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# Influence of *Aloe vera* on the glycosaminoglycans in the matrix of healing dermal wounds in rats

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**Abstract**

The influence of *Aloe vera* (L.) Burman f. on the glycosaminoglycan (GAG) components of the matrix in a healing wound was studied. Wound healing is a dynamic and complex sequence of events of which the major one is the synthesis of extracellular matrix components. The early stage of wound healing is characterized by the laying down of a provisional matrix, which is then followed by the formation of granulation tissue and synthesis of collagen and elastin. The provisional matrix or the ground substance consists of GAGs and proteoglycans (PGs), which are protein–GAG conjugates. In the present work, we have studied the influence of *Aloe vera* on the content of GAG and its types in the granulation tissue of healing wounds. We have also reported the levels of a few enzymes involved in matrix metabolism. The amount of ground substance synthesized was found to be higher in the treated wounds, and in particular, hyaluronic acid and dermatan sulphate levels were increased. The levels of the reported glycohydrolases were elevated on treatment with *Aloe vera*, indicating increased turnover of the matrix. Both topical and oral treatments with *Aloe vera* were found to have a positive influence on the synthesis of GAGs and thereby beneficially modulate wound healing. © 1998 Elsevier Science Ireland Ltd.

*Keywords:* Wound healing; *Aloe vera*; Glycosaminoglycans; Glycohydrolases

## 1. Introduction

Wound healing is a highly complex, but orchestrated cascade of events which can roughly be divided into three overlapping phases—inflammation, granulation tissue formation and remodelling of the extracellular matrix. These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. (Raghow, 1994). Immediately after injury, there is clot formation and the earlier phases of wound repair involves inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans (PGs), which are the heterogenous, non-fibrillar compo-

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nents of the extracellular matrix. These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccharides, the glycosaminoglycans (GAGs). PGs and GAGs have been shown to play important roles in all the above mentioned events of wound healing (Gallo and Bernfield, 1996). For example, they prevent blood coagulation within the vascular space (Kojima et al., 1992; Parkinson et al., 1992), regulate inflammatory cell function (Forrester and Lackie, 1981; Forrester and Wilkinson, 1981) and form the major components of the ground substance (Alison, 1992), on which collagen and elastin fibres are subsequently laid. In addition, GAGs have been shown to be regulators of cellular proliferation, migration and differentiation, and of growth factor activities (Gallo and Bernfield, 1996). The synthesis of these components and their degradation, therefore, are events of great relevance in the wound healing process.

*Aloe vera* (L.) Burman f. has been employed in a host of curative purposes including treatment of skin disorders, and healing of burns and wounds (Grindlay and Reynolds, 1986). The fresh gel, juice or formulated products have been used for medical and cosmetic purposes, as well as for general health. In spite of its wide use as a folk remedy, the biochemical basis of its action or its influence on the various phases of wound healing has not been studied in detail. In this work, we treated full thickness dermal wounds on rats with *Aloe vera* and examined its effects on the content and types of GAGs and the levels of a few glycohydrolases in the matrix of the healing wound.

## 2. Materials and methods

### 2.1. Experimental animals

Male Wistar rats weighing 150–200 g were used in the study. The animals were fed water and commercial feed ad libitum.

### 2.2. Wound creation and sampling

The animals were anaesthetised by an intraperitoneal injection of thiopentone sodium (3 mg/100 g body wt.). The right side of each rat was shaved and an excision wound of size 4 $cm^2$ was made by cutting out a 2 cm × 2 cm piece of skin from the shaven area. The wounds were of full-thickness type, extending down to the subcutaneous tissue. After wound creation, the animals were divided into three groups of six rats each: Group I, untreated controls; Group II, topically treated and Group III, orally treated rats. Animals were sacrificed on the 4th, 8th, 12th and 16th day after wound creation and the entire wound was cut out and stored at −70°C until analysis.

### 2.3. Preparation and administration of Aloe vera gel

Full size mature leaves were cut from the plant and the rind removed. The colourless parenchyma was ground in a blender and centrifuged at $10\,000 \times g$ to remove the fibres. The supernatant was lyophilized and stored at room temperature.

