UNIVERSITI PUTRA MALAYSIA

EFFECTS OF COLLAGEN-CALCIUM ALGINATE COMPOSITE FILM WITH THERAPEUTIC ULTRASOUND MASSAGE ON OPEN WOUND IN RATS AND CATS

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EVALUATION OF COLLAGEN-CALCIUM ALGINATE COMPOSITE FILM WITH THERAPEUTIC ULTRASOUND MASSAGE ON OPEN WOUND IN RATS AND CATS

BY

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

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ABSTRACT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of doctor of philosophy

EVALUATION OF COLLAGEN-CALCIUM ALGINATE COMPOSITE FILM WITH THERAPEUTIC ULTRASOUND MASSAGE ON OPEN WOUND IN RATS AND CATS

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Chair: Associate Professor Dr. Jalila Abu, PhD

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This study was conducted with the hypothesis that collagen-calcium alginate film and therapeutic ultrasound massage are effective wound healing stimulants causing early re-epithelialization and uncomplicated wound healing. The objective of this study was to identify and evaluate wound healing properties of collagen-calcium alginate film and therapeutic ultrasound massage on open wounds in rats. One hundred and twenty healthy Sprague-Dawley rats were used in the study. Ketamine (5 mg/kg) and Xylazine (50 mg/kg) combination was used. A skin wound (2x2cm) was created lateral to the spine between the base of the scapulae and the iliac crest. The rats were randomly divided into four groups (n = 30) namely Group I (control group), Group II (treated with therapeutic ultrasound massage), Group III (treated with collagen-calcium alginate film) and Group IV (treated with collagen-calcium

alginate film with therapeutic ultrasound massage). Evaluation on the effect of biomaterials on open wound was based on clinical observation, haematological, bacteriological, biochemical and histopathological examinations on day 4, 8, 12, 16 and 20 (post-wounding).

The collagen-calcium alginate films with therapeutic ultrasound massage were well accepted and tolerated by animals and did not cause any adverse reactions. Animals in Group IV showed bright red granulation tissue, without malodour and exudates on day 20 post-wounding. This group also had a higher mean percentage of wound epithelialization, wound contraction, and total wound healing was significantly higher ($P<0.05$) compared to the other group. Staphylococcus aureus, Klebsiella spp., Proteus spp. and Pseudomonas spp. were isolated from all animals. Wound of surgical or traumatic origin is invariably colonized by bacteria. Among the common micro-organisms associated with wound infection are Staphylococcus aureus and beta-haemolytic Streptococcus which are considered as transient flora of the skin. Day 8 post-wounding, typical histological appearance of mature fibroblasts with relatively loose type of supporting tissue was noticed. The fibroblast nuclei were elongated in the direction of the collagen fibers with neovacularization in animals in group IV. The predominant nucleoli reflect active protein synthesis in wound healing.

Clinically Collagen-calcium alginate film with therapeutic ultrasound massage was effective when used in rats, it is used in clinical cases involving 8 cats presented at Progressive Animal Welfare society (PAWS).. All the cats. No animals showed any

intolerance and disturb did not the bandage throughout the treatment. Formation of healthy granulation tissue was observed in all the cases without any side effects.

As a conclusion, collagen-calcium alginate film with therapeutic ultrasound massage was an effective wound healing stimulant. This biomaterial combination also was found to be better than Collagen-calcium alginate film or therapeutic ultrasound massage alone. The findings have a commercial application because it is inexpensive alternative to stimulate wound healing in animals where the cost of treatment is a major concern.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Doktor Falsophy

PENILAIAN KOLAGEN KALSIUM ALGINAT FILEM KOMPOSIT DENGAN URUTAN ULTRASOUND TERAPEUTIK PADA PENYEMBUHAN LUKA TERBUKA PADA TIKUS DAN KUCING

Oleh

KHALED. M. A. HUSSIN

July 2014

Pengerusi: Profesor Madya Dr. Jalila Abu, PhD

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Kajian ini dijalankan dengan hipotesis yang filem kolagen-kalsium alginat dan urutan terapeutik ultrabunyi adalah perangsang penyembuhan yang berkesan disebabkan pertumbuhan kulit seperti pada asalnya dan pemulihan luka yang efektif. Objektif kajian ini adalah untuk mengenal pasti dan menilai keberkesanan filem kolagen-kalsium alginat dan urutan terapeutik ultrabunyi dalam merawat luka pada tikus.

Sebanyak 120 tikus jenis 'Sprague-Dawley' telah digunakan dalam kajian ini. Campuran Ketamine (5 mg/kg) dan Xylazine (50 mg/kg) telah digunakan. Satu kawasan luka kulit (2x2 cm) telah buat disisi tulang belakang antara pangkal 'scaplae' dan 'iliac crest'. Tikus dibahagi secara rawak kepada empat kumpulan. Kumpulan I (kumpulan yang dikawal), Kumpulan II (dirawat menggunakan urutan terapeutik ultrabunyi), Kumpulan III (dirawat menggunakan filem kolagen – kalsium alginat) dan Kumpulan IV (dirawat menggunakan urutan terapeutik ultrabunyi serta

menggunakan filem kolagen – kalsium alginate). Ujian keberkesaan biobahan pada luka telah dijalankan secara permerhatian klinikal, hematologi, bakteriologi, biokimia dan histopathologi pada hari 4, 8. 12, 16 dan 20 (selepas luka).

Kolagen-kalsium alginat dengan urutan terapeutik ultrabunyi diterima dengan baik dan sesuai dan tidak menyebabkan reaksi pada kulit. Kumpulan IV mempamerkan kemerahan tisu granulat tanpa bau busuk dan eksudat pada hari 20 selepas luka. Kumpulan ini juga menunjukan peratus tertinggi dalam epiteliasasi luka, pengecutan luka dan pemulihan keseluruhan yang signifikan ($P< 0.05$) berbanding kumpulan yang lain.

S. aureus, Klebsiella spp, Proteus spp. dan Pseudomonas spp. telah dijumpai dalam haiwan di setiap kumpulan. Kadar kecekapan formasi ‘fibroblast’ dan ‘angiogenesis’ di dapati berkesan dalam Kumpulan IV. Ia meransang sembuh awal (hari 8). Ujian klinikal mendapati filem Kolagen-kalsium dengan urutan terapeutik ultrabunyi didapati amat berkesan dalam lapan ekor kuching. Semua kes-kes. Haiwan menunjukkan sebarang sikap tidak bertoleransi dan tidak mengganggu balutan sepanjang tempoh rawatan. Pembentukan tisu granulation sihat diperhatikan dalam semua kes tanpa sebarang kesan sampingan.

Sebagai rumusan, Kolagen-kalsium dengan urutan terapeutik ultrabunyi didapati adalah perangsang penyembuh luka yang berkesan. Campuran biobahan ini juga dapati lebih berkesan berbanding dengan filem Kolagen-kalsium alginat dan urutan terapeutik ultrabunyi yang di jalankan secara berasingan. Hasil kajian ini, mempunyai aplikasi komersial kerana ia adalah alternatif yang murah bagi

merangsang pemulihan luka haiwan di mana harga menjadi aspek penting pada pelanggan.

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APPROVAL SHEETS

I certify that a Thesis Examination Committee has met on **08 July 2014** to conduct the final examination of KHALED. M. A. HUSSIN on his thesis entitled **" EVALUATION OF COLLAGEN-CALCIUM ALGINATE COMPOSITE FILM WITH THERAPEUTIC ULTRASOUND MASSAGE ON OPEN WOUND IN RATS AND CATS"** in accordance with Unversiti Pertanian Malaysia Colleges Act 1971 and the Constitution of the Universiti Pertanian 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:

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;

Guide to Thesis Preparation

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LIST OF ABBREVIATIONS

-	Negative
%	Percentage
+	Positive
AD	Anno Domini
ANOVA	Analysis of variance
BC	Before Christ
BCA	Bicinchonic acid
BSA	Bovine Serum Albumin
°C	Degree Celsius
DPX	A mixture of Distyrene, Plasticier & Xylene
E. coli	*Escherichia coli*
E.C.M	Extracellular matrix
EDTA	Ethylene Diamine Tetra-Acetate
G	G-force
H&E	Haematoxylin & Eosin Haemoglobin
Hb	Haemoglobin
IM	Intramuscular
kDa	Kilodalton
Kg	Kilogram
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
µg	Microgram
MHz	Megahertz

µm	Micrometer
µl	Microlitre
MT	Masson Trichrome
OD	Optical Density
PAWS	Progressive Animal Welfare Society
PCV	Packed Cell Volume
pH	Reciprocal of the hydrogen ion concentration
RBC	Red Blood Cell
SD	Standard Deviation
S. aureus	*Staphylococcus aureus*
TUM	Therapeutic Ultrasound Massage
US 5X	Received Ultrasound Massage applications soon after wounding, and 4, 8, 12 and 16 days later
WBC	White Blood Cell

CHAPTER 1

INTRODUCTION

The largest organ of the body is skin since nearly 15% of the overall body weight is of body's skin. It refers to the complex organ which covers the entire body surface and exists in continuity with the lining of the body openings i.e. mucous membranes (Cribier and Grosshans, 2002; McKee, 1999). Skin has several important roles in the body as it is involved in sensory functions, thermoregulation, and synthesis of vitamin D and storage of nutrients like proteins, carbohydrates, fats and water. It also demonstrates a protective role by acting as a barrier to radiations, desiccation and chemicals (Anderson, 1997; Thilagar *et al.*, 2009). Skin is considered to be the most affected part in case of traumatic injuriyes (Prathiba and Gupta, 2000). Any disruption to standard continuity of the structure of body is termed as a wound. It can be caused by thermal, mechanical or physical agents. In small animal practice, traumatic wounds are commonly encountered and delayed healing of these wounds is not only costly but also disturbing for the affected individuals (Anderson, 1996; Dart *et al.*, 2002b).

Wound healing serves to be a highly complicated but sophisticated organized biological phenomenon. It involves a series of biochemical, molecular, physiological and cellular interactions which are essentially required by the restoration of the anatomical integrity of the injured part. In this way it stops pathogenic microbes to invade the tissues (Raghow, 1994; Soni, 2013) and helps in restoring the typical structural continuity and efficiency (Prathiba and Gupta, 2000).

