



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF KERATIN-GELATIN AND BASIC FIBROBLAST GROWTH
FACTOR-GELATIN COMPOSITE FILM ON OPEN WOUND HEALING IN
DOGS AND CATS**

ARUL JOTHI. N

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By

ARUL JOTHI. N

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

March 2007



**DEDICATED WITH GRATITUDE
TO
MY EXPERIMENTAL DOGS**



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

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FACTOR-GELATIN COMPOSITE FILM ON OPEN WOUND HEALING IN
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ARUL JOTHILN

March 2007

Chairman: Professor Sundararajan Thilagar, PhD

Faculty: Veterinary Medicine

Wound is a disruption of the normal continuity of the skin surface. A prolonged wound healing time is distressing and expensive. Immediate wound coverage is a cornerstone of wound management. Extensive wounds in the skin can be treated using dressing materials and skin grafts. A full-thickness mesh graft can be applied to cover large skin defects. To accelerate wound healing, the use of biomaterials such as keratin, gelatin and basic fibroblast growth factor (bFGF) has increased in recent years.

Feathers contain beta keratin as a major component. Keratin as a structural protein that can be processed from poultry feathers and made into value added products, which benefit wounds healing. Gelatin and bFGF are well known for their wound healing properties. Dermal substitutes are very expensive and used routinely in human. However such materials are not available at reasonable cost to treat extensive wounds in animals.



Keratin hydrolysates from poultry feathers were prepared by controlled alkaline hydrolysis. Following hydrolysis the supernatant solution was decanted and brought to pH 7.0 using sulfuric acid, then 10% solution of pharmaceutical grade gelatin solution was mixed followed by addition of 1% ethylene glycol and 0.35% glutaraldehyde to the solution and finally cast in polythene trays and dried at 50 °C. bFGF-gelatin composite film was prepared by adding pharmaceutical grade gelatin solution 10 %, 1% ethylene glycol and 0.35% glutaraldehyde to basic fibroblast growth factor (0.015µg/cm²) and casted in polythene trays and dried at 50 °C. The film was soaked for 2 minutes in gentamycin (Dutch farm Veterinary pharmaceuticals, Netherlands) and then applied on wound.

This study was conducted with hypothesis that keratin-gelatin and bFGF-gelatin composite films are effective wound healing stimulants causing early re-epithelialization and an uncomplicated wound healing favoring early acceptance of the full thickness skin mesh. The objectives of this study was to identify and evaluate wound healing properties of keratin-gelatin and bFGF-gelatin composite films on open wound and as a feeder layer for early acceptance of full-thickness skin mesh graft in dogs. Following the identification of the better of the two biomaterials, it was used in clinical cases involving 10 cats and two dogs with extensive skin wounds presented to the University Veterinary Hospital University Putra Malaysia. The application of keratin and bFGF on wound healing in experimental dogs and clinical cases in this research was a pilot study undertaken.

Thirty six healthy dogs were used in the study. Under general anaesthesia and aseptic condition, a full-thickness skin wound (approximately 5x 5cm) was created

lateral to the right loin region. Eighteen animals were used for open wound groups divided into 3 groups (n = 6) namely Group I (control group), Group II (treated with keratin-gelatin composite film) and Group III (treated with bFGF-gelatin composite film). Another 18 animals were treated with full-thickness skin mesh graft were also divided into 3 Groups (n = 6) namely Group I (control group), Group II (treated with feeder layer of keratin-gelatin composite film), Group III (treated with feeder layer of bFGF-gelatin composite film). Evaluation of the effect of biomaterials on open wound and the full-thickness skin mesh graft was done based on clinical observation, haematological, bacteriological, biochemical and histopathological examinations on Days 4, 8, 12, 16 and 20 post-operation. Biochemical and histopathological evaluations on the full-thickness skin mesh graft were done on Days 12, 16 and 20 post-operation.