A small quantity of water (1.0 ml in the case of Group III, and a suitable volume to cover the entire area of the wound in the case of Group II) was added to each of 30-mg portions of the lyophilized *Aloe vera* powder, and the resultant gel was administered twice a day, either orally with an oral tube or applied topically on the wound surface. The wounds were left air-exposed.

### 2.4. Estimation of uronic acid

Uronic acid was first extracted from the granulation tissue as described by Schiller et al. (1961). Briefly, tissue was digested with papain (10 mg/g wet wt. of tissue) in 0.5 M acetate buffer, pH 5.5, containing 0.005 M cysteine and 0.005 M disodium salt of EDTA at 65°C for 24 h. An aliquot of this digest was used for the estimation of uronic acid by the spectrophotometric method of Bitter and Muir (1962).

### 2.5. Extraction of GAGs

Total GAGs from the granulation tissue were extracted as described by Smith et al. (1980). Briefly, tissue samples (15–30 mg) were suspended

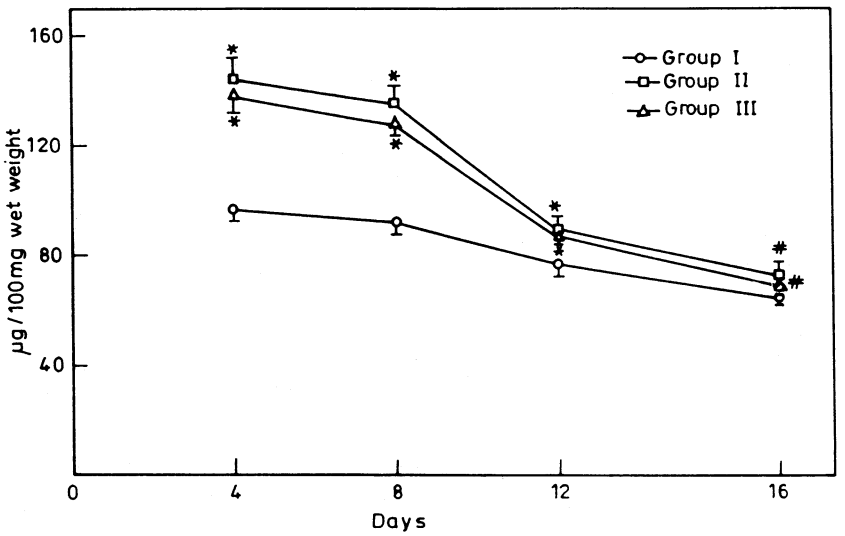


Fig. 1. Uronic acid content of *Aloe vera* treated and untreated control wound granulation tissue. Values are mean ± S.D. for six animals in each group. $^{\#}P < 0.05$; $^{*}P < 0.001$.

in 1% SDS and incubated in a boiling water bath for 10 min. After cooling to room temperature, proteinase K (0.5 mg/ml final concentration) digestion was carried out at 60°C for 18 h. The digested sample was precipitated with an equal volume of 10% TCA and the supernatant was subsequently extracted with cold chloroform/methanol (2:1 v/v). GAGs were precipitated from the extracted aqueous phase by addition of 4–5 volumes of 95% ethanol saturated with 5% potassium acetate. The precipitate was dissolved in water and the amount of GAGs was determined by the estimation of uronic acid.

### 2.6. Estimation of individual GAGs

Individual GAGs were estimated by using GAG degrading enzymes and nitrous acid treatment as described by Breen et al. (1981). The enriched GAG sample was first subjected to hyaluronidase digestion to remove hyaluronic acid. To the remaining fraction, chondroitinase AC and chondroitinase ABC were added to determine the amount of chondroitin sulfate A and C, and dermatan sulfate. Keratan sulfate was removed using endo-$\beta$-D-galactosidase. The remaining GAG was subjected to nitrous acid digestion to determine the amounts of heparin and heparan sulfate.

### 2.7. Enzyme assays

The granulation tissue was minced with scissors and homogenized in 0.25 M sucrose containing 0.2% Triton X-100 at 4°C. $\beta$-Glucuronidase and *N*-acetyl glucosaminidase activities were assayed by the methods described by Kawai and Anno (1971) and Moore and Morris (1982), respectively. $\beta$-Galactosidase and $\beta$-glucosidase were estimated by the method of Conchie et al. (1967).

