J. Vet. Malaysia (2006 18(1): 21 - 26

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

N. Arul Jothi[1], S. Thilagar[1a], A.R. Sheikh Omar[1], M.Y. Kamaruddin[2], Shanthi Ganabadi[1], Y.M Goh[1] and M.Y. Sabri[1]

[1]*Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia*
[2]*Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia*

SUMMARY

Eighteen clinically healthy dogs weighing 10-15 kg body weight were used in this study over a 20-day period. They were allocated randomly into 3 groups of 6 animals each. After the creation of 5 cm x 5cm open wound, Group I was control treated with Gentamycin ointment. Groups II and III were treated with keratin-gelatin and basic fibroblast growth factor (bFGF)-gelatin composite film respectively. On application, the keratin-gelatin and bFGF-gelatin composite film were well accepted by the animals without any adverse reaction. On clinical examination, Group II showed bright beefy red color granulation tissue with angiogenesis when compared to Groups I and III. On bacteriological examination, *Staphylococcus aureus, Pseudomonas, Escherichia coli, Proteus and Klebsiella species* were isolated from all the groups. Mean percentage of epithelialisation, wound contraction and total healing were significantly better in Group II ($P<0.05$). Keratin is a biocompatible protein which does not interfere with the body's normal immunologic response and therefore it can be used in extensive wounds and also in non healing chronic wounds which need a trigger to stimulate the normal healing process. In extensive wounds when there is lack of autologous tissue, biomaterials like keratin-gelatin may be beneficial and can be used.

Keywords: Biomaterial, keratin, bFGF, wound

INTRODUCTION

A wound arises due to disruption of the normal architecture of a tissue. Wound healing occurs as a fundamental response to tissue injury (Sumitra *et al.*, 2005). The main objectives of any wound management are: functional and cosmetic repair, relief of pain and distress to the animal, economic and time efficient procedures and prompt decision making in the event of signs of delayed healing (Anderson, 1996). A major drawback in conventional dressing materials, mainly composed of gauze, is the adherence between the fibres of the gauze materials and the tissue. Separation between the dressing material and the tissue becomes very difficult (Lin *et al.*, 2000).

Immediate wound coverage with native skin is possible only if sufficient donor area is available. Due to lack of autologous tissue, the necrotised areas of large burns must be covered by skin substitutes (Wisser and Steffes, 2003). In large wound cases, if there is no required donor site available, biomaterials like synthetic or natural polymers and their composites can be used as a temporary closure of wounds (Babu *et al.*, 1996). Biomaterials like keratin and bFGF-gelatin composite film can be used. Keratin is a fibrous protein that can be used as an absorbent therapeutic material for skin and for cosmetic applications. Hard keratins are a specialised type of keratin found in feathers, hair, nails, wool and hooves (Barone *et al.*, 2005). Anderson *et al.* (2003) used keratinocyte cultured epithelial auto graft for a dog with severe burn injury. Basic Fibroblast Growth Factors (bFGF) induce mitogenesis and play an important role in wound healing. It is well known that bFGF promote proliferation of almost all cells associated with wound healing (Michiyo *et al.*, 2005). Ono (2002) used bFGF to promote wound healing. This study is done to assess the effects of keratin hydrolysates of poultry feathers prepared by controlled alkaline hydrolysis (Sehgal *et al.*, 1986) and bFGF- gelatin composite films on wound healing in experimental dogs.

MATERIALS AND METHODS

Animal subjects

Eighteen clinically healthy dogs weighing 10-15 kg body weight were used in this study. They were allocated randomly into 3 groups of 6 animals each. All the animals were fed with dry food, and water was provided *ad libitum*. Experimental subjects were given a thorough body wash one day before surgery. Treatment allocation was as described in the following pattern.

1[a]. Corresponding author: Dr S Thilagar; E-mail- *thilagar@vet.upm.edu.my*

Group I: (n=6). Control Group. Gentamycin ointment (Dutch farm Veterinary pharmaceuticals, Netherlands) was applied after creation of 5 cm x 5cm open wound.

GROUP II: (n=6). Keratin-gelatin composite film treated Group. The 5cm x 5cm open wound was treated using 0.2mm thickness of keratin - gelatin film (36 μg/cm^2) soaked for 1-2 minutes in gentamycin (Dutch farm Veterinary pharmaceuticals, Netherlands).