The keratin-gelatin and bFGF-gelatin composite films could easily be applied on wounds. The composite films were well accepted and tolerated by animals and did not show any adverse reactions. Open wounds treated with keratin-gelatin (Group II) showed a bright red granulation tissue, without malodour and exudates on Day 20 post-operation, when compared to other groups. The percentage of wound epithelialization, wound contraction and total wound healing was significantly higher ($P < 0.05$) in keratin-gelatin (Group II) throughout the trail.

The full-thickness skin mesh graft treated with keratin-gelatin (Group II) as a feeder layer showed an early vascularization of the graft, with epithelialization of the interstices. Acceptance of the graft by Day 12 post-operation was complete with hair growth and normal colour of the skin without any evidence of rejection. The graft

acceptance was 90-100% on Day 20 post-operation. In bFGF-gelatin (Group III), all animals showed a dark discolouration of epidermis of the graft without rejection on Days 16 and 20 post-operation. No adverse effects were observed on the hematological values obtained in the treated groups. On bacteriological examination, *Staphylococcus aureus*, *Klebsiella spp.*, *Proteus spp.* and *Pseudomonas spp.* were isolated in all animals in open wound groups. *Staphylococcus aureus* was isolated from one animal each in Groups I and II and *Proteus spp.* from one animal in Group III treated with the full-thickness skin mesh graft. The keratin-gelatin composite film (Group II) favoured tissue DNA, protein and collagen formation, which was essential for wound healing and early acceptance of the full-thickness skin mesh graft. The efficiency of fibroblast formation and angiogenesis was good in the animals treated with keratin-gelatin composite film (Group II) which favoured an early wound healing. In the full-thickness skin mesh graft group, the animals treated with feeder layer of keratin-gelatin composite films, showed normal epidermis thickness on Day 20 post-operation. Keratin-gelatin composite film was effective in clinical cases involving 10 cats and 2 dogs presented at the University Teaching Hospital of Universiti Putra Malaysia.

As per the hypothesis, Keratin-gelatin composite film was an effective wound healing stimulant causing early re-epithelialization and uncomplicated wound healing favoring an early acceptance of the full thickness skin mesh graft. The objective of this study was fulfilled when the use of keratin-gelatin composite film was found to be a better biomaterial when compared to bFGF-gelatin composite film. Keratin-gelatin was effective for wound healing in clinical cases presented at the University Teaching Hospital of Universiti Putra Malaysia. The above findings have

a commercial application because keratin from poultry feathers an inexpensive as a skin substitute to stimulate wound healing in animals where the cost of treatment is a major consideration by clients. Further research is needed at different concentrations of keratin-gelatin and bFGF-gelatin incorporated composite film for wound healing in experimental and clinical cases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**KESAN KERATIN-GELATIN DAN FAKTOR PERTUMBUHAN BASIC
FIBROBLAST - FILEM KOMPOSIT GELATIN KEATAS PEMULIHAN
LUKA TERBUKA DAN SEBAGAI LAPISAN PENYUAPAN FULL-
THICKNESS SKIN MESH GRAFT PADA ANJING**

Oleh

ARUL JOTHILN

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Luka merupakan gangguan kepada keutuhan permukaan kulit normal. Jangkamasa penyembuhan luka yang mengambil masa yang lama adalah menekan dan mahal. Penyembuhan luka dalam masa yang singkat merupakan batu sendi dalam pengendalian luka. Luka yang luas pada kulit boleh dirawat dengan kaedah pembalutan dan dibantu dengan graf kulit. Graf mesy penuh tebal boleh diaplikasi untuk menutup luka kulit yang luas. Dengan graf yang demikian untuk mempercepatkan penyembuhan luka penggunaan biobahan seperti faktor pertumbuhan fibroblast asas (basic fibroblast growth factor – bFGF) telah meningkat kebelakangan ini.