### 2.8. Statistical analysis

Groups II and III were compared with Group I. Students *t*-test was used to identify differences between the groups and data were considered significant at $P < 0.05$.

## 3. Results

Fig. 1 shows the uronic acid content of the granulation tissues of *Aloe vera* treated and untreated control wounds. From the figure it can be seen that the uronic acid content of all the three groups was highest on the 4th day, after which there was a decline in these levels. Both the treated groups had significantly higher levels of uronic acid as compared to the controls on all days. On the 4th day, the topically treated group had about 49% ($P < 0.001$) and orally treated group about 43% ($P < 0.001$) more uronic acid than the untreated controls. However, the levels of uronic acid of the treated groups decreased at a faster rate after the 8th day, but remained still higher than the control values on day 16.

The relative amounts of the various GAGs present on the 4th day in the wound granulation tissues of *Aloe vera* treated and untreated control groups are shown in Fig. 2. Hyaluronic acid formed the major GAG of the wound in all the groups. The hyaluronic acid levels of treated groups were higher than the controls and this increase was about 11% and 20% in the case of topical and oral treatments, respectively. The dermatan sulfate contents of the treated groups were also found to be higher than that of the untreated controls.

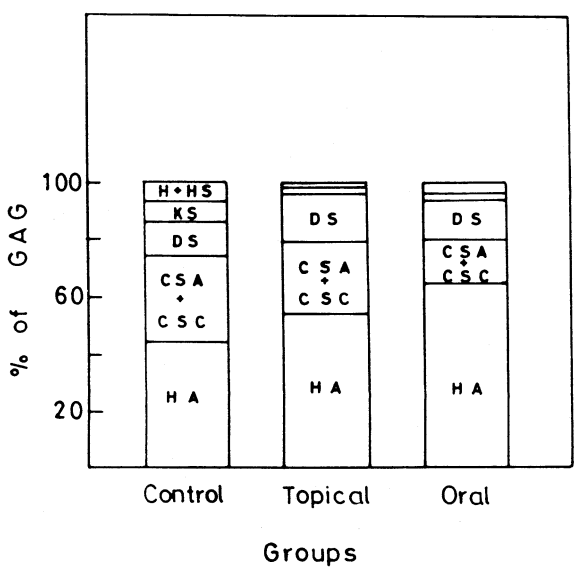


Fig. 2. Percentage of the different GAG fractions from *Aloe vera* treated and untreated control wounds. HA, hyaluronic acid; CSA, chondroitin sulfate A; CSC, chondroitin sulfate C; DS, dermatan sulfate; KS, keratan sulfate; HS, heparan sulfate; H, heparin.

Fig. 3 shows the level of $\beta$-glucuronidase and *N*-acetyl glucosaminidase present in the wound granulation tissues of all three groups. The highest level of these enzymes was seen on day 8. Treated groups showed an increase in the enzyme levels when compared to the untreated controls. Topically and orally treated wounds had about 19% and 15% higher levels of these enzymes, respectively. The levels of $\beta$-glucosidase and $\beta$-galactosidase found in the wound granulation tissue of all the groups are shown in Fig. 4. These enzymes followed a similar pattern to those of the above-mentioned enzymes, with a maximum level on day 8. There was a 20% and 15% increase in the enzyme levels of topically and orally treated rats, respectively, on day 8.

## 4. Discussion and conclusions

In the present work, we have studied the types and amounts of the various GAGs present in the granulation tissue of untreated control and *Aloe vera* treated rat wounds. Significant qualitative and quantitative changes in the GAG content were observed. An increase in the uronic acid content of *Aloe* treated wounds as compared to the controls, represents an enhanced synthesis of GAGs. We observed that the maximum amount of uronic acid was present in the 4th day granulation tissue which is similar to earlier observations that have shown the synthesis of GAG to be at its peak during the first week of wound healing. As the content of collagen in the wound starts increasing, the amount of uronic acid declines (Dunphy and Udupa, 1955). GAGs are important constituents of the extracellular matrix found on cell surfaces, in basement membranes and in association with interstitial collagen and elastin (d'Ardenne, 1992). They are also found free in the ground substance. The GAGs are the first components of the extracellular matrix to be synthesized during wound healing, and form the scaffold for collagen and elastin deposition.