The chief targets of wound management include functional restoration, pain relief, cosmetic repair, employment of efficient techniques and sensible timely decisions if there is any delay in wound healing (Anderson, 1996; Cockbill, 2002). For managing wounds of humans as well as animals, products like growth factors with cellular activity are under development which would be utilized for strengthening single or multiple processes of the wound healing pathway (Cockbill, 2002). Considering the cost-effectiveness, recipient's satisfaction and convenient use of these products, they would prove to be efficient for novel dressings as wells.

The process of wound healing can be divided into three phases.

I: Inflammatory and debridement phase which lasts for 2-5 days after injury. During this phase, monocytes, fibroblasts and neutrophils migrate to the damaged tissue.

II: Proliferative or repair phase which lasts for 2-3 ensuing weeks. During this phase, wound macrophages get activated leading to synthesis of extracellular matrix and collagen, proliferation of fibroblasts and endogenous growth factor production.

III: Maturation phase which duration may take 2 weeks to one year after injury. It involves wound remodeling leading to production and cross-linking of active collagen (DeRossi *et al.*, 2009; Hosgood, 1993). The stages of wound healing are shown in Figure 1.1.

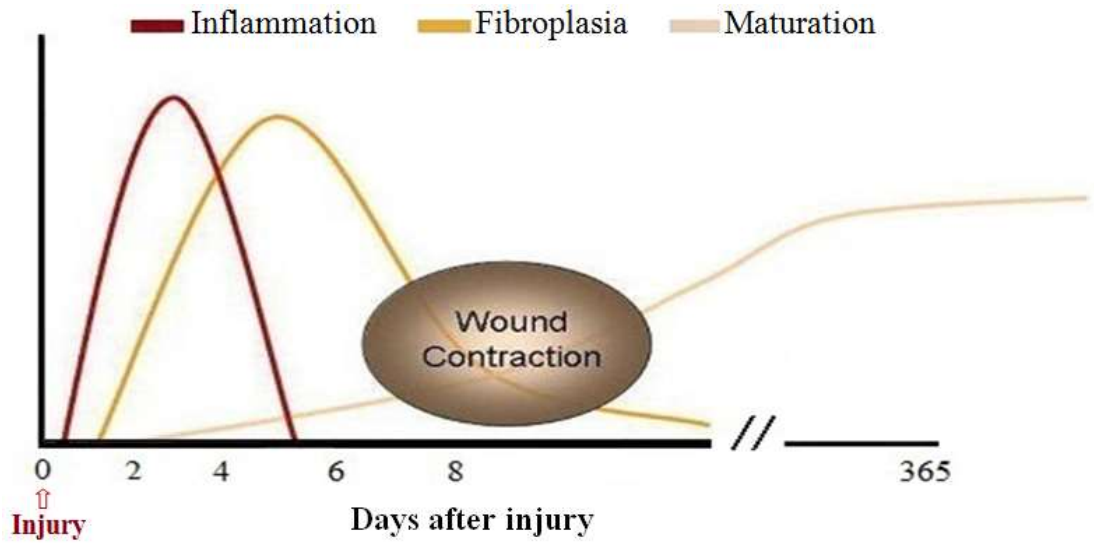

Figure 1.1. Stages of wound healing
(Georgie, 2007)

The usual sequence of the wound healing process is disturbed in case of chronic wounds because of imperfect remodeling of extracellular matrix, continuity of inflammation for long time and inability to re-epithelialise (James and Bayat, 2003). A vital element of wound management is prompt coverage of wound (Singer and Clark, 1999). In addition to this, it is important to change the bandage regularly, especially in wound management in animals. A challenging task in this connection is to keep the bandage safe from contaminants such as urine (Anderson, 2003). Topical antiseptics are toxic to fibroblast and epithelial cells. Topical antibiotics in infected wounds are likely to be washed off by exudates and therefore it is more effective to give systemic. Cleaning solutions or cream made of malic, benzoic and saliscylic acids in propylene glycol have a very low pH of 2.4 and can be an irritant for the surrounding normal (Anderson, 2003). Dry gauze dressing used traditionally has been almost replaced by wound dressings such as hydropolymers, aligates, foams, gels and hydrocolloids. Speedy re-granulation of wounds is important to avoid

additional morbidity or mortality. Certain wounds don't respond to the typical wound healing methods and remain static (Ballard and Baxter, 2000).

Development of engineered skin and tissue has attracted significant level of interest and investment in wound management. In case of wounds of large size, a manufactured skin tissue can be used for wound coverage. However, asingle graft can be as costly as £ 250- £1000 (Ballard and Baxter, 2000). Similarly, the cost of keratinocyte layers lie in the range of $1,000 to $13,000 (Ballard and Baxter, 2000). Besides being very costly, these engineered tissues need extraordinary conditions for storage and careful handling (Anderson, 2003).

Biological materials like natural or synthetic polymers or their composites are also employed for covering wounds temporarily if there is extensive skin damage.(Vedakumari *et al.*, 2013) Biomaterials which are employed for replacing a fraction of an organism or for developing a compatible contact with living tissue can either be natural or synthetic. Examples of natural biomaterials include those derived from omental pedicles (Brockman *et al.*, 1996; Smith *et al.*, 1995; Wang *et al.*, 2012), submucosa, small intestine and porcine (Holt and Mann, 2002). Whereas, examples of synthetic biomaterials include carbon-graphite fibres, bioglass and ceramics (Roush, 2003). Natural biomaterials like calcium alginate and collagen are vital tools in tissue regeneration which is utilized as stimulators of wound healing (Drury and Mooney, 2003).

Collagen is a class of proteins naturally present in animals, particularly in the connective tissues and flesh of vertebrates (Müller, 2003). Collagen is the chief

constituent of connective tissues and since it accounts for 25-35% of the total protein content of the mammal's body, it is considered to be the most abundant protein in them (Di Lullo *et al.*, 2002). The most common collagen producing cell is the fibroblast and is present in abundance in bone, cartilage, intervertebral disc, gut, cornea and blood vessels; however, it exists mainly in fibrous tissues like skin, ligament and tendon.

Collagen is the chief constituent of endomysium in muscles. It constitutes 6% of the weight of tendinous muscles of high strength. The collagen content of muscle tissues ranges from 1-2% (Sikorski, 2001). Hydrolyzed collagen and collagen gels are utilized in cosmetic applications and skin therapies as absorbents (Sharma, 2011). A good source of alginate is seaweed which is used to manufacture calcium alginate dressings (Knill *et al.*, 2004). In case of heavy exudates, these can be used instead of hydrocolloid dressings as calcium alginate dressings are highly absorbent (Jude *et al.*, 2007). Their absorptive capacity makes them ideal tool for not only surface dressing but also for filling cavities after cleaning or draining. The mechanism of action is the same as for hydrocolloid dressings i.e. they develop a natural gel comprising of exudates against the healing wound maintaining flexibility and humidity thus facilitating the tissue growth and healing process. Besides this, the gel also behaves as a barrier to microbial invasion which can lead to secondary infections and obscure wound healing (MSEC, 2013).

The procedure of changing dressingsa is the same as the one which is usually used involving rinsing and washing of wound (MSEC, 2013).

Considering the convenience in removal of calcium alginate dressings, they can lso be used in burn cases. However, they are also employed in ulcer patients, especially for diabetic skin ulcers whose management has proved to be quite challenging with other dressings. While cleaning the wound, the skin is rinsed with saline or simply sloughs off with gel. In contrast to other dressings, it is important to keep the calcium alginate dressings away from normal tissue and apply to damaged tissue only for efficient results. Medical tape and gauze can be used to keep them in a suitable position (MSEC, 2013).

It has been established that therapeutic ultrasound (TUS) application speeds up tissue repair, relieves pain, causes muscle relaxation, enhances local blood flow, modifies scar development and decreases edema (Coakley, 1978; Da Costa Gonçalves *et al.*, 2007; Dyson, 1990; Dyson *et al.*, 1968; Maxwell, 1992; Monte-Raso *et al.*, 2005; Ter Haar, 1978; Young and Dyson, 1990).

Since ultrasound irradiation has been proven to increase skin regeneration (Dyson *et al.*, 1968; Levine and Watson, 2013; Young and Dyson, 1990), it is recommended for treatment of pressure ulcers (Da Costa Gonçalves *et al.*, 2007; Dyson and Suckling, 1978; Roche and West, 1984) and varicose ulcers (Da Costa Gonçalves *et al.*, 2007; Riet *et al.*, 1996). Utilization of ultrasound irradiation for improving the quality and speed of integration of skin grafts has been studied (Da Costa Gonçalves *et al.*, 2007; Ivanov, 1987); however, these activities require further investigation.

Topical products contain wound healing stimulators which cause activation of wound healing cells like macrophages for generating cytokines for early epithelialisation during the course of healing of wound (Swaim, 2003). Wound therapies are the main

focus of researchers exploring advanced wound management (Wendelken *et al.*, 2003). In this regard, utilization of biomaterials as a permanent substitute for skin is still a challenging task (Lin *et al.*, 2000).

The objectives of this research are as follows.

1. to evaluate the clinical effects of collagen-calcium alginate film and therapeutic ultrasound massage for open wounds in experimental animals.
2. to determine the presence of infection and changes in haematological values of animal with open wound treated with collagen-calcium alginate film and therapeutic ultrasound massage in experimental animals.
3. to evaluate the histopathological changes in open wound with collagen-calcium alginate film and therapeutic ultrasound massage in experimental animals.
4. to evaluate the effectiveness of the biomaterials in clinical cases presented at Progressive Animal Welfare society (PAWS).

REFERENCES

Animal Care and Use Program. 2004. Rat husbandry-open cage. http://geiselmed. dartmouth.edu/arc/arc/e18.php . Retrieved 11 June 2013.

Aetna. 2013. Clinical policy bulletin: Wound care. http://www.aetna.com/cpb/ medical/data/200299/0244.html. Retrieved 12 June 2013.

Aksay, I. A., and Weiner, S. 1998. Biomaterial is this real a field of research? Editorial overview. *Current Opinion in Solid State & Materials Science*, 3: 219-220.

Aljady, A., Kamaruddin, M., Jamal, A., and Mohd Yassim, M. 2000. Biochemical study on the efficacy of malaysian honey on inflicted wounds: An animal model. *Medical Journal of Islamic Academy of Sciences*, 13(3): 125-132.

