maximum load of the normal and immediately expanded skin (medium size of PMMA expander) using 6 rats. * denotes significant difference of the tensile strength of the expanded skin in comparison to their corresponding normal (control) specimens at $P < 0.05$ 105

6.5 The stress-strain curve of both normal skin flap (A) and immediately expanded skin flap (B) of metatarsal area of a rat 105

6.6 Maximum load of the normal and immediately expanded skin flap of the all groups (small, medium and big size of PMMA expander) using 6 dogs. * denotes significant difference of the tensile strength of the expanded skin in comparison to their corresponding normal (control) specimens within groups at $P <$
0.05. There was no significant difference among expanded skin
of different groups at $P < 0.05$

6.7 The stress-strain curve of both normal skin flap (A) and
immediately expanded skin flap (B) of metacarpal area of a dog

6.8 Maximum load of the normal and gradually expanded skin flap
of the all group (round and rectangle-shaped tissue expander)
using 6 dogs. * denotes significant difference of the tensile
strength of the expanded skin in comparison to their
corresponding normal (control) specimens within groups at $P < 0.05$. There was no significant difference between expanded
skin of different groups at $P < 0.05$

6.9 The stress-strain curve of both normal skin flap (A) and
gradually expanded skin flap (B) of metacarpal area of a dog
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Institutional Animal Care and Use Committee approval (rats)</td>
</tr>
<tr>
<td>B</td>
<td>Institutional Animal Care and Use Committee approval (dogs)</td>
</tr>
<tr>
<td>C</td>
<td>Short form of Glasgow pain scale used for pain assessment in dogs.</td>
</tr>
<tr>
<td>D</td>
<td>Histopathology Procedure</td>
</tr>
<tr>
<td>E</td>
<td>Avidin Biotin Complex (ABC) Immunoperoxidase Staining</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB</td>
<td>International Pound</td>
</tr>
<tr>
<td>µg</td>
<td>Micro gram</td>
</tr>
<tr>
<td>ABC</td>
<td>Avidin biotin complex</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>Cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>G</td>
<td>Gram</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>I.V</td>
<td>Intravenous</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IP</td>
<td>Immuno Peroxidase</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>Mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MI</td>
<td>Milliliter</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>pH</td>
<td>Potential for hydrogen ion</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly methyl methacrylate</td>
</tr>
<tr>
<td>S.C</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistic for social sciences</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of the study

Skin can be defined as the largest organ in the mammalian organism that protects body against many external factors which include physical, chemical and biological. There are several skin functions of vital importance such as defense, thermoregulation, excretion, resorption, metabolism and sensation. The major defensive function of the skin is the hemostasis maintenance when uncontrolled loss of water, ions and serum proteins is prevented (Darlenski et al., 2011).

Skin consists of three different layers: epidermis, dermis and subcutis. Each layer varies in cellular composition resulting in various structure and function. The outermost layer of skin is the epidermis in which the veins and capillaries are absent. The epidermis thickness is variable according to different body site as well as epidermis water content. The epidermis is composed of five sublayers: stratum basale (basal cell layer), stratum spinosum (prickle cell layer), stratum granulosum (granular cell layer), stratum lucidum (clear layer) and stratum corneum (horny cell layer).

The dermis is the second layer of skin which is located beneath the epidermal layer and is subdivided into two sublayers: the upper papillary layer and the lower reticular layer. The dermis mainly consists of collagen and elastin fibers with few cells when compared to the epidermis. Subcutis, or hypodermis is an elastic, fatty layer located under the dermis where the massive amount of fat cells act as a shock absorber for blood vessels and nerve endings (Igarashi et al., 2007). Various body sites have different skin characteristics such as pH, temperature, moisture, topological and microbiological features. In addition, laxity and extensibility also differ in certain body parts in spite of the same collagen concentrations. For instance, skin of chest and abdominal regions is less lax when compared with high lax groin skin, denoting that the overall elastic modulus is also influenced by the structure of both dermis and hypodermis. Furthermore, there are different mechanical properties at different body regions as a result of distribution of skin ligament. For example, skin is anchored by many skin ligaments to the sites having underlying muscle movement such as head, neck, upper trunk and limbs, while irregular skin ligament patterns have been observed in abdomen and buttocks leading to volume changes of these sites to allow adipose tissue deposition (Wong et al., 2016).
Small proximal limb wounds or other wounds over different body regions are easier to manage than large defects of skin, distal limb wounds and wounds close to orifices which are difficult to repair as there is lack of spare skin necessary for simple wound closure. In addition, functional problems as a result of wound distortion in area near the anus and eyes are anticipated in spite of free skin availability (Yool, 2012). The management of defects on the extremities or relatively large defects can be achieved by tissue transfer from other sites. The amount of skin available for transfer varies between sites on the same animal and between breeds. Little skin can be mobilized in the extremities, whereas advancing adjacent tissue often can close large defects over the trunk (Fossum et al., 2013).