Bulu yang mengandungi beta keratin digunakan sebagai komponen utama. Keratin sebagai protein berstruktur boleh diproses daripada bulu ayam, itik dan dijadikan produk tambahnilai yang berguna dalam penyembuhan luka. Gelatin dan bFGF sedia diketahui bermanfaat dalam penyembuhan luka.. Kulit gentian adalah mahal dan

hanya digunakan untuk manusia dan hingga kini tidak terdapat bahan dengan harga berpatutan yang boleh digunakan untuk merawat luka yang luas dalam haiwan.

Dengan yang demikian, kajian ini adalah bertujuan mengenal pasti dan menilai keupayaan penyembuhan keratin-gelatin dan filem komposit bFGF-gelatin pada luka terbuka sebagai lapisan pemakanan (feeder layer) sebagai penerimaan awal untuk graf mesy sepenuh tebal kulit dalam anjing. Selepas mengenalpasti biomaterials yang lebih baik antara dua bahan tersebut bahan berkenaan telah digunakan untuk merawat kes-kes luka mendalam (anjing dan kucing) yang dirujuk kepada Hospital Pengajaran Universiti, Universiti Putra Malaysia.

Tiga puluh enam ekor anjing yang sihat telah digunakan dalam kajian ini. Dengan menggunakan teknik aseptik yang sesuai dan anaesthesia umum, luka sebagai sepenuh tebal kulit, berukuran 5 x 5 cm dibedah berhampiran sisi pinggang. Lapan belas ekor anjing telah digunakan untuk kumpulan luka terbuka yang dibahagikan kepada tiga kumpulan kecil (n=6). Kumpulan I merupakan kumpulan kawalan; kumpulan II sebagai kumpulan yang dirawat dengan filem komposit keratin-gelatin; manakala kumpulan III pula dirawat dengan filem komposit bFGF-gelatin. Disamping itu, lapan belas ekor anjing yang lain pula dirawat dengan graf mesy sepenuh tebal kulit yang dibahagikan kepada tiga kumpulan (n=6).

Kumpulan I dan Kumpulan II dirawat dengan lapisan pemakanan filem komposit keratin-gelatin dan Kumpulan III dirawat dengan lapisan pemakanan filem komposit bFGF-gelatin. Penilaian ke atas kesan penggunaan biomaterial pada luka terbuka dan graf mesy sepenuh tebal kulit dilakukan dengan berasaskan pemerhatian klinikal,

pemeriksaan hematologi, bakteriologi, biokimia dan histopatologi pada hari-hari ke-4, 8, 12, 16, dan 20 tarikh pembedahan. Filem-filem komposit keratin-gelatin dan bFGF-gelatin mudah digunakan pada permukaan luka. Filem-filem komposit tersebut telah diterima dan ditoleransi oleh haiwan dan tidak menunjukkan sebarang kesan yang memudaratkan. Selepas dua puluh hari selepas tarikh pembedahan, luka terbuka Kumpulan II menunjukkan granulasi tisu yang berwarna merah cerah, tidak berbau (malodour) serta bereksudat berbanding dengan kumpulan-kumpulan lain. Sepanjang tempoh kajian, peratusan pembentukan epitelium pada luka, pencerutan dan jumlah pemulihan luka adalah signifikan ($P < 0.05$) bagi kumpulan keratin-gelatin (Kumpulan II).

Bagi kumpulan graf mesy sepenuh tebal kulit yang dirawat dengan keratin-gelatin (kumpulan II) sebagai lapisan pemakanan menunjukkan pembentukan awal kapilari darah pada graf di celah-celah epitelium. Penerimaan graf pada hari ke-12 kelihatan sempurna dengan pertumbuhan bulu dan penukaran warna kulit kepada warna asal. Peratusan penerimaan adalah 90-100% pada hari ke-20. Kesemua haiwan di kumpulan bFGF-gelatin (Kumpulan III) pula menunjukkan graf epidermis berwarna gelap tanpa penolakan pada hari ke-16 dan ke-20.

