Immunohistochemistry of EGF Receptor and Fibronectin in Wounds Healing Treated with Chitosan and Taurine-Chitosan

Kitosan ve Taurin-Kitosan Uygulamasında Yara İyileşmesinde EGF Reseptör ve Fibronektin'in İmmünohistokimyasal Olarak Gösterilmesi

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SUMMARY

Objective: The purpose of this study is to observe the pattern and distribution of epidermal growth factor receptor (EGF-r) and fibronectin in mice skin wounds treated with chitosan and taurine-chitosan gel.
Methods: The mice were incised on their dorsums and divided into two groups: Group 1: Chitosan gel applied group, Group 2: Taurine-Chitosan gel applied group. Gels were locally applied on the animals. The sections were excised at the 6th hour, and on the 1st, 3rd, 5th and 7th days following incision. They were then stained for EGF-r and fibronectin with immunohistochemical methods.
Results: Immunostaining revealed a positive area of EGF-r staining persisting up to the 3rd day especially in group 2. The positive area was located between the injured tissue, wound exudates, fibroblasts, smooth muscle cells and the vascular cells. During the early phase of healing in the dermis, fibronectin was detected earlier in the basement membrane and collagen bundles especially in group 2. On the 5th and 7th days, the fibronectin staining decreased markedly, but strong reactivity persisted in the dermal-epidermal junctions.
Conclusion: Reepithelization and repair of wounds in the dermis were found to be more rapid in the group treated with taurine-chitosan gel than the group treated with chitosan gel. *(Gazi Med J 2011; 22: 6-13)*

Key Words: EGF-r, fibronectin, chitosan, taurine, wound healing

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ÖZET

Amaç: Çalışmanın amacı kitosan ve taurin-kitosan jel uygulanan fare deri yarasında epidermal büyüme faktörü reseptörü (EGF-r) ve fibronektinin dağılımlarını gözlemektir.
Yöntemler: Dorsal bölgeden kesi yarası oluşturulan farelerden iki grup oluşturuldu; 1. grup: Kitosan uygulanan grup, 2. grup: Taurin-Kitosan uygulanan grup. Etken maddeler deneklere lokal olarak uygulandı. Yara oluşumunu izleyen 6. saat, 1., 3., 5. ve 7. günlerde doku örnekleri alınarak EGF-r ve fibronektin ile immünohistokimyasal olarak boyandı.
Bulgular: Boyama sonucunda taurin-kitosan uygulanan gruplarda daha belirgin olmak üzere, 3. güne değin yaygın EGF-r tutulumu saptandı. Pozitif tutulum yara dokusu, yara dudakları, fibroblast, düz kas hücreleri ve damar hücrelerinde belirgindi. Fibronektin ise yine 2. grupta daha belirgin olmak üzere iyileşmenin erken evrelerinde dermis bazal membranlarında ve kollagen liflerinde izlendi. 5. ve 7. günlerde fibronektin tutulumunun belirgin olarak azaldığı ancak dermis-epidermis bağlantı bölgesinde güçlü reaktivitenin sürdüğü gözlendi.
Sonuç: Yara bölgesinde reepitelizasyon ve dermal onarımın taurin-kitosan jel uygulanan grupta, kitosan uygulanan gruba karşın daha hızlı olduğu belirlenmiştir. *(Gazi Med J 2011; 22: 6-13)*

Anahtar Sözcükler: EGF-r, fibronektin, kitosan, taurin, yara iyileşmesi

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INTRODUCTION

EGF is a polypeptide that stimulates epithelial cells and mesenchymal cells to migrate and proliferate (1, 2). EGF regulates cellular processes by binding the EGF receptors on the cell surface (3, 4). EGF, platelet-derived growth factor (PDGF), transforming growth factor alpha (TGFα) and transforming growth factor beta (TGFβ) are known to play a central role in different phases of wound healing. EGF is expressed and distributed in the early skin wounds (5). Experiments on cultured cells have shown that binding to the EGF-r tyrosine kinase triggers cellular growth (6).

Fibronectin is a ubiquitous glycoprotein of the extracellular matrix that plays an important role as a mediator of cell-cell and cell-matrix adhesion. Integrin regulates a wide variety of cellular processes essential to epithelial repair, including spreading, migration, proliferation and survival (7-9). Fibronectin is a potent chemo-attractant, mainly involved in tissue repair processes such as wound healing. This glycoprotein has a vital function in the initial phase of wound healing. Its expression is highly up-regulated after wounding (10).

Chitosan (1-4)-2-amino-2-deoxy-β-D-glucan] is an N-acetylated form of chitin, which is in various stages of deacytylation and depolymerization (11, 12). Being a non-toxic, biocompatible and biodegradable polymer, chitosan has been widely used for pharmaceutical and medical applications (12, 13). Positive chitosan is made from its polyelectrolyte complexes with various glycosaminoglycans such as chondroitin sulphates and hyaluronic acid (14). It can be used safely in biological materials (13, 15).

