UNIVERSITI PUTRA MALAYSIA

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

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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

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

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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 ^{0}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/cm^{2}) and casted in polythene trays and dried at 50 ^{0}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 JOTHI.N

March 2007

Pengerusi: **Profesor Sunderarajan Thilagar, PhD**

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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 berkenan 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 dirawati dengan filem komposit bFGF-gelatin. Disamping itu, lapan belas ekor anjing yang lain pula dirawati dengan graf mesy sepenuh tebal kulit yang dibahagikan kepada tiga kumpulan (n=6).

Kumpulan I dan Kumpulan II dirawati 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 histopatalogi 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 epitilium 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 dirawati dengan keratin-gelatin (kumpulan II) sebagai lapisan pemakanan menunjukkan pembentukan awal kapilari darah pada graf di celah-celah epitilium. 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 dirawati dengan filem komposit keratin-gelatin (Kumpulan II) yang membantu proses penyembuhan awal luka. Haiwan daripada Kumpulan graf mesy sepenuh tebal kulit dirawati dengan lapisan pemakanan filem komposit keratin-gelatin menunjukkan ketebalan epidermis yang normal pada hari ke-20 selepas pembedahan. Filem komposit keratin-gelatin juga baleh 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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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 :

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