Bacaan hematologi ke atas kumpulan-kumpulan dalam kajian tidak menunjukkan sebarang kesan yang memudaratkan. Beberapa spesis bakteria berjaya diasingkan seperti *Staph. aureus*, *Klebsiella spp.*, *Proteus spp.*, dan *Pseudomonas spp.* apabila ujian bakteriologi dilakukan keatas kesemua haiwan dari kumpulan luka terbuka. *Staphylococcus aureus* pula diasingkan daripada seekor anjing masing-masing dari

kumpulan I dan II, manakala *Proteus spp.* diasingkan daripada seekor anjing dari kumpulan III.

Filem komposit keratin-gelatin (Kumpulan I) membantu pembentukan tisu DNA, protein dan kolagen yang penting dalam penyembuhan luka dan penerimaan awal graf mesy sepenuh tebal kulit. Kesempurnaan pembentukan fibroblast dan angiogenesis adalah amat ketara pada haiwan yang dirawat dengan filem komposit keratin-gelatin (Kumpulan II) yang membantu proses penyembuhan awal luka. Haiwan daripada Kumpulan graf mesy sepenuh tebal kulit dirawat dengan lapisan pemakanan filem komposit keratin-gelatin menunjukkan ketebalan epidermis yang normal pada hari ke-20 selepas pembedahan. Filem komposit keratin-gelatin juga boleh dikatakan ubat gentian yang berkesan bagi kes klinikal (anjing dan kucing) yang dirujuk ke Hospital Pengajaran Universiti, Universiti Putra Malaysia.

Penyelidikan ini telah mencapai objektifnya dimana boleh disimpulkan bahawa filem komposit keratin-gelatin merupakan pendorong kepada penyembuhan luka yang sempurna di peringkat awal bagi luka yang mendalam pada anjing. Selain itu, ia juga merupakan lapisan pemakanan yang sesuai untuk graf mesy sepenuh tebal kulit untuk meningkatkan kadar penerimaan graf. Keratin-gelatin juga boleh dikatakan pengubatan gantian yang lebih murah untuk merawat luka yang dalam pada haiwan. Mengambil kira ia merupakan kajian yang pertama dalam bidang veterinar, maka penyelidikan seterusnya diperlukan dengan memberi tumpuan yang berbeza terhadap keratin-gelatin dan bFGF-gelatin dimasukkan dalam filem komposit untuk penyembuhan luka.

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I certify that an Examination Committee has met on 14th June 2007 to conduct the final examination of Arul Jothi.N on her Doctor of Philosophy thesis entitled “Effects of Keratin-Gelatin and Basic Fibroblast Growth Factor-Gelatin Composite Film on Open Wound Healing in Dogs and Cats.” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ARUL JOTHI. N

Date :

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vii
ACKNOWLEDGEMENTS	xi
APPROVAL	xiii
DECLARATION	xv
LIST OF TABLES	xix
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxix
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	8
2.1 History of wound healing	8
2.2 Structure of the skin	9
2.3 Biomaterials	10
2.3.1 Historical development of biomaterials	10
2.4 Open wound	18
2.5 Phases of wound healing	18
2.5.1 Inflammatory phase	19
2.5.2 Granulation formation/proliferative or fibroblastic phase	20
2.5.3 Wound contraction	23
2.5.4 Maturation / remodeling phase	25
2.6 Chronic/ Non-healing wound	25
2.7 Treatment of wound	26
2.8 Clinical observation of the wound	27
2.9 Wound planimetry	28
2.10 History of skin grafts	30
2.10.1 Full-thickness skin mesh graft	31
2.11 Skin substitutes	33
2.12 Haematological evaluation	34
2.13 Bacteriological evaluation	35
2.14 Biochemical analysis	38
2.15 Histopathological examination	41