Altomare, M., Nascimento, A. P., Romana-Souza, B., Amadeu, T. P., and Monte-Alto-Costa, A. 2009. Ultrasound accelerates healing of normal wounds but not of ischemic ones. *Wound Repair and Regeneration*, 17(6): 825-831.

Andersen, t., Strand, B., Formo, K., Alsberg, E., and Christensena, B. E. 2012. Alginates as biomaterials in tissue engineering. *Journal of Carbohydrate Chemistry*, 37: 227-258.

Anderson, D., Stanley, M., and White, R. 2003. Canine keratinocyte culture and use of a cultured epidermal autograft in a dog. *Veterinary and Comparative Orthopaedics and Traumatology*, 16(4): 255-259.

Anderson, D. M. 1996. Wound management in small animal practice. *In Practice*, 18(3): 115-128.

Anderson, D. M. 1997. Practical approach to reconstruction of wounds in small animal practice part 1. *In Practice*, 19(9): 463-471.

Anderson, D. M. 2003. Wound dressings unravelled. *In Practice*, 25(2): 70-83.

Arora, P. D., Narani, N., and McCulloch, C. A. 1999. The compliance of collagen gels regulates transforming growth factor-β induction of α-smooth muscle actin in fibroblasts. *The American Journal of Pathology*, 154(3): 871-882.

Arul, V., Gopinath, D., Gomathi, K., and Jayakumar, R. 2005. Biotinylated ghk peptide incorporated collagenous matrix: A novel biomaterial for dermal wound healing in rats. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 73(2): 383-391.

Arul, V., Kartha, R., and Jayakumar, R. 2007. A therapeutic approach for diabetic wound healing using biotinylated ghk incorporated collagen matrices. *Life Sciences*, 80(4): 275-284.

Babu, P. R., Sastry, T., Rose, C., and Rao, N. M. 1997. Hydrogels based on gelatin poly (hydroxyethyl methacrylate) and poly (butyl acrylate) graft copolymer impregnated with fibrin. *Journal of Applied Polymer Science*, 65(3): 555-560.

Baetge, E. 2001. You are in health. Scientists can now grow skin from hair follicles-cutting out the need for painful skin grafts. *BBC News on Line June*: 21,2001.

Baharestani, M. M. 2008. Quality of life and ethical issues. *Wound Care Essentials: Practice Principles. Springhouse, PA: Springhouse Pub*: 4-12.

Ballard, K., and Baxter, H. 2000. Developments in wound care for difficult to manage wounds. *British Journal of Nursing*, 9(7): 405-412.

Barnea, Y., Amir, A., Leshem, D., Zaretski, A., Weiss, J., Shafir, R., and Gur, E. 2004. Clinical comparative study of aquacel and paraffin gauze dressing for split-skin donor site treatment. *Annals of Plastic surgery*, 53(2): 132-136.

Baum, C. L., and Arpey, C. J. 2005. Normal cutaneous wound healing: Clinical correlation with cellular and molecular events. *Dermatologic Surgery*, 31(6): 674-686.

Behrens, B. J., and Michlovitz, S. L. 2006. Physical agents: Theory and practice (2nd ed.), FA Davis Company Filadelfia.

Belloa, O. S., Emikpeb, B. O., Olaifac, A. K., and Olaifaa, F. E. 2013. Investigation into the healing properties of walnut (tetracarpidium conophorum) leaf and onion (allium cepa) bulb residues in clarias gariepinus. *Archivos de Medicina Veterinaria* 45(3): 291-297.

Berry, D. P., Harding, K. G., Stanton, M. R., Jasani, B., and Ehrlich, H. P. 1998. Human wound contraction: Collagen organization, fibroblasts, and myofibroblasts. *Plastic and Reconstructive Surgery*, 102(1): 124-131.

Bichsel, A., James, C. W., and Gurk-Turner, C. 2000. Fluoroquinolone drug class update. *Baylor University Medical Center Proceedings*, 13(3): 289-292.

Bohling, M. W., Henderson, R. A., Swaim, S. F., Kincaid, S. A., and Wright, J. C. 2004. Cutaneous wound healing in the cat: A macroscopic description and comparison with cutaneous wound healing in the dog. *Veterinary Surgery*, 33(6): 579-587.

Bohling, M. W., Henderson, R. A., Swaim, S. F., Kincaid, S. A., and Wright, J. C. 2006. Comparison of the role of the subcutaneous tissues in cutaneous wound healing in the dog and cat. *Veterinary Surgery*, 35(1): 3-14.

Bowler, P., Duerden, B., and Armstrong, D. 2001. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2): 244-269.

Bowler, P., Jones, S., Walker, M., and Parsons, D. 2004. Microbicidal properties of a silver-containing hydrofiber® dressing against a variety of burn wound pathogens. *Journal of Burn Care & Research*, 25(2): 192-196.

Boyce, S. T., and Warden, G. D. 2002. Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. *American Journal of Surgery*, 183(4): 445-456.

Brett, D. 2008. A review of collagen and collagen-based dressings. *Wounds*, 20: 347-353.

Brockman, D. J., Pardo, A. D., Conzemius, M. G., Cabell, L. M., and Trout, N. J. 1996. Omentum-enhanced reconstruction of chronic nonhealing wounds in cats: Techniques and clinical use. *Veterinary Surgery*, 25(2): 99-104.

Brown, G. L., Nanney, L. B., Griffen, J., Cramer, A. B., Yancey, J. M., Curtsinger 3rd, L., Holtzin, L., Schultz, G. S., Jurkiewicz, M. J., and Lynch, J. B. 1989. Enhancement of wound healing by topical treatment with epidermal growth factor. *The New England Journal of Medicine*, 321(2): 76-79.

Burton, J. L., Etherington, D. J., and Peachey, R. D. 1978. Collagen sponge for leg ulcers. *British Journal of Dermatology*, 99(6): 681-685.

Byl, N., McKenzie, A., Wong, T., West, J., and Hunt, T. 1993. Incisional wound healing: A controlled study of low and high dose ultrasound. *The Journal of Orthopaedic and Sports Physical Therapy*, 18(5): 619.

Campoccia, D., Doherty, P., Radice, M., Burn, P., Abatangelo, G., and Williams, D. F. 1998. Semisynthetic resrbsorbable material form hyaluronan esterification. *Biomaterials*, 19: 2101-2127.

Caruso, D. M., Foster, K. N., Blome-Eberwein, S. A., Twomey, J. A., Herndon, D. N., Luterman, A., Silverstein, P., Antimarino, J. R., and Bauer, G. J. 2006. Randomized clinical study of hydrofiber dressing with silver or silver sulfadiazine in the management of partial-thickness burns. *Journal of burn care & research*, 27(3): 298-309.

Cetinkale, O., Demir, M., Sayman, H., Ayan, F., and Onsel, C. 1997. Effects of allopurinol, ibuprofen and cyclosporin a on local microcirculatory disturbances due to burn injuries. *Burns*, 23(1): 43-49.

Chen, M. M., Lam, A., Abraham, J. A., Schreiner, G. F., and Joly, A. H. 2000. Ctgf expression is induced by tgf- β in cardiac fibroblasts and cardiac myocytes: A potential role in heart fibrosis. *Journal of Molecular and Cellular Cardiology*, 32(10): 1805-1819.

Chin, G. A., Diegelmann, R. F., and Schultz, G. S. 2005. Cellular and molecular regulation of wound healing. *Wound Healing*, 33: 17-39.

Chithra, P., Sajithlal, G. B., and Gowri, C. 1998. Influence of aloe vera on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *Journal of ethnopharmacology*, 59(3): 179-186.

Clark, R. 1996. Wound repair: Overview and general considerations. *The molecular and cellular biology of wound repair,* 2nd ed, Plenum Press. p. 3-50.

Clark, R. A. F. 1985. Cutaneous tissue repair: Basic biologic considerations. I. *Journal of the American Academy of Dermatology*, 13(5): 701-725.

Clewlow, J. 2003. A review of the history of veterinary wound management http:// www.worldwidewounds.com/2003/july/Clewlow/Vet-History-Review.html. Retrieved 12 Joun 2013.

Coakley, W. T. 1978. Biophysical effects of ultrasound at therapeutic intensities. *Physiotherapy*, 64(6): 166-169.

Cochrane, C. A., Pain, R., and Knottenbelt, D. C. 2003. In-vitro wound contraction in the horse: Differences between body and limb wounds. *Wounds*, 15(6): 175-181.

Cockbill, S. 2002. The healing process. *Hospital Pharmacist-London*, 9(9): 255-260.

Coles, E. H. 1967. Leukocytes. *Veterinary Clinical Pathology*: pp 31-63. W.B. Saunders Company, Philadelphia, London.

Corr, D. T., Gallant-Behm, C. L., Shrive, N. G., and Hart, D. A. 2009. Biomechanical behavior of scar tissue and uninjured skin in a porcine model. *Wound Repair and Regeneration*, 17(2): 250-259.

Cribier, B., and Grosshans, E. 2002. Histologie de la peau normale et lésions histopathologiques élémentaires. *Encyclopedia Medical Chiropractor (Dermatologie)* 16(A10): 85-98.

Cutler, N. R., George, R., Seifert, R. D., Brunelle, R., Sramek, J. J., McNeill, K., and Boyd, W. M. 1993. Comparison of quantitative methodologies to define chronic pressure ulcer measurements. *Advances in Skin & Wound Care*, 6(6): 20-31.

Cutting, K. F. 2001. A dedicated follower of fashion? Topical medications and wounds. *British Journal of Nursing-London-Mark Allen Publishing Limited*, 10(2): 9-16.

Cuzzell, J. Z. 1988. The new ryb colour code. *American Journal of Nursing*, 31: 314-319.

Da Costa Gonçalves, A., Barbieri, C. H., Mazzer, N., Garcia, S. B., and Thomazini, J. A. 2007. Can therapeutic ultrasound influence the integration of skin grafts? *Ultrasound in Medicine & Biology*, 33(9): 1406-1412.

Dart, A. J., Cries, L., Jeffcott, L. B., Hodgson, D. R., and Rose, R. J. 2002a. The effect of equine recombinant growth hormone on second intention wound healing in horses. *Veterinary Surgery*, 31(4): 314-319.