GROUP III: (n=6). The bFGF-gelatin composite film treated Group. The 5cm x 5cm open wound was treated using bFGF-gelatin composite film (0.015 μg/cm^2) soaked for 1-2 minutes in gentamycin (Dutch farm Veterinary pharmaceuticals, Netherlands).

Wound dressing, clinical observation, wound planimetery and bacteriological evaluation of wound for all groups were done on days 4, 8, 12, 16 and day 20. Elizabethan collar was applied to prevent self-mutilation of the surgical site.

Surgical protocol

On day 0, animals were pre-medicated with Acepromazine (0.1mg/kg body wt S/C) and Atrophine sulphate (0.05mg/kg body wt, Troy Laboratories, Australia). Induction was done with 2.5 % Thiopentone sodium (12.5 mg/kg body weight IV, Troy Laboratories, Australia) and maintained with Halothane (Troy Laboratories, Australia) in oxygen delivered via T-piece circuit using cuffed endotracheal tube.

Animals were positioned in lateral recumbency and the lower lateral aspect of the lumbosacral region in the upper flank area was prepared for aseptic surgery for creation of open wound. The skin was disinfected with hibiscrub, containing 0.6% chlorohexidin then with 70 % alcohol solution and with 2% iodine solution (Druecke *et al.*, 2004)

Using a sterile millimetre ruler and cotton tipped applicator dipped in sterile methylene blue, 5cm x 5cm square was drawn on the skin. A full thickness skin defect in which all tissue down to and including the panniculus muscle was excised using no. 15 scalpel blade.

Clinical observation

The colour of the wound bed gives an indication of the phase of healing. Colour coding taken into account are red, yellow, black and pink areas as the main variables in a wound. Shiny beefy red or red indicates healthy granulation tissue. Yellow colour is due to fibrous tissue or necrotic slough, black is eschar or necrotic tissue and pink or purple means re-epithelialisation has begun. (James and Bayat, 2003)

Odour: Mal-odour of the wound is commonly associated with infection or attributed to poor hygiene or from a dressing that has not been changed regularly. Presence of necrotic tissue gives off an offensive repulsive odour, and anaerobes typically produce a distinctive or putrid odour (James and Bayat, 2003).

Exudate: Exudates can be scored as no exudates, moderate or excessive. Type of discharge can be described as serous (clear fluid without blood), pus or debris. Serosanguinous and sanguinous indicates thin watery pale red to pink fluid and bloody to bright red respectively. Purulent discharge is thick, cloudy, and yellow or tans (James and Bayat, 2003).

. A more accurate method of determining the wound area in healing studies is tracing of the wound margins onto a clear plastic film. In this method, a double layer sterile plastic sandwich bag is placed on the wound for tracing wound margins; the layer that contacts with the wound can be removed and disposed and the outer layer is placed on the graph sheet and the area is measured by square counting procedure. The number of squares (0.25 cm^2) that lay completely (N_c) and partially (N_p) inside the tracing are counted and area is determined using the following formula: $A_{c+p} = (N_c + 0.40 \times N_p) \times 0.25$. (Richard *et al.*, 2000). The wound margin at the border between normal skin and the wound and the outlined area is considered to be the total wound area. Next, the leading edge of advancing epithelium is traced. The area between these two margins is considered to be the area for epithelialisation. The area within the margin of advancing epithelium is the area of open or unhealed wound (Bohling *et al.*, 2004).

Calculation of wound healing

Percentage of epithelialisation

$$\text{Percentage of epithelialisation day}_n = \frac{\text{Area of epithelium day}_n \times 100}{\text{Total wound area day}_n}$$

Percentage of wound contraction

Step 1: Total wound on day $_n$ as % of original

$$= \frac{\text{Total wound area day}_n \times 100}{\text{Original wound area day}_0}$$

Step 2: % wound contraction day$_n$ = 100- total wound on day $_n$ as % of original

Percentage of total wound healing was calculated by

Step 1: open wound day $_n$ as % of original

$$= \frac{\text{Open wound area day}_n \times 100}{\text{Original wound area (day}_0\text{)}}$$

Step 2: % of total wound healing day$_n$

= 100- open wound day$_n$ as % of original

(Bohling *et al.*, 2004).