Taurine is a type of sulphur-containing amino acid that enhances cell proliferation, viability, inflammation and collagenogenesis (16, 17). Taurine, which has antioxidant effects, is known to prevent lipid peroxidation and neutrophil activation (18, 19). Taurine has been shown to prevent oxidative damage and free radical formation in several tissues (20-22).

The purpose of this study is to determine EGF-r and fibronectin immunohistochemically after treatment with chitosan and chitosan taurin gel during wound healing. Tissue samples are evaluated with the light microscope.

MATERIALS AND METHODS

This study was performed on 60 male Swiss-Albino mice weighing 32±1.0g. Each mouse received water and food ad libitum before the operation. The animals were anesthetized and their dorsal surfaces were shaved and cleaned with tincture of iodine. Only one standard 3 cm-long wound was made through the whole thickness of skin on the dorsum of each mouse. The wound was sutured by 3 silk stitches (5-0). After surgery, each animal was placed in an individual cage (16, 23).

The mice were divided into two groups; each group being further divided into five subgroups: 1st Group: Chitosan gel was applied (30 μl to each wound every day at10 a.m.). 2nd Group: Taurine-chitosan was applied (30μl to each wound every day at 10 a.m.)

Preparation of Gels

Preparation of chitosan gels

Preparation of the gel dosage form of chitosan (1.5%) was carried out as follows: A weighed amount of polymer was carefully added to the required amount of 1% acetic acid solution. It was gently stirred for 1 minute and then kept at room temperature overnight before the application. The pH of the gel was 4.5 (23).

Preparation of taurine-chitosan gel: 50 mM Taurine gel dosage form was prepared in a chitosan gel (1.5%). The preparation of the gel dosage form of chitosan (1.5%) was carried out as follows: A weighed amount of polymer was carefully added to the required amount of 1% acetic acid. It was gently stirred for 1 minute and then kept at room temperature overnight before the application. The pH of the gel was 4.5. Taurine gel dosage form was prepared by adding taurine (50 mM) to the gel form. These dosage forms were divided into small fractions and stored at 4°C before daily applications (23).

Immunohistochemical Procedure

At the 6th hour, and on the 1st, 3rd, 5th and 7th days following incision, wound tissues from each group were extracted (5, 24, 25). Biopsy samples from each skin wound were fixed in neutral formalin and processed for paraffin embedding. Sections of 5 μm thickness were processed for polylysine-coated microscope slides.

All subgroups were divided into two groups: The first and second groups were processed immunohistochemically for EGF-r and fibronectin, respectively.

Oncogene Research Product (Lot: D06525) and Zymed (Lot: 81144808) kits were used for EGF-r and fibronectin staining, respectively.

EGF-r Staining: Sections were deparaffinized by xylene rehydrated through a graded series of ethanol. To remove endogenous peroxidase activity, the slides were incubated for 10 min in a 3% H_2O_2 and then washed with water and phosphate buffer saline (PBS). A specific binding of antibodies was avoided by 30 min preincubation with normal goat serum (Lot: D6172, Oncogene Research). The sections were incubated overnight at +4°C with the primary antibody (Polyclonal anti-rabbit Ig, Lot: D0357-1, Oncogene Research) and washed three times with PBS. Subsequently, the appropriate biotinated secondary antibody (Anti-rabbit total Ig, Lot: D06173, Oncogene Research) was applied for 30 min. Then, enzyme (Lot: D06170, Oncogene Research) was applied for 30 min. The color reaction was performed in a tablet DAB (Lot: D03981, Oncogene Research) 0.1% H_2O_2, 20 μl PBS, pH 7.4. Finally the sections were counterstained with Mayer's hematoxylene.

Fibronectin Staining: Sections were deparaffinized through xylene rehydrated through a graded series of ethanol. To remove endogenous peroxidase activity, the slides were incubated for 10 min in a 10% H_2O_2 and then washed with water and tris buffer saline (TBS). A specific binding of antibodies was avoided by 30 min preincubation with normal goat serum (Lot: 81144808, Zymed). The sections were incubated at 45 min at +4°C with the primary antibody (Polyclonal anti-rabbit IgG, Lot: 81144808, Zymed) and washed with TBS. Subsequently the appropriate biotinated secondary antibody (Anti-rabbit total Ig, Lot: 81144808, Zymed) was applied for 20 min. This was performed with the enzyme complex (Lot: 81144808, Zymed) for 10 min. The color reaction was performed for 3 min in a tablet DAB (Lot: 81144808, Zymed), 0.1% H_2O_2, 20 μl PBS, pH 7.6. Finally the sections were counterstained with Mayer's hematoxylene.

The sections were examined photo-light microscope (Olympus, BH2, Japan).