Dart, A. J., Cries, L., Jeffcott, L. B., Hodgson, D. R., and Rose, R. J. 2002b. Effects of 25% propylene glycol hydrogel (solugel) on second intention wound healing in horses. *Veterinary Surgery*, 31(4): 309-313.

Davidson, J. 1998. Animal models for wound repair. *Archives of Dermatological Research*, 290(1): S1-S11.

Debus, E. S., Schmidt, K., Ziegler, U. E., and Thiede, A. 2000. The role of growth factors in wound healing. *Zentralblatt für Chirurgie*, 125(1): 49-55.

DeLapp, N. W., and Dieckman, D. K. 1990. Effect of basic fibroblast growth factor (bfgf) and insulin-like growth factors type i (igf-i) and type ii (igf-ii) on adult human keratinocyte growth and fibronectin secretion. *Journal of Investigative Dermatology*, 94(6): 777-780.

Demling, R. H., and Desanti, L. 2001. Effects of silver on wound management. *Wounds*, 13(1): 4-15.

Demling, R. H., Niezgoda, J. A., Haraway, G. D., and Mostow, E. 2004. Small intestinal submucosa wound matrix and full-thickness venous ulcers: Preliminary results. *Wounds*, 16(1): 18-22.

DeRossi, R., Coelho, A. C. A. d. O., Mello, G. S. d., Frazílio, F. O., Leal, C. R. B., Facco, G. G., and Brum, K. B. 2009. Effects of platelet-rich plasma gel on skin healing in surgical wound in horses. *Acta cirúrgica brasileira*, 24(4): 276-281.

Desmoulière, A., Redard, M., Darby, I., and Gabbiani, G. 1995. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *The American Journal of Pathology*, 146(1): 56-66.

Di Lullo, G. A., Sweeney, S. M., Körkkö, J., Ala-Kokko, L., and San Antonio, J. D. 2002. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type i collagen. *Journal of Biological Chemistry*, 277(6): 4223-4231.

Diegelmann, R. F., and Evans, M. C. 2004. Wound healing: An overview of acute, fibrotic and delayed healing. *Frontiers in Bioscience*, 9(1): 283-289.

Doillon, C., Silver, F., Olson, R., Kamath, C., and Berg, R. 1988. Fibroblast and epidermal cell-type i collagen interactions: Cell culture and human studies. *Scanning Microscopy*, 2(2): 985-992.

Doillon, C. J., DeBlois, C., Côté, M.-F., and Fournier, N. 1994. Bioactive collagen sponge as connective tissue substitute. *Materials Science and Engineering: C*, 2(1): 43-49.

Doillon, C. J., and Silver, F. H. 1986. Collagen-based wound dressing: Effects of hyaluronic acid and firponectin on wound healing. *Biomaterials*, 7(1): 3-8.

Doillon, C. J., Whyne, C. F., Berg, R. A., Olson, R. M., and Silver, F. H. 1984. Fibroblast-collagen sponge interactions and the spatial deposition of newly synthesized collagen fibers in vitro and in vivo. *Scanning Electron Microscopy*(Pt 3): 1313-1320.

Doughty, D. B., and Holbrook, R. 2007. Lower-extremity ulcers of vascular etiology. *Acute and Chronic Wounds: Current Management Concepts. 3rd ed. St. Louis, MO: Mosby*: 258-306.

Driver, V. R., Landowski, M. A., and Madsen, J. L. 2007. Neuropathic wounds: The diabetic wound. *Acute and Chronic Wounds:Current Management Concepts .3rd ed. St. Louis, MO: Mosby/Elsevier.*: 307-336.

Drury, J. L., and Mooney, D. J. 2003. Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials*, 24(24): 4337-4351.

Dunn, J. 2000. Disorders of leucocyte number. *Bsava manual of canine and feline haematology and transfusin medicine*, J. D. M, A.Mackin.J.D, ed.: pp 93-104. UK. Lookers.

Dyke, M. E. V., Cheryl, B. R., Scott, T. F., Arlene, S. J. J., and Robert, S. A. 2001. Soluble keratin peptide. http:www.patentalert.com/docs/000/2000//352.shtml. Retrieved 10 Joun 2013.

Dyson, M. 1990. Role of ultrasound in wound healing. *Kloth LC, Miller KH. Wound healing. Philadelphia: FA Davis*: 259-285.

Dyson, M., Pond, J., Joseph, J., and Warwick, R. 1968. The stimulation of tissue regeneration by means of ultrasound. *Clinical Science*, 35(2): 273-285.

Dyson, M., and Suckling, J. 1978. Stimulation of tissue repair by ultrasound: A survey of the mechanisms involved. *Physiotherapy*, 64(4): 105-108.

Dyson, M., Young, S., Pendle, C. L., Webster, D. F., and Lang, S. M. 1988. Comparison of the effects of moist and dry conditions on dermal repair. *Journal of investigative dermatology*, 91(5): 434-439.

Eaglstein, W. H., and Falanga, V. 1997. Chronic wounds. *The Surgical Clinics of North America*, 77(3): 689-700.

Emsen, I. M. 2007. The effect of ultrasound on flap survival: An experimental study in rats. *Burns*, 33(3): 369-371.

Enoch, S., and Leaper, D. J. 2008. Basic science of wound healing. *Surgery* 26(2): 31-37.

Esmaeelinejad, M., and Bayat, M. 2013. Effect of low-level laser therapy on the release of interleukin-6 and basic fibroblast growth factor from cultured human skin fibroblasts in normal and high glucose mediums. *Journal of Cosmetic and Laser Therapy*, 15(6): 310-317.

Fish, D. N., and North, D. S. 2001. Gatifloxacin, an advanced 8-methoxy fluoroquinolone. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 21(1): 35-59.

Flanagan, M. 1997. A practical framework for wound assessment. 2: Methods. *British journal of Nursing (Mark Allen Publishing)*, 6(1): 6-11.

Fowler, D. 1993. Principles of wound healing. *Surgical complications and wound healing in the small animal practice*, J. Harari, ed.: pp70-71. W.B.Saunders Company.

Gajendrareddy, P. K., Sen, C. K., Horan, M. P., and Marucha, P. T. 2005. Hyperbaric oxygen therapy ameliorates stress-impaired dermal wound healing. *Brain, Behavior, and Immunity*, 19(3): 217-222.

Geer, D. J., Swartz, D. D., and Andreadis, S. T. 2004. In vivo model of wound healing based on transplanted tissue-engineered skin. *Tissue engineering*, 10(7-8): 1006-1017.

Georgie, H. 2007. Stages of wound healing. http://www.dechra.co.uk/Therapy-Areas /Woundcare/Stages-of-Wound-Healing.aspx. Retrieved 16 June 2013.

Ghalbzouri, A. E., Hensbergen, P., Gibbs, S., Kempenaar, J., Schors, R. V. D., and Pones, M. 2004. Fibroblasts facilitate re-epthelialization in wound human skin equivalents. *Laboratory Investigation*, 84: 102-112.

Gibson, W. T., Scott, I. R., Saunders, H. J., Brunskill, J. E., and Harding, C. R. 1984. Markers of epidermal differentiation expressed by rat keratinocytes cultured by a modified feeder layer technique. *European Journal of Cell Biology*, 33(1): 75-83.

Gogia, P. P. 1995. Clinical wound management. http://moon.ouhsc.edu/llepak/ wounds/healing/tissue%20healing.pdf. Retrieved 14 June 2013.

Goldbaum, E. 2001. New skin promotes wound healing. *Journal of the Federation of American Societies of Experimental Biology*, 32(26): 1-7.

Gomez, J. H., Schumacher, J., Lauten, S. D., Sartin, E. A., Hathcock, T. L., and Swaim, S. F. 2004. Effects of 3 biologic dressings on healing of cutaneous wounds on the limbs of horses. *Canadian Journal of Veterinary Research*, 68(1): 49-55.

Gottrup, F., and Leaper, D. 2004. Wound healing: Historical aspect. *European wound management*. *Association Journal*, 4(2): 21-26.

Gottrup, F., Nix, D. P, and Bryant, R. A. 2006. The multidisciplinary team approach. *Acute and Chronic Wounds: Current Management Concepts. St. Louis, MO: Mosby/Elsevier*: 23-38.

Govindarajan, R. A., Pushpangadan, P., Kumara, B., and Vijayakumar, M. 2007. Ethnopharmacological approaches to wound healing—exploring medicinal plants of india. *Journal of Ethnopharmacology*, 114(2): 103-113.

Graham, A. 1998. The use of growth factors in clinical practice. *Journal of Wound Care*, 7(9): 464-466.

Guptaa, B. 2010. Textile-based smart wound dressings. *Indian Journal of Fibre & Textile Research*, 35(6): 174-187.

Hart, J., Silcock, D., Gunnigle, S., Cullen, B., Light, N. D., and Watt, P. W. 2002. The role of oxidised regenerated cellulose/collagen in wound repair: Effects in vitro on fibroblast biology and in vivo in a model of compromised healing. *The International Journal of Biochemistry & Cell biology*, 34(12): 1557-1570.

Heness, G., and Ben-Nissan, B. 2004. Biomaterials- classifications and behaviour of different types of biomaterials.Abstracted from "innovative bioceramics" in materials forum, . The Institute of Materials Engineering Australasia Ltd, 27: 104-114. .

Heredero, S. F. X., Hamann, C., Martin, O. J. M., Arias, R. C., and Menchero, C. S. 1996. Experimental burn models. *Annal of Burns and Fire Disasters*, 9(2): 1-5.

Hoath, S. B., and Leahy, D. 2003. The organization of human epidermis: Functional epidermal units and phi proportionality. *Journal of Investigative Dermatology*, 121(6): 1440-1446.

Hoath, S. B., and Leahy, D. G. 2006. The human stratum corneum as extended, covalently cross-linked biopolymer: Mathematics, molecules, and medicine. *Medical Hypotheses*, 66(6): 1191-1198.

Holt, T. L., and Mann, F. A. 2002. Carbon dioxide laser resection of a distal carpal pilomatricoma and wound closure using swine intestinal submucosa in a dog. *Journal of the American Animal Hospital Association*, 39(5): 499-505.

Hopkinson, I. 1992a. Growth factors and extracellular matrix biosynthesis. *Journal of Wound Care*, 1: 47-50.