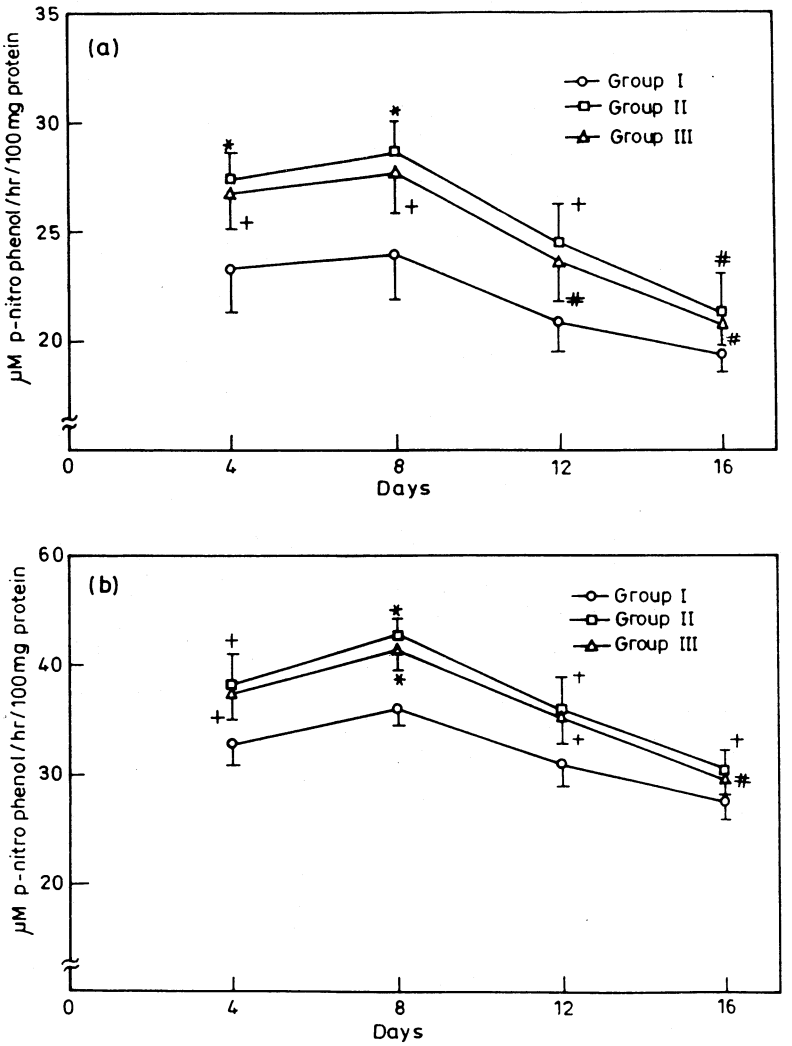


Fig. 3. Levels of $\beta$-glucuronidase (a) and *N*-acetyl glucosaminidase (b) activities in *Aloe vera* treated and untreated control wound granulation tissues. Values are expressed as mean $\pm$ S.D. for six animals in each group. $^{\#}P < 0.05$; $^{+}P < 0.01$; $^{*}P < 0.001$.

Determination of the amounts of the various individual GAGs from the three groups revealed a change in their relative amounts. It was observed that hyaluronic acid formed the major part of the total wound granulation tissue GAGs and that the levels of hyaluronic acid and dermatan sulfate of Groups II and III (treated) wounds were significantly higher than that of Group I (untreated control).

Hyaluronic acid has an important role to play in the early wound healing process and earlier reports have shown that there is an increase in the expression of hyaluronic acid in wound granulation tissue (Gallo and Bernfield, 1996). Changes in the levels of hyaluronate affect cellular proliferation and the orderly deposition of structural matrix (Toole, 1976). Hyaluronic acid may extend its regulatory effects by two possible mechanisms. It forms a pericellular coat attached to the surface of the cells (Underhill and Toole, 1979; Knudson and Toole, 1985). Such a coat would create an anionic boundary around the fibroblasts that