Tissue expansion is the perfect strategy which generates extra skin of the same color, texture, and hair bearance of the adjacent healthy skin, so that scars and risk of rejection can be minimized (Tepole et al., 2012). In the early 1900s, the orthopedic surgeons performed the early attempts of living tissues expansion, but the first clinical application was experienced by Neumann in 1957 when post auricular skin tissue expansion was achieved by subcutaneous placement of a rubber balloon (Neumann, 1957). Decades later, expansion process was advanced for breast reconstruction by Radovan (Radovan, 1982). Ever since the useful clinical application of skin tissue expansion has been performed routinely for repair of skin defects (Fochtmann et al., 2013).

Conventional chronic tissue expansion is achieved by subcutaneous implantation of tissue expander for relatively long period of time leading to increased epidermal thickness, dermal thinning, bone resorption, vascular capsule formation and angiogenesis that improves flap viability. An increase in surface area during conventional tissue expansion could be primarily attributed to the biological tissue creep (Johnson et al., 1993). The amount of chronically expanded tissue for 6 week period is about 135% increase in the skin surface area (Shan & Baker, 1991).

Conventional tissue expansion was modified first by Sasaki in 1985 resulting into immediate intraoperative tissue expansion technique. Successful soft-tissue reconstruction using this technique has been reported by several authors, on the other hand its effectiveness has been confirmed and questioned using animal models in a controlled studies. Mechanical creep is a mechanism by which skin tissue can be acutely expanded relying on structural and morphological features of the skin collagen and elastin fibers. Sasaki protocol of rapid tissue expansion was three minute expansion followed by three minute rest cycle which is repeated three times with the maximal volume of the selected expander (Raposio et al., 2000).
The most important advantage of tissue expansion process of the adjacent donor tissue is that it creates an additional tissue of similar color and texture of the recipient site tissue as well as both sensation and hair bearing tissue are recruited by tissue expansion. Reconstruction of the breast, neck, and the trunk are the main tissue expansion applications with usually accepted adverse effects (Kotb & Soliman, 2007). Many qualitative and quantitative tissue defects of the nose, forehead, temple, scalp and massive abdominal wall defects can be overcome by ideal tissue replacement provided by the exceptional reconstructive expansion technique. With limited donor sites, tissue expansion helps to reconstruct extensive areas of burns and correct male-pattern baldness (MPB) as among the most valuable uses of tissue expansion (El-Moghazy, 2003). There are many disadvantages of tissue expansion such as long duration of the expansion process (3-25 weeks) which varies according to site, age, the amount of skin required, so that many times of hospital visits are needed during this time to inflate the expander by a percutaneous injection in case of using traditional tissue expander. Other disadvantages include: deformity problem as a result of expander appearance, high complication rate (40%) such as infection, hematoma, exposure of the expander or tubing, and failure of the implant, resulting in a leak and deflation and few body regions that cannot be expanded (Hughes, 1987).

Usually major complications like infection and extrusion lead to failure of the expansion process, while minor complications are those that do not result in failure. They are in the form of erythema, leakage, hematoma, valve dysfunction and wound dehiscence, infusion port extrusion, impending exposure, scar hypertrophy, and unusually wide scars. The complications rate was observed to be the highest in the extremities especially in regions below the knee and elbow joints (Hawary, 1998; Fochtmann et al., 2013).

1.2 Statement of the problems

1. Primary closure after excision of large skin tumors, scars, and burns in small animals seems to be difficult due to loss of considerable amounts of skin especially in areas of limited tissue mobility. Traditional techniques such as split-skin grafting as well as complex reconstructions (free flaps) which were associated with poor clinical and cosmetic outcomes are used to close these wounds.

2. Surgical coverage of these defects cannot be easily performed without sufficient skin tissue. Expansion of tissue using current techniques to create additional amount of skin was also associated with significant complications in animals.
1.3 Research hypothesis

Immediate or gradual skin tissue expansion techniques using rigid or gradual self-inflating implant in laboratory and small animals can produce additional skin tissue that is clinically, histologically and physically viable. Careful planning of tissue expansion as well as attentive post-operative care can reduce the complications rate of tissue expansion.

1.4 Objectives:-

To assess clinically the expanded tissue following immediate or gradual skin tissue expansion.

1. To study the Histological aspects of the cutaneous tissue following immediate or gradual skin tissue expansion.
2. To evaluate the mechanical properties of the expanded tissue caused by immediate or gradual skin tissue expansion.
3. To test the presence and evaluate the expression of the vascular endothelial growth factor (VEGF) in the immediately and gradually expanded skin tissue.
REFERENCES