Hopkinson, I. 1992b. Molecular components of the extracellular matrix. *Journal of Wound Care*, 1: 52-54.

Hosgood, G. 1993. Wound healing: The role of platelet-derived growth factor and transforming growth factor beta. *Veterinary Surgery*, 22(6): 490-495.

Hosgood, G. 2003. Wound repair and specific tissue response to injury. *Textbook of small animal surgery, Vol.1 3rd ed*: 66-86. Saunders Philadelphia.

HyeJin, S. 2008. Effect of 3-electrode electrical stimulation on current delivery and healing in chronic wounds, phd thesis, Loma Lind University.

Hynes, R. O. 1992. Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell*, 69(1): 11-25.

Inan, S., Oztukcan, S., Vatansever, S., Turel Ermertcan, A., Zeybek, D., Oksal, A., Giray, G., and Muftuoglu, S. 2006. Histopathological and ultrastructural effects of glycolic acid on rat skin. *Acta histochemica*, 108(1): 37-47.

Ioannides, A. 1999. The hippocratic wound healer. http://www.theedicaldirectory. org/essays/hippocratic.shtml. Retrieved 12 June 2013

Ishiguro, N., Yabe, Y., Shimizu, T., Iwata, H., and Miura, T. 1994. Basic fibroblast growth factor has a beneficial effect on the viability of random skin flaps in rats. *Annals of Plastic Surgery*, 32(4): 356-360.

Ivanov, V. V. 1987. Use of ultrasonics in the fixation of autologous skin transplants. *Vestnik Khirurgii Imeni II Grekova*, 139(8): 110-111.

Jaakkola, P., Kontusaari, S., Kauppi, T., Määttä, A., and Jalkanen, M. 1998. Wound reepithelialization activates a growth factor-responsive enhancer in migrating keratinocytes. *The Federation of American Societies for Experimental Biology Journal*, 12(11): 959-969.

James, A. L., and Bayat, A. 2003. Basic plastic surgery techniques and principles: Chronic wound management. *The International Medical Journal for Students (Student BMJ)*, 11: 406-407.

Jimenez, P. A., and Rampy, M. A. 1999. Keratinocyte growth factor-2 accelerates wound healing in incisional wounds. *The Journal of Surgical Research*, 81(2): 238-242.

Johnston, D. 1990. Wound healing in skin. *The Veterinary clinics of North America. Small animal practice*, 20(1): 1-25.

Johnstone, C. C., Farley, A., and Hendry, C. 2005. The physiological basics of wound healing. *Nursing Standard*, 19(43): 59-65.

Jones, S. A., Bowler, P. G., Walker, M., and Parsons, D. 2004. Controlling wound bioburden with a novel silver-containing hydrofiber® dressing. *Wound Repair and Regeneration*, 12(3): 288-294.

Jørgensen, B., Price, P., Andersen, K. E., Gottrup, F., Bech-Thomsen, N., Scanlon, E., Kirsner, R., Rheinen, H., Roed-Petersen, J., and Romanelli, M. 2005. The silver-releasing foam dressing, contreet foam, promotes faster healing of critically colonised venous leg ulcers: A randomised, controlled trial. *International Wound Journal*, 2(1): 64-73.

Jude, E., Apelqvist, J., Spraul, M., and Martini, J. 2007. Prospective randomized controlled study of hydrofiber® dressing containing ionic silver or calcium alginate dressings in non-ischaemic diabetic foot ulcers. *Diabetic Medicine*, 24(3): 280-288.

Karlsmark, T., Agerslev, R., Bendz, S., Larsen, J., Roed-Petersen, J., and Andersen, K. E. 2003. Clinical performance of a new silver dressing, contreet foam, for chronic exuding venous leg ulcers. *Journal of wound care*, 12(9): 351-354.

Karodi, R., Jadhav, M., Rub, R., and Bafna, A. 2009. Evaluation of the wound healing activity of a crude extract of rubia cordifolia l.(indian madder) in mice. *International journal of Applied research in natural products*, 2(2): 12-18.

Kaufman, T., Levin, M., and Hurwitz, D. J. 1984. The effect of topical hyperalimentation on wound healing rate and granulation tissue formation of

experimental deep second degree burns in guinea-pigs. *Burns*, 10(4): 252-256.

Khadeer, S. B., Suresh, K. R. V., Haragopal, V., Srilatha, C., Sastry, T. P., and Vidyavathi, M. 2011. Effects of fish scales extracted collagen biocasings on cutaneous wound healing in dogs. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(2): 36-49.

Kimura, T., and Doi, K. 2004. Experimental skin graft in hairless descendants of mexican hairless dogs. *Indian Journal of Dermatology*, 49(02): 55-61.

Kirfel, G., and Herzog, V. 2004. Migration of epidermal keratinocytes: Mechanisms, regulation, and biological significance. *Protoplasma*, 223(2-4): 67-78.

Klein, C. E., Dressel, D., Steinmayer, T., Mauch, C., Eckes, B., Krieg, T., Bankert, R. B., and Weber, L. 1991. Integrin alpha 2 beta 1 is upregulated in fibroblasts and highly aggressive melanoma cells in three-dimensional collagen lattices and mediates the reorganization of collagen i fibrils. *The Journal of cell biology*, 115(5): 1427-1436.

Knill, C., Kennedy, J., Mistry, J., Miraftab, M., Smart, G., Groocock, M., and Williams, H. 2004. Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. *Carbohydrate Polymers*, 55(1): 65-76.

Kuroyanagi, Y., Kenmochi, M., Ishihara, S., Takeda, A., Shiraishi, A., Ootake, N., Uchinuma, E., Torikai, K., and Shioya, N. 1993. A cultured skin substitute composed of fibroblasts and keratinocytes with a collagen matrix: Preliminary results of clinical trials. *Annals of plastic Surgery*, 31(4): 340-351.

Lansdown, A. 2002. Silver i: Its antibacterial properties and mechanism of action. *Journal of wound care*, 11(4): 125-130.

Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Percoraro, R. E., Rodeheaver, G., and Robson, M. C. 1994. Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair and Regeneration*, 2(3): 165-170.

Levine, D. A., and Watson, T. B. 2013. Therapeutic ultrasound. 19(3): 328-341.

Lin, F. H., Chen, T. M., Chen, K. S., Wu, T. H., and Chen, C. C. 2000. An animal study of a novel tri-layer wound dressing material-non-woven fabric grafted with n-isopropyl acrylamide and gelatin. *Materials Chemistry and Physics*, 64(3): 189-195.

Lingen, M. W. 2001. Role of leukocytes and endothelial cells in the development of angiogenesis in inflammation and wound healing. *Archives of Pathology & Laboratory Medicine*, 125(1): 67-71.

Lowe, A. S., Walker, M. D., Cowan, R., and Baxter, G. D. 2001. Therapeutic ultrasound and wound closure: Lack of healing effect on x-ray irradiated

wounds in murine skin. *Archives of Physical Medicine and Rehabilitation*, 82(11): 1507-1511.

Lucy O'Hara, L. R. N. 2005. Documenting wound measurements consistency the key. http://www.woundcare.org/newsvol3n2/ar.5htm. Retrieved 17 June 2013.

Luna, L. G. 1968. Manual of histologic staining methods of the armed forces institute of pathology, McGraw-Hill New York.

Madhero, A., and Komorniczak, M. 2012. Layers of the skin. https://en.wikipedia. org/wiki/File:Skin layers.png. Retrieved 25 may 2013.

Magnusson, B., Cross, S., Winckle, G., and Roberts, M. 2006. Percutaneous absorption of steroids: Determination of in vitro permeability and tissue reservoir characteristics in human skin layers. *Skin Pharmacology and Physiology*, 19(6): 336-342.

Martin, P. 1997. Wound healing--aiming for perfect skin regeneration. *Science*, 276(5309): 75-81.

Maruguchi, T., Maruguchi, Y., Suzuki, S., Matsuda, K., Toda, K.-I., and Isshiki, N. 1994. A new skin equivalent: Keratinocytes proliferated and differentiated on collagen sponge containing fibroblasts. *Plastic and Reconstructive Surgery*, 93(3): 537-544.

Maxwell, L. 1992. Therapeutic ultrasound: Its effects on the cellular and molecular mechanisms of inflammation and repair. *Physiotherapy*, 78(6): 421-426.

McGee, G. S., Davidson, J. M., Buckley, A., Sommer, A., Woodward, S. C., Aquino, A. M., Barbour, R., and Demetriou, A. A. 1988. Recombinant basic fibroblast growth factor accelerates wound healing. *Journal of Surgical Research*, 45(1): 145-153.

McKee, P. H. 1999. Pathology of the skin (3nd edition), London: Mosby. pp142-149.

McNees, P. 2006. Skin and wound assessment and care in oncology. *Seminars in Oncology Nursing*, 22(3): 130-143.

Middelkoop, E., Van Den Bogaerdt, A., Lamme, E., Hoekstra, M., Brandsma, K., and Ulrich, M. 2004. Porcine wound models for skin substitution and burn treatment. *Biomaterials*, 25(9): 1559-1567.

Miller, M.-C., and Nanchahal, J. 2005. Advances in the modulation of cutaneous wound healing and scarring. *BioDrugs*, 19(6): 363-381.

Mollineau, W., Adogwa, A., and Garcia, G. 2009. The gross and micro anatomy of the accessory sex glands of the male agouti (dasyprocta leporina). *Anatomia, histologia, embryologia*, 38(3): 204-207.

Monte-Raso, V. V., Barbieri, C. H., Mazzer, N., and Fasan, V. S. 2005. Can therapeutic ultrasound influence the regeneration of peripheral nerves? *Journal of Neuroscience Methods*, 142(2): 185-192.

Moreo, K. 2005. Understanding and overcoming the challenges of effective case management for patients with chronic wounds. *The Case Manager*, 16(2): 62-67.

Morgan, P. W., Binnington, A. G., Miller, C. W., Smith, D. A., Valliant, A., and Prescott, J. F. 1994. The effect of occlusive and semi-occlusive dressings on the healing of acute full-thickness skin wounds on the forelimbs of dogs. *Veterinary Surgery*, 23(6): 494-502.

Morris, J. S., and Dunn, J. K. 1992. Haematology. Getting the best from the laboratory. *In Practice*, 14(2): 67-72.