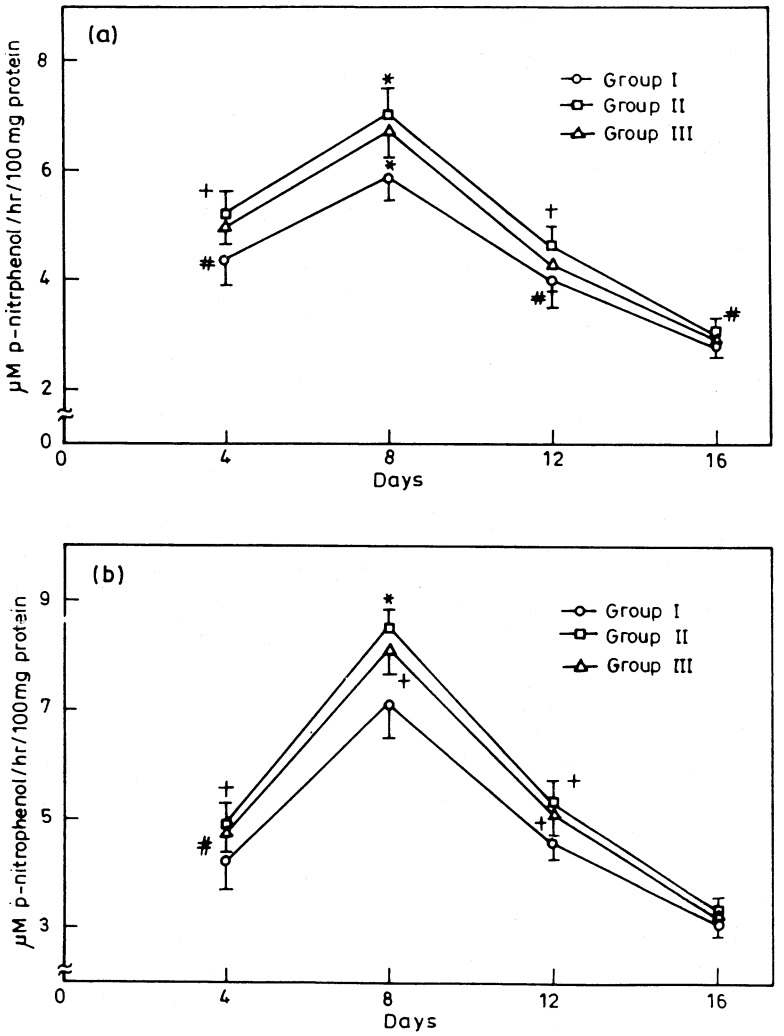


Fig. 4. Levels of $\beta$-glucosidase (a) and $\beta$-galactosidase (b) activities in *Aloe vera* treated and untreated control wound granulation tissues. Values are expressed as mean $\pm$ S.D. for six animals in each group. $^{\#}P < 0.05$; $^{+}P < 0.01$; $^{*}P < 0.001$.

could result in the attraction or localization of cationic growth factors and thus provide the fibroblasts with a greater accessibility to growth factors. A second mechanism may be the result of cell surface binding of hyaluronic acid to fibroblasts. This promotes protein tyrosine phosphorylation and phospholipid breakdown. Such second messenger generation may provide the intracellular signals required for cellular functions (Turley, 1989). Hyaluronic acid has also been shown to stimulate collagen synthesis in fetal fibroblast cultures (Mast et al., 1993). Fetal wounds which exhibit scarless wound healing have been found to contain 100% hyaluronic acid in the wound matrix (De Palma et al., 1989). Its interaction with keratinocytes also has an important role in the process of epithelialization (Okasala et al., 1995). Since hyaluronic acid may provide a more fluid and malleable matrix that facilitates greater cell mobility and more facile early remodeling, the increased amounts of hyaluronic acid in *Aloe* treated wounds may result in the formation of a more stable scar.

The increase in the relative amounts of dermatan sulphate in treated wounds also plays an important role in the faster healing of wounds by *Aloe vera*. Dermatan sulphate PGs are closely associated with collagen fibres (Fleischmajer et al., 1991). They have been shown to influence collagen fibril formation in vitro and may therefore contribute to the organisation and strength of the fibrillar network in vivo (Scott, 1988). The accumulation of dermatan sulphate in *Aloe* treated wounds might result in the improved formation of a fully resistant scar.