Table 1: Percentage of epithelialisation (mean ± S.D)

Days	Control mean ± S.D	Keratin-Gelatin mean ± S.D	bFGF-gelatin mean ± S.D
0	0	0	0
4	11.50 ± 4.11[a]	21.34 ± 9.20[b]	17.87 ± 7.73[b]
8	28.00 ± 3.16[a]	51.52 ± 11.04[c]	40.06 ± 11.71[b]
12	45.80 ± 6.04[a]	76.75 ± 4.03[b]	46.23 ± 1.24[a]
16	60.94 ± 1.65[a]	86.52 ± 4.40[b]	59.43 ± 1.83[a]
20	80.94 ± 1.67[a]	98.99 ± 0.74[c]	86.57 ± 1.21[b]

Values with different superscripts within a row differed significantly at $P < 0.05$

Table 2: Percentage of wound contraction (mean ± S.D)

Days	Control mean ± S.D	Keratin-Gelatin mean ± S.D	bFGF-gelatin mean ± S.D
0	0	0	0
4	10.62 ± 4.10[a]	17.95 ± 12.23[a]	17.52 ± 9[a]
8	29.53 ± 4.57[a]	34.00 ± 6..31[ab]	37.00 ± 6.30[b]
12	50.76 ±10.05	56.91 ± 14.63[a]	58.38 ± 0.82[a]
16	70.72 ± 1.72[ab]	75.22 ± 6.09[b]	69.71 ± 3.49[a]
20	76.89 ± 3.65[a]	88.23 ± 2.62[c]	80.14 ± 1.83[b]

Values with different superscripts within a row differed significantly at $P < 0.05$

Table 3: Percentage of total wound healing (mean ± S.D)

Days	Control mean ± S.D	Keratin-Gelatin mean ± S.D	bFGF-gelatin mean ± S.D
0	0	0	0
4	28.72 ±12.46[a]	38.99 ± 6.68[a]	40.59 ± 7.45[a]
8	51.80 ± 6.36[a]	70.44 ± 7.37[ab]	66 ± 3.19[b]
12	67.68 ± 9.75	87.80 ± 12.46[a]	73.53 ± 3.72[a]
16	83.59 ± 2.8[ab]	96.64 ± 1.58[b]	86.20 ± 3.46[a]
20	89.45 ± 3.65[a]	99.55 ± 0.40[c]	96.98 ± 0.56[b]

Values with different superscripts within a row differed significantly at $P < 0.05$

Sterile cotton swabs were rolled over the wounds and streaked on blood and MacConkey agar plates. The colony growth was examined and identified by biochemical test after an incubation period of 24 hours.

Data analysis

Wound epithelialisation, contraction, and total wound healing across treatment groups were compared using repeated measures analysis of variance method for days 0, 4,8,12, 16 and 20. Significant means were elucidated using Duncan's multiple range test. All statistical tests were conducted at 95% level of confidence.

RESULTS

On application, the keratin-gelatin and bFGF-gelatin composite film adhered uniformly to the wound site. No unabsorbed remnants were noticed during the next application. In Groups I and III, the wound colour was red up to day 12 and pink in colour from day 16 and 20. Group II showed bright beefy red colour up to day 12 and it was red from day16 and 20. Mal-odour was observed up to day 12 in Group I and III. In Group II, mild mal-odour was observed up to day 8. Serous discharge was noticed up to day 12 in Group I. No exudates were seen in Group II throughout the study. Mild serous discharge was noticed up to day 8 in Group III.

Percentage of epithelialisation was consistently better in Group II throughout the trial. The difference between Groups II and III compared to Group I was evident as early as day 4. The percentage of epithelialisation improved dramatically from 21% (day 4) to almost 99% on day 20 in Group II. This was almost twice the value noted for Group I until day 12. Group III on the other hand, demonstrated better than average epithelialisation throughout the trial (Table 1).

Percentage of wound contraction was better in Group III up to day 12; however, it was best in Group II on day 16 and 20. The difference between Groups II and III compared to Group I was evident on day 8. Percentage of wound contraction improved dramatically from 75 % (day 16) to almost 88 % on day 20 in Group II, but Group III

showed improvement from 17% (day-4) to almost 80% on day 20. On the other hand, at the end of the trail, Group II showed better wound contraction (Table 2).