Moulin, V., Auger, F. A., Garrel, D., and Germain, L. 2000. Role of wound healing myofibroblasts on re-epithelialization of human skin. *Burns*, 26(1): 3-12.

MSEC. 2013. Best uses for calcium alginate dressings. http://skin-wound-care.medical-supplies-equipment-company.com/calcium-alginate-dressings-391.htm. Retrieved 25 may 2013.

Muehlberger, T., Moresi, J. M., Schwarze, H., Hristopoulos, G., Laenger, F., and Wong, L. 2005. The effect of topical tretinoin on tissue strength and skin components in a murine incisional wound model. *Journal of the American Academy of Dermatology*, 52(4): 583-588.

Müller, W. E. G. 2003. The origin of metazoan complexity: Porifera as integrated animals. *Integrative and Comparative Biology*, 43(1): 3-10.

Muncaster, D. 2001. The physiology of wound healing and wound assessment. *British Journal of Perioperative nursing: The Journal of the National Association of Theatre Nurses*, 11(8): 362-370.

Mustoe, T. 2004. Understanding chronic wounds: A unifying hypothesis on their pathogenesis and implications for therapy. *The American Journal of Surgery*, 187(5): S65-S70.

National Pressure Ulcer Advisory Panel. 2007. Npuap staging report. http://www.npuap.org/positn6.html. Retrieved 25 may 2013.

Noorjahan, S., and Sastry, T. 2004a. An in vivo study of hydrogels based on physiologically clotted fibrin–gelatin composites as wound-dressing materials. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 71(2): 305-312.

Noorjahan, S. E., and Sastry, T. P. 2004b. Hydrogels based on physiologically clotted fibrin–gelatin composites. *Journal of Polymer Science Part A: Polymer Chemistry*, 42(9): 2241-2252.

Noormah, M. D. 2010. Health technology assessment report: Antiseptics for skin preparations prior to procedures, Putrajaya, Malaysia.

Nunes, P. S., Albuquerque-Júnior, R. L., Cavalcante, D. R., Dantas, M. D., Cardoso, J. C., Bezerra, M. S., Souza, J. C., Serafini, M. R., Quitans-Jr, L. J., and Bonjardim, L. R. 2011. Collagen-based films containing liposome-loaded usnic acid as dressing for dermal burn healing. *BioMed Research International*, 2011.

O'Meara, S., Cullum, N., Majid, M., and Sheldon, T. 2000. Systematic reviews of wound care management:(3) antimicrobial agents for chronic wounds;(4) diabetic foot ulceration. *Health Technology Assessment (Winchester, England)*, 4(21): 1-237.

Ono, I. 2002. The effects of basic fibroblast growth factor (bfgf) on the breaking strength of acute incisional wounds. *Journal of Dermatological Science*, 29(2): 104-113.

Otterlei, M., Sundan, A., Skjåk-Bræk, G., Ryan, L., Smidsrød, O., and Espevik, T. 1993. Similar mechanisms of action of defined polysaccharides and lipopolysaccharides: Characterization of binding and tumor necrosis factor alpha induction. *Infection and Immunity*, 61(5): 1917-1925.

Palmieri, B. 1992. Heterologous collagen in wound healing: A clinical study. *International Journal of Tissue Reactions*, 14: 21-25.

Parker, L. 2000. Applying the principles of infection control to wound care. *British Journal of Nursing*, 9(7): 394-404.

Paul, C. N. 2008. Skin substitutes in burn care. *Wounds*, 20(7): 203-205.

Percival, S., Bowler, P., and Russell, D. 2005. Bacterial resistance to silver in wound care. *Journal of hospital infection*, 60(1): 1-7.

Pieper, B. 2007. Mechanical forces: Pressure, shear, and friction. *Bryant, RA (Ed.) Acute and Chronic Wounds. Nursing Management St. Louis, MO: Mosby/Elsevier*: 205-234.

Pinches, M. 2006. Getting results in clinical pathology 2. Pros and cons of in-clinic haematological testing. *In Practice*, 28(3): 144-146.

Pizzo, P. P. 1997. Bilateral. http://www.engr.sjsu.edu/WofMatE/Biomaterials.htm. Retrieved 16 June 2013.

Plassmnn, P., Melhuish, J. M., and Harding, K. G. 2005. Problems of assessing wound size. http://www.com.glam.uk/pages/staff/pplassna/medimaging/ projects/wounds/MAVIS/Review.doc . Retrieved 16 June 2013.

Pope, E. R. 1993. Skin healing.Disease maechanisms in *Small animal surgery*, M. J. Bojrab, ed.: pp 151-155. Philadelphia, London. Lea and Febiger

Prathiba, V., and Gupta, P. D. 2000. Cutaneous wound healing: Significance of proteoglycans in scar formation. *Current Science*, 78(6): 1-5.

Raghow, R. 1994. The role of extracellular matrix in postinflammatory wound healing and fibrosis. *Federation of American Society for Experimental Biology Journal*, 8(11): 823-831.

Ramakrishnan, M., Pratibha, V., Rao, S. K., Jayaraman, V., Babu, M., and Gupta, P. D. F. 1995. Expression of keratin in post-burn scars and keloids. *Annals of Burns and Fire Disasters*, 8(4): 123-128.

Rangaraj, A., Harding, K., and Leaper, D. 2011. Role of collagen in wound management. *Wounds UK*, 7(2): 54-63.

Ransford, A., Morley, T., Edgar, M., Webb, P., Passuti, N., Chopin, D., Morin, C., Michel, F., Garin, C., and Pries, D. 1998. Synthetic porous ceramic compared with autograft in scoliosis surgery a prospective, randomised study of 341 patients. *Journal of Bone & Joint Surgery, British Volume*, 80(1): 13-18.

Rebar, A. H., MacWilliams, P. S., Feldman, B. F., Metzger, F. L., Pollock, R. V. H., and Roche, J. 2005. Interpretation of the hemogram: Introduction, white cells, red cells, platelets *a guide to hematology in dogs and cats* (eds). A guide to hematology in dogs and cats. Teton newmedia, jackson wy (www.Tetonnm.Com). Retrieved 23 june 2013.

Reher, P., Doan, N., Bradnock, B., Meghji, S., and Harris, M. 1998. Therapeutic ultrasound for osteoradionecrosis: An *in vitro* comparison between 1mhz and 45khz machines. *European Journal of Cancer*, 34(12): 1962-1968.

Richard, J.-L., Daures, J.-P., Parer-Richard, C., Vannereau, D., and Boulot, I. 2000. Of mice and wounds: Reproducibility and accuracy of a novel planimetry program for measuring wound area. *Wounds*, 12(6): 148-154.

Riet, G., Kessels, A. G., and Knipschild, P. 1996. A randomized clinical trial of ultrasound in the treatment of pressure ulcers. *Physical Therapy*, 76(12): 1301-1312.

Roche, C., and West, J. 1984. A controlled trial investigating the effect of ultrasound on venous ulcers referred from general practitioners. *Physiotherapy*, 70(12): 475-477.

Rojas, I.-G., Padgett, D. A., Sheridan, J. F., and Marucha, P. T. 2002. Stress-induced susceptibility to bacterial infection during cutaneous wound healing. *Brain, Behavior, and Immunity*, 16(1): 74-84.

Romanelli, M., gaggio, g., Colucci, M., Rizzello, F., and Piagges, A. 2002. New therapeutic approaches in wound healing.Technological advances in wound *Wounds: A Compendium of Clinical Research and Practice*, 14(2): 58-66.

Roush, J. K. 2003. Biomaterials and surgical implants. . *Textbook of small animal surgery*, Saunders, Philadelphia: Vol.1. 3rd ed pp141-148.

Rowett, H. G. Q. 1974. The rat as a small mammal (3rd ed), John Murray Limited.

Rubin, C., Bolander, M., Ryaby, J. P., and Hadjiargyrou, M. 2001. The use of low-intensity ultrasound to accelerate the healing of fractures. *The Journal of Bone & Joint Surgery*, 83(2): 259-259.

Schäffer, M. R., Tantry, U., and Barbul, A. 2004. Wound fluid inhibits wound fibroblast nitric oxide synthesis. *Journal of Surgical Research*, 122(1): 43-48.

Schiro, J. A., Chan, B., Roswit, W. T., Kassner, P. D., Pentland, A. P., Hemler, M. E., Eisen, A. Z., and Kupper, T. S. 1991. Integrin α 2 β 1(vla-2) mediates reorganization and contraction of collagen matrices by human cells. *Cell*, 67(2): 403-410.

Schubert, V. 1997. Measuring the area of chronic ulcers for consistent documentation in clinical practice. *Wounds*, 9(5): 153-159.

Sharma, P. 2011. Cosmeceuticals: Regulatory scenario in us, europe & india *International Journal of Pharmacy &Technology*, 3(4): 1512-1535.

Shirtliff, M. E., Calhoun, J. H., and Mader, J. T. 2002. Gatifloxacin efficacy in treatment of experimental methicillin-sensitive staphylococcus aureus-induced osteomyelitis in rabbits. *Antimicrobial Agents and Chemotherapy*, 46(1): 231-233.

Shmon, C. 2003. Assessment and preparation of the surgical patient and the operating team. *Textbook of small animal surgery*, D. Slatter, ed., WB Saunders Philadelphia,, USA: 167-168.

Shukla, A., Rasik, A., Jain, G., Shankar, R., Kulshrestha, D., and Dhawan, B. 1999. In vitro and in vivo wound healing activity of asiaticoside isolated from *centella asiatica*. *Journal of Ethnopharmacology*, 65(1): 1-11.

Sidhu, G. S., Mani, H., Gaddipati, J. P., Singh, A. K., Seth, P., Banaudha, K. K., Patnaik, G. K., and Maheshwari, R. K. 1999. Curcumin enhances wound healing in streptozotocin induced diabetic rats and genetically diabetic mice. *Wound Repair and Regeneration*, 7(5): 362-374.

Sikorski, Z. E. 2001. Chemical and functional properties of food components, CRC Press. p.242.

Silverthorn, D. U., Johnson, B. R., and Ober, W. C. 2012. Human physiology. Pearson/Benjamin Cummings.

Singer, A. J., and Clark, R. 1999. Cutaneous wound healing. *The New England Journal of Medicine*, 341(10): 738-746.