Glycohydrolases play an important role in the destruction of connective tissue matrix during the inflammatory process (Weissman, 1967). We have therefore determined the levels of a few glycohydrolases in the wound granulation tissue. These enzymes are mainly of lysosomal origin and may be endo- or exo-glycosidases which depolymerise and degrade GAGs. $\beta$-Glucuronidase and *N*-acetyl glucosaminidase act alternatively on the $\beta$-linkage of glucuronic acid and *N*-acetyl glucosamine in GAGs to release monosaccharides. $\beta$-Glucosidase and $\beta$-galactosidase hydrolyse the terminal non-reducing $\beta$-glucosyl residues of glycolipids and $\beta$-galactosyl residues of GAG, respectively. We find that on treatment of wounds with *Aloe vera*, there is a significant increase in the amounts of these glycohydrolases, which may result in increased turnover of the matrix. Increase in $\beta$-glucuronidase activity in wounds has been reported earlier (Levvy et al., 1948) and this increase reflects catabolic events especially in connection with degradation of PGs (Raekallio, 1970).

From the above, we find that *Aloe vera* has a significant influence on the PGs and GAGs in a healing wound. Treatment of frostbite and electrical injury with *Aloe* has been shown to result in reduced tissue loss and faster healing (Heggars et al., 1993; Miller and Koltai, 1995). There was also less morbidity in the case of frostbite wounds (Heggars et al., 1993). This wound healing property of *Aloe* may be attributed in part to the gel polysaccharides. Recent work has shown mannose-6-phosphate, a constituent of *Aloe* to have wound healing and inflammation reducing properties (Davis et al., 1994). Acemannan, another component of *Aloe*, has been used effectively in wound dressing gels (Roberts and Travis, 1995).

It may therefore be concluded from this work, that the wound healing activity of *Aloe vera* involves its influence on the GAG component of the wound matrix.

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## References

Alison, M.R., 1992. The response to injury: Repair and regenerative responses. In: McGee, J.O'D., Isaacson, P.G., Wright, N.A. (Eds.), Oxford Textbook of Pathology, Vol. 1. Principles of Pathology. Oxford University Press, London.

Bitter, T., Muir, H.M., 1962. A modified uronic acid carbazole reaction. Analytical Biochemistry 4, 330–334.

Breen, M., Knepper, P.A., Weinstein, H.G., Blacik, L.J., Lewandowski, D.G., Baltrus, B.M., 1981. Microanalysis of glycosaminoglycans. Analytical Biochemistry 113, 416–422.

Conchie, J., Gelman, A. L, Levvy, G.A., 1967. Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4 and C-6 specificity of $\beta$-glucosidase, $\beta$-galactosidase. Biochemical Journal 103, 609–615.

d'Ardenne, A.J., 1992. Ground substance. In: McGee, J.O'D., Isaacson, P.G., Wright, N.A. (Eds.), Oxford Textbook of Pathology, Vol. 1. Principles of Pathology. Oxford University Press, London.

Davis, R.H., Donato, J.J.D., Hartman, G.M., Haas, R.C., 1994. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. Journal of the American Podiatric Medical Association 84, 77–81.

De Palma, R.L., Krummel, T.M., Durham, L.A., Michna, B.A., Thomas, B.L., Nelson, J.M., Diegelmann, R.F., 1989. Characterization and quantitation of wound matrix in the fetal rabbit. Matrix 9, 224–231.

Dunphy, J.E., Udupa, K.N., 1955. Chemical and histochemical sequences in the normal healing of wounds. New England Journal of Medicine 253, 847–851.

Fleischmajer, R., Fischer, L.W., McDonald, E.D., Jacobs, L., Perlisch, J.S., Termine, J., 1991. Decorin interacts with fibrillar collagen of embryonic and adult human skin. Journal of Structural Biology 106, 82–90.

Forrester, J.V., Lackie, J.M., 1981. Effect of hyaluronic acid on neutrophil adhesion. Journal of Cell Science 50, 329–344.

Forrester, J.V., Wilkinson, P.C., 1981. Inhibition of leukocyte locomotion by hyaluronic acid. Journal of Cell Science 48, 315–331.