Percentage of total wound healing was consistently better in Group II throughout the trial. The difference between Groups II and III compared to Group I was evident from day 8. Percentage of total wound healing improved dramatically from 39% (day 4) to almost 99.5% on day 20 in Group II. On the other hand Group II, demonstrated better than average total wound healing throughout the trial (Table 3).

On bacteriological examination, *Staphylococcus aureus, Pseudomonas, Escherichia coli, Proteus and Klebsiella species* were isolated from all the Groups.

DISCUSSION

In Groups II and III, application of keratin-gelatin and bFGF-gelatin composite film was well tolerated by the animals. Both the films are easy to apply on the wound without any adverse reaction and were well accepted by all the animals. Keratins contain no cellular materials so they do not elicit any immune response (Dyke, 2005). Keratin-gelatin composite film is of animal origin, as such no reaction and rejection were observed and was well accepted without any adverse reaction. Application of keratin- gelatin and bFGF-gelatin composite film did not show any adhesion of the gauze during wound dressing.

The colour of the wound bed in Groups I and III was red while Group II showed bright beefy red color, which indicates healthy granulation tissue with neovascularisation, (James and Bayat, 2003) and resistance to infection until the epithelial barrier is re-established (Pope, 1993; Hosgood, 2003). The bright red color observed is due to micro vascular network throughout the granulation tissue (Tonnesen *et al.*, 2000). Basic fibroblast growth factor set the stage for angiogenesis during the first three days of wound repair (Schaffer *et al.*, 2004) and plays an important role in granulation tissue formation and the wound healing process (Takehara, 2000), but in the present study Group III showed less angiogenesis compared to Group II; this may be due to the fact that only 0.05 µg/square centimeter of bFGF-gelatin composite film was incorporated in the film and this may be too low to stimulate anticipated wound healing since it is dose dependent (Ono, 2002). Granulation in all the cases was flat without any exuberant nature; granulation tissue with a smooth surface facilitates migration of epithelial cells (Pope, 1993). On days 16 and 20, the granulation tissue was observed to be pink in colour in Group I and III which indicates the final stage of wound healing. In Group II the granulation tissue was red in colour due to neovascularisation (James and Bayat, 2003).

Mal-odour was observed up to day 12 in Groups I and III; however, In Group II, mild mal-odour was observed up to day 8 which may be due to infection (James and Bayat, 2003). The presence of bacterial infection was the common cause for the mal-odour observed, because all the wounds were infected. Serous discharge was noticed up to day 12 in Group I and mild serous discharge was noticed up to day 8 in Group III; subsequently, the discharge was reduced because healthy vascular granulation tissue is resistant to infection (Pope, 1993; Hosgood, 2003). No exudates were seen in Group II throughout the study because of the absorbent property of keratin. Keratin powder can be used as a therapeutic absorbent for skin applications (Jacksen *et al.*, 2004).

Wound epithelialisation of Group II was consistently better than Group I, and also better then Group III because the re-epithelialisation process in a full-thickness wound is accelerated by the presence of keratinocyte growth factor and its proliferation is essential for optimal wound healing (Ghalbzouri *et al.*, 2004). On day 4, epithelialisation was significantly better in Groups II and III, compared to Group I, because keratinocyte from the wound margins began to migrate to form new epithelium (Kirfel and Herzog, 2004). Keratinocyte plays an important role in the renewal of epidermis (Ghalbzouri *et al.*, 2004).

The mean percentage of wound epithelialisation in Group II was maximum between days 4 and 8 (30.18 %) but in Groups III and I, it was between days 16 and 20 (27% and 20 %). A similar finding was reported by Swaim *et al.* (1993), Bohling *et al.* (2004). Keratinocyte growth factor is a potent specific growth factor for epithelial cells and assists in re-epithelialisation (Werner *et al.*, 1994). The percentage of epithelialisation was significantly higher in Group II, quantity of bFGF (0.015 µg/cm^2) used was too low to produce significant results when compared to keratin, because Ono, (2001) reported that a dose of about 1 gram per cm is needed for significant wound healing. Basic fibroblast growth factor is expensive when compared to keratin which can be prepared from poultry feathers.