Singh, A., Halder, S., Chumber, S., Misra, M. C., Sharma, L. K., Srivastava, A., and Menon, G. R. 2004. Meta-analysis of randomized controlled trials on hydrocolloid occlusive dressing versus conventional gauze dressing in the healing of chronic wounds. *Asian Journal of Surgery*, 27(4): 326-332.

Smith, B. A., Hosgood, G., and Hedlund, C. S. 1995. Omental pedicle used to manage a large dorsal wound in a dog. *Journal of Small Animal Practice*, 36(6): 267-270.

Smith, R. A., Blanchard, C. R., and Lankford Jr, J. 1994. Nonantigenic keratinous protein material. http://www.patentstorm.us/patents/5358935.html. Retrieved 12 June 2013.

Soni, R. 2013. Effect of ethanolic extract of cinnamomum tamala leaves on wound healing in stz induced diabetes in rats. *Asian Journal of Pharmaceutical and Clinical Research*, 6(4): 39-42.

Sotozono, C., Inatomi, T., Nakamura, M., and Kinoshita, S. 1995. Keratinocyte growth factor accelerates corneal epithelial wound healing in vivo. *Investigative Ophthalmology & Visual science*, 36(8): 1524-1529.

Stevens, A., Lowe, J. s., and Deakin, P. J. 2000. Supporting connective tissue. Wheater's functional histology. *A text and colour atlas,ed.* Young, b and heath, j.W, pp 70, Churchill Livingstone, Edinburgh, London.

Subrahmanyam, M. 1998. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. *Burns*, 24(2): 157-161.

Sullivan, T. P., Eaglstein, W. H., Davis, S. C., and Mertz, P. 2001. The pig as a model for human wound healing. *Wound Repair and Regeneration*, 9(2): 66-76.

Sussman, C., and Bates-Jensen, B. M. 2001. Wound care: A collaborative practice manual for physical therapists and nurses, pp 26-51 Aspen Publishers, Inc.

Suzuki, S., and Ikada, Y. 2012. Introduction. Biomaterials for surgical operation, Springer Science+Business Media, LLC: 1-5.

Swaim, S. F. 2003. Skin grafts. *Textbook of small animal surgery, ed douglas slatter*, Vol.1 3rd ed, pp 327-329 W.B. Saunders. Phiadelphia.

Swaim, S. F., and Henderson, R. A. 1997. Small animal wound management (2^{nd} ed.), Maryland: Williams and Wilkins.

Takei, M., Fukuda, H., Yasue, T., Hosaka, M., and Oomori, Y. 1998. Inhibitory activities of gatifloxacin (am-1155), a newly developed fluoroquinolone, against bacterial and mammalian type ii topoisomerases. *Antimicrobial Agents Chemother*, 42(10): 2678-2681.

Takenaka, H., Kishimoto, S., Tooyama, I., Kimura, H., and Yasuno, H. 1997. Protein expression of fibroblast growth factor receptor-1 in keratinocytes during wound healing in rat skin. *Journal of Investigative Dermatology*, 109(1): 108-112.

Tan, M. K., Adli, H., Sharifah, D., Tumiran, M. A., Abdulla, M. A., and Yusoff, K. M. 2012. The efficacy of gelam honey dressing towards excisional wound

healing. *Evidence-Based Complementary and Alternative Medicine*, 2012: 1-6.

Tarun, K., and Gobia, N. 2012. Calcium alginate/pva blended nano fibre matrix for wound dressing. *Indian Journal of Fibre & Textile Research*, 37(6): 127-132.

Ter Haar, G. 1978. Basic physics of therapeutic ultrasound. *Physiotherapy*, 64(4): 100-103.

Thilagar, S., Jothi, N. A., Omar, A., Kamaruddin, M., and Ganabadi, S. 2009. Effect of keratin-gelatin and bfgf-gelatin composite film as a sandwich layer for full-thickness skin mesh graft in experimental dogs. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 88(1): 12-16.

Thomas, D., Goode, P., LaMaster, K., Tennyson, T., and Parnell, L. 1999. A comparison of an opaque foam dressing versus a transparent film dressing in the management of skin tears in institutionalized subjects. *Ostomy/wound management*, 45(6): 22-4, 27-8.

Thomas, S. 2000. Alginate dressings in surgery and wound management-part *Journal of Wound Care*, 9(2): 56-60.

Tonks, A., Cooper, R., Jones, K., Blair, S., Parton, J., and Tonks, A. 2003. Honey stimulates inflammatory cytokine production from monocytes. *Cytokine*, 21(5): 242-247.

Tonks, A., Cooper, R., Price, A., Molan, P. C., and Jones, K. 2001. Stimulation of tnf-α release in monocytes by honey. *Cytokine*, 14(4): 240-242.

Tonnesen, M. G., Feng, X., and Clark, R. A. 2000. Angiogenesis in wound healing. *Journal of Investigative Dermatology Symposium Proceedings*, 5(1): 40-46.

Torrance, A. 2000. Overview of haematological diagnostic techniques. *Manual of canine and feline haematology and transfusion medicine*. 1st ed. Glocester: Bsava, A. M.Day, ed.: pp 3-17. UK: Loookers.

Tsala, D. E., Amadou, D., and Habtemariam, S. 2013. Natural wound healing and bioactive natural products. *Phytopharmacology*, 4(3): 532-560.

Tsuboi, R., and Rifkin, D. B. 1990. Recombinant basic fibroblast growth factor stimulates wound healing in healing-impaired db/db mice. *The Journal of Experimental Medicine*, 172(1): 245-251.

Turton, J., and Hooson, J. 2003. Target organ pathology: A basic text, Informa Healthcare, pp456.Taylor & Francis Ltd, 1 Gunpowder Square, London.

Ueno, C., Hunt, T. K., and Hopf, H. W. 2006. Using physiology to improve surgical wound outcomes. *Plastic and Reconstructive Surgery*, 117(7S): 59S-71S.

Unger, P. 2008. Sound evidence: Low-frequency, noncontact, non-thermal, ultrasound therapy: A review of the literature. *Ostomy/Wound Management*, 54(1): 4.

Van Zuijlen, P. P., Angeles, A. P., Suijker, M. H., Kreis, R. W., and Middelkoop, E. 2004. Reliability and accuracy of techniques for surface area measurements of wounds and scars. *The International Journal of Lower Extremity Wounds*, 3(1): 7-11.

Varaprasad, K., Mohan, Y. M., Vimala, K., and Mohana Raju, K. 2011. Synthesis and characterization of hydrogel-silver nanoparticle-curcumin composites for wound dressing and antibacterial application. *Journal of Applied Polymer Science*, 121(2): 784-796.

Vedakumari, W. S., Prabu, P., Babu, S. C., and Sastry, T. P. 2013. Fibrin nanoparticles as possible vehicles for drug delivery. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830(8): 4244-4253.

Vogt, P. M., Lehnhardt, M., Wagner, D., Jansen, V., Krieg, M., and Steinau, H. 1998. Determination of endogenous growth factors in human wound fluid: Temporal presence and profiles of secretion. *Plastic and Reconstructive Surgery*, 102(1): 117-123.

Waldron, D. R., and Pope, N. Z. 2003. Superficial skin wound. Textbook of small animal surgery, D. Slatter, ed.: pp.259-273. Saunders, Philadelphia.

Wang, C., Li, C., Deng, G., Xu, X., Shu, L., Liu, X., and Chen, Q. 2012. Value of the pedicle omentum transfer for the healing of large skin wound in dogs. *International Journal of Applied Research in Veterinary Medicine*, 10(4): 300-304.

Wendelken, M. E., Markowitz, L., Patel, M., and Alvarez, O. M. 2003. Original research objective, noninvasive wound assessment using b-mode ultrasonography. *Wounds*, 15(11): 351-360.

Werner, S., Breeden, M., Hübner, G., Greenhalgh, D. G., and Longaker, M. T. 1994. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. *Journal of Investigative Dermatology*, 103(4): 469-473.

Wilgus, T. T., Bergdall, V. K., Tober, K. l., Hill, K. J., Mitra, S., Flavahan, N. A., and Oberyszyn, T. M. 2004. The impact of cycolooxygenase-2 mediated inflammation on scarless fetal wound healing. *American Journal of Pathology*, 165(3): 753-761.

Winkler, J. T., Swaim, S. F., Sartin, E. A., Henderson, R. A., and Welch, J. A. 2002. The effect of a porcine-derived small intestinal submucosa product on wounds with exposed bone in dogs. *Veterinary Surgery*, 31(6): 541-551.

Winter, G. D. 1962. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature*, 193: 293-294.

Wisser, D., and Steffes, J. 2003. Skin replacement with a collagen based dermal substitute, autologous keratinocytes and fibroblasts in burn trauma. *Burns*, 29(4): 375-380.

Young, S., and Dyson, M. 1990. The effect of therapeutic ultrasound on angiogenesis. *Ultrasound in Medicine & Biology*, 15(3): 251-259.

Young, S. R., Dyson, M., Hickman, R., Lang, S., and Osborn, C. 1991. Comparison of the effects of semi-occlusive polyurethane dressings and hydrocolloid dressings on dermal repair: 1. Cellular changes. *Journal of investigative dermatology*, 97(3): 586-592.

Zawacki, B., and Jones, R. 1967. Standard depth burns in the rat: The importance of the hair growth cycle. *British Journal of Plastic Surgery*, 20: 347-354.

APPENDICES

Appendix A approved of the proposed animal utilization protocol

UPM

UNIVERSITI PUTRA MALAYSIA

FAKULTI PERUBATAN VETERINAR
(FACULTY OF VETERINARY MEDICINE)

Date:	24 May 2012
Ref:	UPM/FPV/PS/3.2.1.551/AUP-R158
Project Title:	Evaluation of wound healing using collagen and calcium alginate film, and therapeutic ultrasonic massage tor chronic wounds in rats
Principle Investigator:	Assoc. Prof. Dr. Kalthum Hashim
Associates:	Prof. Dr. Noordin Mohamed Mustapha Dr. Jalila Abu
Student:	Khaled M.A Hussin
Committee decision:	The committee has reviewed and approved the proposed animal utilization protocol
AUP No:	12R158/ May 12 – April 13
Project Classification:	Chronic
Category of invasiveness:	B
Source of animals:	A Saphire Enterprise
Housing:	Experimental rooms, Faculty of Veterinary Medicine
Duration:	May 2012 – April 2013

(Assoc. Prof. Dr. Abdul Rahim Mutalib)
Chairman, Animal Care and Use Committee
Faculty of Veterinary Medicine
Universiti Putra Malaysia

Fakulti Perubatan Veterinar, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
603-8946 0000 603-894 8280 603-8946 8333 http://www.vet.upm.edu.my

Appendix B1

Blood agar base

Beef heart infusion	375g
Tryptose	10g
Sodium chloride	5g
Agar	15g
Distilled water	1000ml

Autoclaving was done at 121˚C for 15 minute.