3	CLINICAL EVALUATION OF KERATIN-GELATIN AND BASIC FIBROBLAST GROWTH FACTOR–GELATIN COMPOSITE FILM ON OPEN WOUND AND AS A FEEDER LAYER FOR FULL-THICKNESS SKIN MESH GRAFT	
3.1	Introduction	43
	3.1. Recent trend in wound treatment	43
3.2	Materials and Methods	45
	3.2.1 Composite film preparation	45
	3.2.2 Animals	47
	3.2.3 Anaesthesia	48
	3.2.4 Surgical protocol	49
	3.2.5 Clinical observation	54
3.3	Results	58
	3.3.1 Clinical observation	58
3.4	Discussion	81
4	A HAEMATOLOGICAL AND BACTERIOLOGICAL EVALUATION OF OPEN WOUND AND FULL-THICKNESS SKIN MESH GRAFT TREATED WITH KERATIN-GELATIN AND bFGF-GELATIN COMPOSITE FILM	
4.1	Introduction	91
	4.1.1 Haematological study	91
	4.1.2 Bacteriological study	92
4.2	Materials and Methods	93
	4.2.1 Blood sampling technique	93
	4.2.2 White blood cell count	94
	4.2.3 Differential count	94
	4.2.4 Serum total protein and albumin estimation	94
	4.2.5 Bacteriological examination	95
	4.2.6 Statistical Analysis	96
4.3	Results	96
	4.3.1 White blood cell count	96
	4.3.2 Differential count	99
	4.3.3 Total serum protein	109
	4.3.4 Serum albumin	111
	4.3.5 Bacteriological examination	114
4.4	Discussion	114
5	BIOCHEMICAL AND HISTOPATHOLOGICAL EVALUATION FOR OPEN WOUND AND FULL - THICKNESS SKIN MESH GRAFT WITH FEEDER LAYER	
5.1	Introduction	118
	5.1.1 Biochemical analysis	118
	5.1.2 Histopathological evaluation	119
5.2	Materials and Methods	120
	5.2.1 Tissue sample collection	120
	5.2.2 DNA and Protein estimation	121

	5.2.3 Collagen estimation	124
	5.2.4 Histopathological evaluation	125
	5.2.5 Statistical Analysis	128
5.3	Results	129
	5.3.1 DNA content	129
	5.3.2 Protein content	133
	5.3.3 Collagen content	135
	5.3.4 Histopathological evaluation	136
5.4	Discussion	163
6	CLINICAL EVALUTION ON EFFECTS OF KERATIN-GELATIN COMPOSITE FILM ON WOUND HEALING IN DOGS AND CATS	
6.1	Introduction	169
6.2	Materials and Methods	173
	6.2.1 Preparation of keratin-gelatin composite film	173
	6.2.2 Animals	173
	6.2.3 Open wound	173
	6.2.4 Open wound treatment	174
	6.2.5 Clinical observation	174
	6.2.6 Wound planimetry	175
	6.2.7 Bacteriological examination	175
6.3	Results	175
	6.3.1 Open wound treatment	175
	6.3.2 Clinical observation	175
	6.3.3 Wound planimetry	184
	6.3.4 Bacteriological examination	184
6.4	Discussion	187
7	GENERAL DISCUSSION AND CONCLUTIONS	189
	REFERNCES	201
	APPENDICES	217
	BIODATA OF THE STUDENT	226
	LIST OF PUBLICATIONS	227