The percentage of wound contraction on post wound days 4, 8, 12 and 16 of Group II showed no significant difference from Group I; this may be because of an inherent property of fibroblasts that appears early in the process of wound contraction which after some time do not contract as forcefully as those that appear later (Bohling *et al.*, 2004). Schaffer *et al.* (2004) reported that there is increased fibroblast activity of the body until post-wound day 14. On days 8, 16 and 20, Group III was significantly better than Group I since basic fibroblast growth factor effectively accelerated wound fibroblast proliferation (Kawai *et al.*, 2000) and has a characteristic myofibroblastic appearance which plays a critical role in closure and healing (Cheng *et al.*, 1999).

The mean percentage of wound contraction of Group II on day 20 was significantly better than other two groups; in open wounds, contraction becomes an important feature and epithelialisation assumes a more predomi-

nant role. However, the two processes are independent of each other (Prost, 2003). All the groups showed maximum rates of contraction in the period between 8 and 12 post-wound days; similar findings have been reported by Swaim *et al.* (1993); Bohling *et al.* (2004); Aljady *et al.* (2000) and Baie and Sheikh (2000).

The percentage of total wound healing of Group II was significantly better then Group I and consistently better than Group III because of more rapid epithelialisation; there is a corresponding reduction in area of exposed granulation tissue in the wound. (Bohling *et al.*, 2004). On day 20, among Groups II and III there was no significant difference but healing was significantly better than in Group I. Basic FGF is well known for promoting the proliferation of almost all cells associated with wound healing (Michiyo *et al.*, 2005). Keratinocyte produces bFGF which participates in wound healing (Ono, 2002). All the groups showed maximum mean percentage of total wound healing from days 4 to 8; this is in concurrence with the results of Bohling *et al.* (2004) but differs from the findings of Swaim *et al.* (1993). *Staphylococcus aureus, Psudomonous, Klebsiella* sp *Escherichia coli* is the common infection in wounds (Kumar *et al.*, 2006). All wounds can be contaminated regardless of precautions taken (Pope, 1993).

The present study shows that keratin-gelatin composite film can be used as a wound healing stimulant to promote healing of extensive wounds in animals. Keratins are a family of structural proteins that provide the durable overcoat to the skin and protect the deeper cells. It is a biocompatible protein that does not interfere with the body's normal immunologic response and can be used in non healing chronic wounds which require a trigger to stimulate the normal healing process.

CONCLUSION

Keratin can be a better wound healing biomaterial in dogs. It can be used as a less expensive skin substitute in order to stimulate and promote wound healing in animals .

ACKNOWLEDGEMENT

The authors would like to thank Dean Faculty Veterinary Medicine, Staff at the Universiti Veterinary Hospital, Head of Department of Veterinary Clinical Studies for the facilities provided, and Dr. Zunita Zakaria and Prof. Rasedee Abdullah for help rendered during the study and Waltham canine food distributor for sponsoring the canine food.

REFERENCES

Aljady, A.M., Kamaruddin, M.Y., Jamal, A. M. and Yassim, M.Y.M. (2000). Biochemical study on the efficacy of Malaysian honey on inflicted wounds: an animal model. *Med. J. Islamic Aca. of Sci.* **13 (3)**:125-132.

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

Anderson, D.M., Stanley, M.A. and White, R.A.S. (2003). Canine keratinocyte culture and use of cultured epidermal autograft in a dog. *Vet. and Compara. Orthope. Traumatol.* **16** : 255 – 259.

Babu, P.R., Sastry, T. P., Rose, C and Rao, N.M. (1996). Hydrogels based on gelatin poly (hydroxyethyal methacrylate) and poly (butyl acrylate) graft copolymer impregnated with fibrin. *J. of App. Poly. Sci.* **65**: 555-560.

Baie, S. H and Sheikh, K.A. (2000). The wound healing properties of properties of *channa striatus*-cetrimide cream–wound contraction and glycosaminoglycan and measurement. *J. Ethnopharmacol.* **73**: 15 – 30

Barone, J.R., Liebner, C.F. and Schmidt, W.F. (2005). A feather in the cap of poultry producers. *Zootec. Intern.* **6**: 24-28.

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 dog. *Vet. Surg.* **33**: 579-587.

Cheng, B., Fu, X., Sheng, Z., Gu, X., Sun, T. and Sun, X. (1999). The effects of basic fibroblast growth factor on myofibroblasts and its significance on wound healing. *Zhonghua Yi Xue Za Zhi* **82(17)** : 1187 - 1191.