Gallo, R.L., Bernfield, M., 1996. Proteoglycans and their role in wound repair. In: Clark, R.A.F. (Ed.), The Molecular and Cellular Biology of Wound Repair, 2nd ed. Plenum, New York.

Grindlay, D., Reynolds, T., 1986. The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. Journal of Ethnopharmacology 16, 117–151.

Heggars, J.P., Pelley, R.P., Robson, M.C., 1993. Beneficial effects of *Aloe* in wound healing. Phytotherapy Research 7, S48–S52.

Kawai, Y., Anno, K., 1971. Mucopolysaccharide degrading enzymes from the liver of squid *Ommastre phesloni* I. Hyaluronidase. Biochimica et Biophysica Acta 242, 428–436.

Kojima, T., Shworak, N.W., Rosenberg, R.D., 1992. Molecular cloning and expression of two distinct cDNA-encoding heparan sulfate proteoglycan core proteins from a rat endothelial cell line. Journal of Biological Chemistry 267, 4870–4877.

Knudson, C.B., Toole, B.P., 1985. Changes in the pericellular matrix during differentiation of limb bud mesoderm. Developmental Biology 112, 308–318.

Levvy, G.A., Kerr, L.M.H., Campbell, J.G., 1948. Beta glucuronidase and cell proliferation. Biochemical Journal 42, 462–468.

Mast, B.A., Diegelmann, R.F., Krummel, T.M., Cohen, I.K., 1993. Hyaluronic acid modulates proliferation, collagen and protein synthesis in cultured fetal fibroblasts. Matrix 13, 441–446.

Miller, M.B., Koltai, P.J., 1995. Treatment of experimental frostbite with pentoxifylline and *Aloe vera* cream. Archives of Otolaryngology, Head and Neck Surgery 121, 678–680.

Moore, J.C., Morris, J.E., 1982. A simple automated colorimetric method for determination of *N*-acetyl-$\beta$-D-glucosaminidase. Annals of Clinical Biochemistry 19, 157–159.

Okasala, O., Salo, T., Tammi, R., Hakkinen, L., Jalkanen, M., Inki, P., Larjava, H., 1995. Expression of proteoglycans and hyaluronan during wound healing. Journal of Histochemistry and Cytochemistry 43, 125–135.

Parkinson, J.F., Koyama, T., Bang, N.Y., Preissner, K.T., 1992. Thrombomoduline: An anticoagulant cell surface proteoglycan with physiologically relevant glycosaminoglycan moiety. Advances in Experimental Medicine and Biology 313, 177–188.

Raekallio, J., 1970. Enzyme histochemistry of wound healing. Progress in Histochemistry and Cytochemistry 1, 51–52.

Raghow, R., 1994. The role of extracellular matrix in postinflammatory wound healing and fibrosis. FASEB Journal 8, 823–831.

Roberts, D.B., Travis, E.L., 1995. Acemannan-containing wound dressing gel reduces radiation-induced skin reactions in C3H mice. International Journal of Radiation Oncology Biology Physics 32, 1047–1052.

Schiller, S., Slover, G., Dorfman, A.A., 1961. A method for the separation of acid mucopolysaccharides: Its application to the isolation of heparin from the skin of rats. Journal of Biological Chemistry 236, 983–987.

Scott, J.E., 1988. Proteoglycan fibrillar collagen interactions. Biochemical Journal 252, 313–323.

Smith, R.L., Gilkerson, E., Kohatsu, N., Merchant, T., Schurman, D.J., 1980. Quantitative microanalysis of synovial fluid and articular cartilage glycosaminoglycans. Analytical Biochemistry 103, 191–200.

Toole, B.P., 1976. Morphogenetic role of glycosaminoglycans (acid mucopolysaccharides) in brain and other tissues. In: Barondes, S.H. (Ed.), Neuronal Recognition. Plenum, New York, pp. 275–329.

Turley, E.A., 1989. The role of cell-associated hyaluronan-binding protein in fibroblast behaviour. Ciba Foundation Symposium 143, 121–133.

Underhill, C.B., Toole, B.P., 1979. Binding of hyaluronate to the surface of cultured cells. Cell Biology 82, 475–484.

Weissman, G., 1967. The role of lysosomes in inflammation and disease. Annual Review of Medicine 18, 97–112.