LIST OF TABLES

Table		Page
1.1	Colour code of the wound	55
2.1	Clinical observation of the open wound	60
3.1	Percentage of epithelialization of the open wound (Mean \pm S.D)	67
3.2	Percentage of contraction of the open wound (Mean \pm S.D)	69
3.3	Percentage of total wound healing of the open wound (Mean \pm S.D)	70
3.4	Clinical observation of the full-thickness skin mesh graft	74
4.1	WBC (blood) counts $\times 10^9$ /L of the open wound (Mean \pm S.D)	96
4.2	WBC (blood) counts $\times 10^9$ /L of the full-thickness skin mesh graft (Mean \pm S.D)	97
4.3	Segmented neutrophil counts $\times 10^9$ /L of the open wound (Mean \pm S.D)	99
4.4	Segmented neutrophil counts $\times 10^9$ /L of the full-thickness skin mesh graft (Mean \pm S.D)	99
4.5	Lymphocyte counts $\times 10^9$ /L of the open wound (Mean \pm S.D)	101
4.6	Lymphocyte counts $\times 10^9$ /L of the full-thickness skin mesh graft (Mean \pm S.D)	102
4.7	Monocyte counts $\times 10^9$ /L of the open wound (Mean \pm S.D)	104
4.8	Monocyte counts $\times 10^9$ /L of the full-thickness Skin mesh graft (Mean \pm S.D)	104
4.9	Eosinophil counts $\times 10^9$ /L of the open wound (Mean \pm S.D)	106

4.10	Eosinophil counts x 10 ⁹ /L of the full-thickness skin mesh graft (Mean ± S.D)	107
4.11	Total serum protein values g/L of the open wound (Mean ± S.D)	109
4.12	Total serum protein values g/L of the full-thickness skin mesh graft (Mean ± S.D)	109
4.13	Serum albumin values g/L of the open wound (Mean ± S.D)	111
4.14	Serum albumin values g/L of the full-thickness skin mesh graft (Mean ± S.D)	112
5.1	Biochemical analysis of DNA (mg/gram), Protein (mg/50mg) and Collagen (µg/mg) content of the open wound (Mean ± S.D)	131
5.2	Biochemical analysis of DNA (mg/gram), Protein (mg/50mg) and Collagen (µg/mg) content of the grafted skin tissue from full-thickness skin mesh graft (Mean± S.D)	132
5.3	Fibroblast numbers in five randomly selected fields of the open wound (Mean ± S.D)	139
5.4	Histopathological assessment of the graft tissue from the full-thickness skin mesh graft (H&E stain 10x)	145
5.5	Epidermis thickness (µm) of the grafted skin from the full-thickness skin mesh graft (Mean ± S.D)	150
6.1	Details of the clinical wound cases treated	171 & 172
6.2	Clinical observation of the cases after application of the keratin-gelatin film	177
6.3	Results of wound planimetry	185 & 186

LIST OF FIGURES

Figure		Page
1.1	Basic fibroblast growth factor-gelatin composite film (A). Keratin-gelatin composite film (B)	46
2.1	Preparation of the recipient site: Sterile millimetre ruler is used to measure 5cm x 5cm (A). Sterile Methylene blue is used to mark the site (B). Full-thickness skin defect created and removing subcutaneous fat (C). Subcutaneous injection of normal saline in the recipient bed (D)	50
3.1	Preparation of the donor graft: Donor graft is placed dermal side up on a steel board (A). Cobblestone appearance of the dermis after subcutaneous tissue removal (B). Preparation of the mesh graft (C)	52
3.2	Application of feeder layer to the recipient bed and closer of the donor site: Application of feeder layer to the recipient bed (A). Graft sutured to the recipient bed with tacking sutures placed through the mesh incisions (B). Donor site closed with simple continues suture (C)	53
3.3	Drawing of an open wound margin traced on graph. M_1 =original wound outer margin. The area within the margin M_1 is the total wound area (A). M_2 =margin between wound and open wound. The area within the margin of M_2 is the open wound area. Area of wound epithelium $C = A-B$	56
3.4	Open wound on Day 0 post-operation treated with composite film: Control-Group I (A). Keratin-gelatin composite film-Group II (B). bFGF-gelatin composite film-Group III (C)	61
3.5	Open wound on Day 4 post-operation. Control-Group I (A). Keratin-gelatin composite film-Group II (B).bFGF-gelatin composite film-Group III (C)	62
3.6	Granulating open wound on Day 8 post-operation:Control-Group I (A). Keratin-gelatin composite film-Group II (B). bFGF-gelatin composite film-Group III (C).	63
3.7	Open wound on Day 12 post-operation showing healthy granulation bed. Control-Group I (A). Keratin-gelatin composite film-Group II, with shiny red colour of the wound (B). bFGF-gelatin composite film-Group III (C)	64