Cool agar (50˚C) and add 53ml defibrinated bovine blood and pour into sterile Petri plate, 20ml/plate under sterile condition.

Appendix B2

MacConkey agar

Peptone	17g
Proteose-peptone	3g
Lactose	10g
Bile Salts	1.5g
Agar	13.5g
Neutral red	0.03g
Crystal violet	0.001g
Distilled water	1000ml

Autoclaving was done at 121˚C for 15 minute.

Cool agar (50˚C) and pour into sterile Petri plate, 20ml/plate under sterile condition.

Appendix B3

Gram stain

1. Stock crystal violet

Crystal violet	10.0g
Ethyl alcohol	100.0ml

2. Stock oxalate solution

Ammonium oxalate	1.0g
Distilled water	100.0ml

Crystal violet working solution: Mix 20 ml of solution 1 with 80 ml of stock.

3. Gram's Iodine Solution

Iodine crystal	1.0g
Potassium iodide	2.0g

Dissolve the above reagents in 10 ml of distilled water then add distilled water up to 200.0ml and store in dark bottle.

4. Decolourizer

Acetone	3 ml
Ethyl alcohol	97 ml

5. Counter Stain

Basic Fuchsin	3.0g
Ethyl alcohol	100.0ml

Procedure:

STEP 1: Flood the entire slide with crystal violet and stand for about 60 seconds. Wash the slide for 5 second with water.

STEP 2: Flood the slide with the iodine solution for about a minute. Rinse the slide with water for 5 seconds.

STEP 3: Add the ethanol drop by drop until the blue-violet color is no longer emitted. Rinse the slide with water for 5 seconds.

STEP 4: Flood the slide with the safranin stand for about a minute to allow the bacteria to incorporate the saffranin (Gram negative bacteria, take on a pink color). Rinse the slide with water for 5 seconds.

STEP 5: Blot the slide gently with tissue paper or allow it air dry before viewing it under the microscope.

Appendix C1

Haematoxylin and Eosin staining

Harris haematoxylin

Haematoxylin	1 gram
Absolute alcohol	10.0 ml
Ammonium or potassium sulphate	20 grams
Mercuric oxide	0.5 gram
Distilled water	200 ml

The haematoxylin was dissolved in alcohol and the ammonium in water with aid of heat; both the haematoxylin and the alum solution were combined and boiled. The addition of mercuric oxide made the solution dark purple in colour. The solution was filtered before be fore using or storing the solution.

Eosin counters stain for haematoxylin 1% stock alcohol eosin

Eosin Y (water soluble)	1 Gram
Distilled water	20.0 ml
95% alcohol	80.0 mll

Working eosin solution

Eosin stock solution	1 part
80 % alcohol	20.0 ml

Just before use, 0.5 ml of glacial acid was added to each 100 ml of stain and stirred.

Staining technique

1. De-wax section in xylene for 5 minutes.
2. Rehydrate in 100% alcohol and 70% each for 8 minutes.
3. Wash in running water by dipping up and down for 1-2 minutes.
4. Stain in Haematoxylin for 1-2 minutes.
5. Wash in running water until no trace of stain observed on the tissues.
6. Decolorize in 1 % acetic alcohol by dipping 3 times.
7. Wash in running water for 10 minutes for bluing.
8. Stain in eosin for 30 seconds.
9. Dehydrate with 95% alcohol.
10. Clean in xylene.
11. Mount with coverslip using DPX.

Appendix C2

Masson's trichrome stain

1. Bouin's solution

Picric acid saturated aqueous solution	750.00 ml
Formalin 37-40%	250.00 ml
Glacial acetic acid	50.00 ml

2. Weigerts' iron haematoxylin solution

Solution A:

Haematoxylin crystals	1.00 grams
Alcohol 95%	100.00 ml

Solution B:

Ferric chloride 29 % aqueous	4.00 ml
Distilled water	95.00ml
Concentrated Hydrochloric acid	1.00ml

Working solution

Equal parts of solution A and solution B

3. Biebrich Scarlet- Acid Fuchsin Solution

Biebrich Scarlet, aqueous 1%	90.00 ml
Acid Fuchsin aqueous 1 %	10.00 ml
Glacial acetic acid	1.00 ml

4. Phosphomolybdic-phosphotungstic Acid

Phosphomolybdic acid	5 grams
Phosphotungstic acid	5 grams
Distilled water	200 ml

5. Aniline blue solution

Aniline blue	2.5 grams
Glacial acetic acid	2.00 ml
Distilled water	100.00 ml

6. 1 % Acetic asid

Glacial acetic acid	1.00 ml
Distilled water	100.00 ml

Masson's trichrome (MT) Staining technique

1. De-wax section in xylene for 5 minutes.
2. Rehydrate in 100% alcohol and 70% each for 8 minutes.
3. Wash in running water by dipping up and down for 1-2 minutes.
4. Slides submerged in Bouin's fluid for 1 hour at 56˚C.
5. Wash in running water until no traces of stain observed on the tissues.
6. Stain in Weigert's haematoxylin for 5 minutes.
7. Rinsed thoroughly in running tap water until no traces of stain observed on the tissues.
8. Stain in Biebrich scarlet-acid fuchsin for 15 minutes.
9. Rinsed thoroughly in distilled water.
10. Submerged in Phosphomolbdic/Phosphotungstic acid 10 to 15 minutes.
11. Submerged in Aniline blue stain for 5 to 10 minutes.
12. Rinsed thoroughly in distilled water.
13. The slides were placed in 1 % Acetic acid for 3 to 5 minutes.
14. Dehydrate with 95 % alcohol.
15. Clean in xylene.
16. Mount with cover slip using DPX.

Appendix D

The results of wound planimetry

Case No	Characteristics	Wound healing (%)				
		1st (day 3)	2nd (day 6)	3rd (day 9)	4th (day 12)	5th (day 15)
1	Epithelialization	10.67	27.68	26.67	95.00	NR
	Contraction	4.67	18.96	80.68	85.43	
	Wound healing	24.45	51.74	92.71	96.39	
2	Epithelialization	12.78	49.56	71.35	92.24	NR
	Contraction	48.78	68.62	79.38	80.42	
	Wound healing	45.90	79.13	90.45	97.12	
3	Epithelialization	14.56	42.00	59.82	74.89	NR
	Contraction	14.89	27.57	42.67	73.12	
	Wound healing	24.84	54.98	75.39	79.24	
4	Epithelialization	35.08	66.79	75.41	79.44	86.89
	Contraction	37.78	60.22	69.67	72.48	81.44
	Wound healing	58.14	68.11	85.13	89.78	95.87
5	Epithelialization	69.44	76.24	86.36	96.00	NR
	Contraction	20.13	31.09	43.92	48.34	
	Wound healing	81.24	85.89	92.95	96.32	
6	Epithelialization	11.60	39.57	56.14	69.14	81.30
	Contraction	11.80	23.67	39.89	70.32	84.53
	Wound healing	22.78	51.41	71.13	78.02	95.90
7	Epithelialization	8.96	38.89	61.54	79.45	NR
	Contraction	37.22	62.53	73.49	93.95	
	Wound healing	42.13	70.15	89.15	97.94	
8	Epithelialization	86.56	93.89	95.92	98.89	NR
	Contraction	65.12	76.09	81.67	84.70	
	Wound healing	91.89	95.13	96.80	99.03	

NR- not reported.

BIODATA OF STUDENT

Khaled was born in Benghazi, Libya on April 5, 1977. He is the 1st child of Mr. Masood A. Hussin. Khaled is happily married to Ms. Asma Saleh and they are blessed with two sons and one daughter, Tiam, Yazan and Aytin. He pursued his higher secondary school from, Asr El- gamaher school higher secondary school, Benghazi. After school education, in 1996 pursued his Bachelor of Veterinary Science from Omar Al- Mukhtar University, El Beida, Libya. After completion of B.V.Sc., he started work with the private sector in different veterinary fields for 18 months. In June 2004, he continued studying for a Master of veterinary medicine in University Putra Malaysia (UPM). Soon after completion of his Master study, he was transferred to the Department of Veterinary Surgery and Radiology as a Lecturer at Omar Al- Mukhtar University, El Beida, Libya in 2006 and served for two years. To pursue his Doctor of Philosophy degree (PhD) programmed in Veterinary Surgery, with scholarship. He joined the faculty of, Veterinary Medicine, University Putra Malaysia (UPM) in the year 2008. So far, he has published two citation journal papers during his PhD candidature and two others currently under review. He also published two papers from his MVM work and several publications, conferences and seminar thereafter.

LIST OF PUBLICATIONS

1. Khaled, M. A., Jalila, A., Kalthum, H., Noordin, M., and Asma, S. W. 2014. Effects of biomaterials collagen-calcium alginate film with therapeutic ultrasound for management on wound healing in a rat. Accepted for publication at *Journal of Animal and Veterinary Advances.*

2. Khaled, M. A., Jalila, A., Kalthum, H., Noordin, M., and Asma, S. W. 2014. Collagen-calcium alginate film dressing with therapeutic ultrasound to treat open wound in rats. *Research Journal of Biological Sciences.*9 (2): 57-61.

3. Khaled, M. A., Jalila, A., Kalthum, H., Noordin, M., and Asma, S. W. 2014. Clinical evaluation of collagen - calcium alginate film along with therapeutic ultrasound massage for wound healing in cats. (Under review).

4. Khaled, M. A., Jalila, A., Kalthum, H., Noordin, M., and Asma, S. W. 2014. Macroscopic evaluation of wounds healing progress treated with collagen-calcium alginate film dressing with therapeutic ultrasound.Malaysia International Biological Symposium (*i*-SIMBIOMAS) 28-29 October.