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

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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

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.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Loqman Mohamad Yusof, PhD
Faculty : Veterinary Medicine

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

Pengembangan tisu adalah teknik yang digunakan untuk menyebabkan tubuh menumbuhkan tisu tambahan, membolehkan liputan pelbagai luka kompleks**.** Pada tahun 1957, Neumann merupakan yang pertama menghuraikan pengembangan tisu lembut klinikal apabila belon getah berisi udara ditempel secara subkutaneus untuk membina semula telinga selepas ablasi traumatik**.** Teknik ini telah diperbaiki oleh Radovan dan Argenta pada tahun 1970 apabila mereka menggunakan larutan garam untuk mengembangkan belon silikon secara beransur-ansur melalui liang pengisian subkutis**.** Peranti ini adalah berbentuk yang sangat standard dan bentuk serta saiz yang berbeza sedia ada untuk memenuhi pelbagai indikasi. Suatu model pengembungan-sendiri dihuraikan oleh Austad dan Rose pada tahun 1982. Tujuan kajian ini adalah untuk mengkaji kesan kadar segera dan beransur-ansur pengembangan tisu kulit anggota badan di dalam tikus dan anjing menggunakan satu teknik pengembangan**.** Objektif khusus kajian ialah: (1) menilai secara klinikal pengembangan tisu melalui pengembangan segera atau pengembangan beransur-ansur. (2) mengkaji aspek histologi tisu kulit selepas pengembangan segera atau beransur-ansur. (3) menilai ciri-ciri mekanikal pengembangan tisu disebabkan pengembangan segera atau beransur-ansur tisu kulit. (4) menguji kehadiran dan menilai ekspresi faktor pertumbuhan endotelium vaskular (VEGF) di dalam tisu kulit tikus dan anjing selepas pengembangan segera atau beransur-ansur.

Pengembangan tisu segera telah dikaji menggunakan pengembang tisu dengan beberapa saiz berbeza yang berbentuk empat segi tepat yang tetap diperbuat daripada polimetil metakrilat (PMMA) yang ditempel melalui pembedahan di bawah lapisan tisu subkutis di kawasan metakarpus bagi anjing dan kawasan metatarsal bagi tikus. Pengembang tisu pengembung-kendiri hidrogel berbentuk bulat atau empat segi tepat juga ditempel melalui pembedahan di tapak yang sama di dalam haiwan anjing individu yang berbeza untuk menilai pengembangan tisu kulit secara beransur-ansur. Pengembangan segera tisu kulit dari kawasan metatarsus dilakukan ke atas 4 kumpulan tikus (6 ekor/ kumpulan) menggunakan 4 saiz pengembang PMMA yang berbeza. Pengembangan segera kulit di kawasan metakarpal diaruh di dalam 3 kumpulan anjing (6 ekor/ kumulan) menggunakan tiga saiz berbeza pengembang PMMA. Pengembangan segera dibiarkan berlangsung selama 14 hari dari masa implantasi pengembang tisu di dalam tikus dan anjing. Untuk mengaruh pengembangan tisu kulit secara beransur-ansur di kawasan metakarpal, dua kumpulan anjing (6 ekor/ kumpulan) digunakan. Pengembangan tisu kulit dilakukan menggunakan pengembang berbentuk bulat atau empat segi tepat, dan dibiarkan berlangsung selama 30 hari.

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 dikaitkan 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, neovaskularisasi, penjajaran 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 signufikan 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 emapt 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.

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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)

_____
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Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____
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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

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

IB	International Pound
µg	Micro gram
ABC	Avidin biotin complex
ANOVA	Analysis of variance
Cm	Centimeter
G	Gram
H&E	Hematoxylin and Eosin
I.V	Intravenous
IACUC	Institutional Animal Care and Use Committee
IP	Immuno Peroxidase
IP	Intraperitoneal
Kg	kilogram
Mg	Milligram
Ml	Milliliter
PBS	Phosphate Buffered Saline
pH	Potential for hydrogen ion
PMMA	Poly methyl methacrylate
S.C	Subcutaneous
SPSS	Statistic for social sciences
UPM	Universiti Putra Malaysia

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.

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