Druecke, D., Lamme, E.N., Hermann, S., Piper, J., May, P.S., Steinau, H.U., and Steinstraesser, L. (2004). Modulation of scar tissue formation using different dermal regeneration templates in the treatment of experimental full-thickness wound. *Wound Rep. and Regene.* **12 (5)** : 518.

Dyke, V.M.E.(2005).Biomaterials development. http://www1.wfubmc.edu/regenmed/faculty/Mark+Van+Dyke Accessed on 6 October 2005.

Ghalbzouri, A. E., Hensbergen, P., Gibbs, S., Kempenaar, J., Schors, R.V.D. and Ponec, M. (2004). Fibroblasts facilitate re-epithelialization in wounded human skin equivalents. *Lab. Invest.* **84**: 102-112.

Hosgood, G. (2003). Wound repair and specific tissue response to injury. In: Textbook of Small Animal Surgery. Slatter, D. (Ed.), Vol.1, 3rd ed. Saunders. pp.327-329.

Jacksen, S., Arlene, J., Dyke, V.M.E., Timmans, S.F., Blanchard, C.R. and Robert, A.S. (2004). Keratin-based powder and hydrogel for pharmaceutical applications. http://appft.1.uspto.gov/netacgi/nph+Parsen. Accessed on 25 July 2005.

James, A. and Bayat, A. (2003). Basic plastic Surgery techniques and principles: Chronic wound management. *Student BMJ* **11**: 406 – 407.

Kawai, K., Suzuki, S., Tabata, Y., Ikada, Y. and Nishimura, Y. (2000). Accelerated tissue regeneration through incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis. *Biomaterials* **21**: 489 – 499.

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

Kumar, M.S., Sripriya, R., Raghavan, H.V. and Sehgal, P.V. (2006). Wound healing potential of Cassia fistula on infected Albino Rat Model. *J. Surg. Research* **131**: 283-289.

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 Chem. and Phy.* **64** :189-195.

Michiyo, M., Takeshi, K., Hiroharu, I .H., Yasuhiko, T., Yoshito, I. and Shigehiko, S. (2005). Effects of bFGF-gelatin composite film incorporated into a gelatin sheet on wound healing. *J. Biomat. Sci. Poly. Ed.* **16 (7)** : 893-907.

Ono, I. (2002). The effects of basic fibroblast growth factor (bFGF-gelatin composite film) on the breaking strength of acute incisional wounds. *J. Dermatol. Sci.* **29:** 104-113.

Pope, E.R. (1993). Skin healing. In: Disease Mechanisms in Small Animal Surgery. Joseph Bojrab, M.(Ed.). 2nd ed. Philadelphia, London. pp. 152- 155.

Prost, C.W. (2003). Wound healing and specific tissue regeneration. In: Textbook of Small Animal Surgery. Slatter, D. (Ed.), Vol.1, 3rd ed. Saunders. pp. 53-63.

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

Schaffer, M.R., Tantry,U and Barbul, A. (2004). Wound fluid inhibits wound fibroblast nitric oxide synthesis. *J. Surg. Res.* **122**: 43-48.

Sehgal, P.K., Sastry, T.P., Kumar, M. (1986). Studies on solubilized keratins from poultry feathers. *Leather Science* **33 (12)**: 333 – 344.

Sumitra, M., Manikandan, P. and Suguna, L. (2005). Efficacy of Butea monosperma on dermal wound healing in rats. *Intern. J. Biochem & Cell Biol.* **37**: 566-573.

Swaim, S.F., Bradley, D.M., Spano, J.S., McGuire, J.A., Hoffman, C.E. and Trachy, R.E. (1993). Evaluation of multipeptide-copper complex medications on open wound healing in dogs. *J. Amer. Anim. Hosp. Asso.* **29:** 519 – 525.

Takehara, K.(2000). Growth regulation of skin fibroblasts. *J. Dermatol. Sci.* **24 (1)** : 70-77.

Tonnesen, M.G., Feng, X and Clark, R.A.F.(2000). Angiogenesis in wound healing. *J. Invest. Dermatol. Symposium Proceedings* **5**: 40- 46.

Werner, S., Breeden, M., Hubner, 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. *J. Invest. Dermatol.* **103**: 469 - 473.

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