3.8	Open wound on Day 16 post-operation with epithelialization. Control-Group I (A). Keratin-gelatin composite film-Group II (B). bFGF-gelatin composite film-Group III (C)	65
3.9	Open wound on Day 20 post-operation showing the final stage of wound healing. Control-Group I (A). Keratin-gelatin composite film-Group II (B). bFGF-gelatin composite film-Group III (C)	66
3.10	Percentage of epithelialization of the open wound. Significantly high in Group II animals on Days 4-20 post-operation	68
3.11	Percentage of the open wound contraction. Significantly high in Group II animals on Day 16 and 20 post-operation	69
3.12	Percentage of total wound healing of the open wound is high in Group II animals on Days 4-20 post-operation	71
3.13	Full-thickness skin mesh graft on Day 0 post-operation. Control-Group I (A). Keratin-gelatin composite film-Group II (B). bFGF-gelatin composite film-Group III (C)	75
3.14	Full-thickness skin mesh graft on Day 4 post-operation. Control-Group I (A). Keratin-gelatin composite film-Group II, with bluish red color of the graft (B). bFGF-gelatin composite film-Group III (C)	76
3.15	Full-thickness skin mesh graft on Day 8 post-operation showing epithelialization of mesh openings: Control-Group I (A). Keratin-gelatin composite film-Group II with more reddish nature of the graft (B). bFGF-gelatin composite film-Group III (C)	77
3.16	Full-thickness skin mesh graft on Day 12 post-operation. Control-Group I with epithelialization of mesh openings (A). Keratin-gelatin composite film-Group II showing contraction of mesh openings with hair growth (B). bFGF-gelatin composite film-Group III showing dark discoloration of epidermis (C)	78
3.17	Full-thickness skin mesh graft on Day 16 post-operation. Control-Group I, contraction of mesh openings (A). Keratin-gelatin composite film-Group II, showing contraction of mesh openings with hair growth (B). bFGF-gelatin composite film-Group III, showing dark discoloration of epidermis (C)	79

3.18	Full-thickness skin mesh graft on Day 20 post-operation. Control-Group I, showing the acceptance of the graft with hair growth (A). Keratin-gelatin composite film-Group II, showing graft with hair and normal skin colour (B). bFGF-gelatin composite film-Group III, showing dark eschar on the graft surface (C)	80
4.1	WBC (blood) counts x 10 ⁹ /L of the open wound. Elevation on Day 8 post-operation in Group I and II animals	97
4.2	WBC (blood) counts x 10 ⁹ /L of the full-thickness skin mesh graft	98
4.3	Segmented neutrophil counts x 10 ⁹ /L of the open wound. Elevation on Day 8 post-operation in all the Groups	100
4.4	Segmented neutrophil counts x 10 ⁹ /L of the full-thickness skin mesh graft	100
4.5	Lymphocyte counts x 10 ⁹ /L of the open wound	102
4.6	Lymphocyte counts x 10 ⁹ /L of the full-thickness skin mesh graft	103
4.7	Monocyte counts x 10 ⁹ /L of the open wound. Significantly high on Day 8 post-operation in Group I animals	105
4.8	Monocyte counts x 10 ⁹ /L of the full-thickness skin mesh graft	105
4.9	Eosinophil counts x 10 ⁹ /L of the open wound	107
4.10	Eosinophil counts x 10 ⁹ /L of the full-thickness skin mesh graft	108
4.11	Total serum protein values g/L of the open wound. Significantly high in Group II and III animals on Day 12 post-operation	110
4.12	Total serum protein values g/L of the full-thickness skin mesh graft	110
4.13	Serum albumin values g/L of the open wound	112
4.14	Serum albumin values g/L of the full-thickness skin mesh graft	113