RESULTS

EGF-r

The wounds treated with chitosan gel (Figure 1A) and taurine-chitosan gel (Figure 1B) revealed a positive area of EGF-r staining 6 hours after incision. In the chitosan gel applied group, basal cells

of epidermis and wound exudates had a strong immunoreactivity. However, in the taurine-chitosan gel group, weak to moderate immunoreactivity was seen in the basal and intermediate layers, strong staining was observed in the upper layer and keratinized region. In the dermis, the fibroblasts, vascular endothelial and smooth muscle cells were moderately stained by EGF-r.

Epithelial margins surrounding the partial and full thickness incision wounds displayed strong immunoreactivity on the 1st postoperative day. EGF-r was uniformly present in epithelial cells. However, generally no significant differences between the distribution and pattern of positive staining were observed between the chitosan group and the taurine-chitosan group (Figure 2 A, B).

In both groups, the tissue distribution of EGF-r was similar, but stronger staining was observed in the taurine-chitosan group than the chitosan group. This difference was revealed on the 3rd day (Figure 3 A, B).

On the 5th postoperative day, in the chitosan group, EGF-r staining appeared to be weak in the epidermis, and moderate to strong in the dermis. In the taurine-chitosan group staining remained intensely positive in the dermis and weak to moderate in keratinocytes within the epidermis (Figure 4 A, B).

On the 7th postsurgical day, as the process of reepithelization continued during the late postsurgical period, the majority of marginal and non-marginal epithelia exhibited moderately positive staining for EGF-r in the chitosan group. EGF-r remained diminished in the taurine-chitosan group with weak to moderate staining in the epidermis. The wound displayed a thicker dermal layer with a more randomized collagen bundle organization. In the chitosan group

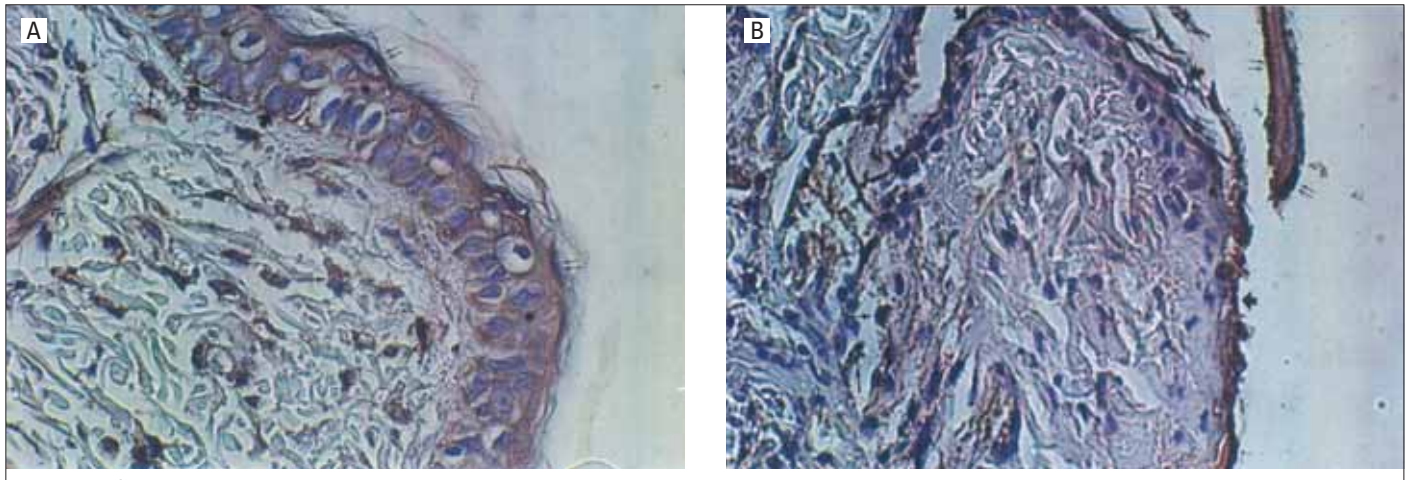

Figure 1. A) The wound formed after 6 hours incision chitosan gel treated group, EGF-r immunoreactivity observed surround the wound area. The membranous immunoreactivity (⇒) was observed strongly at the base of basal cells and moderate cytoplasmic immunoreactivity (*) detected in all cells. It was remarkable strongly reactivity in keratinization area (↑↑). At the dermis some fibroblasts show strongly immunoreactivity but some of them have weak immunoreactivity (↑). And also smooth muscle cells show weak immunoreactivity (>>)
B) The wound formed after 6 hours incision taurin-chitosan gel treated group, at the surrounding epithelial tissue the basal and lower layer showed strongly and moderate EGF-r immunoreactivity (*). The remarkable immunoreactivity was observed at the surface and keratinization area (⇒). The endothelium of dermis blood vessels shows variable EGF-r immunoreactivity (↑)

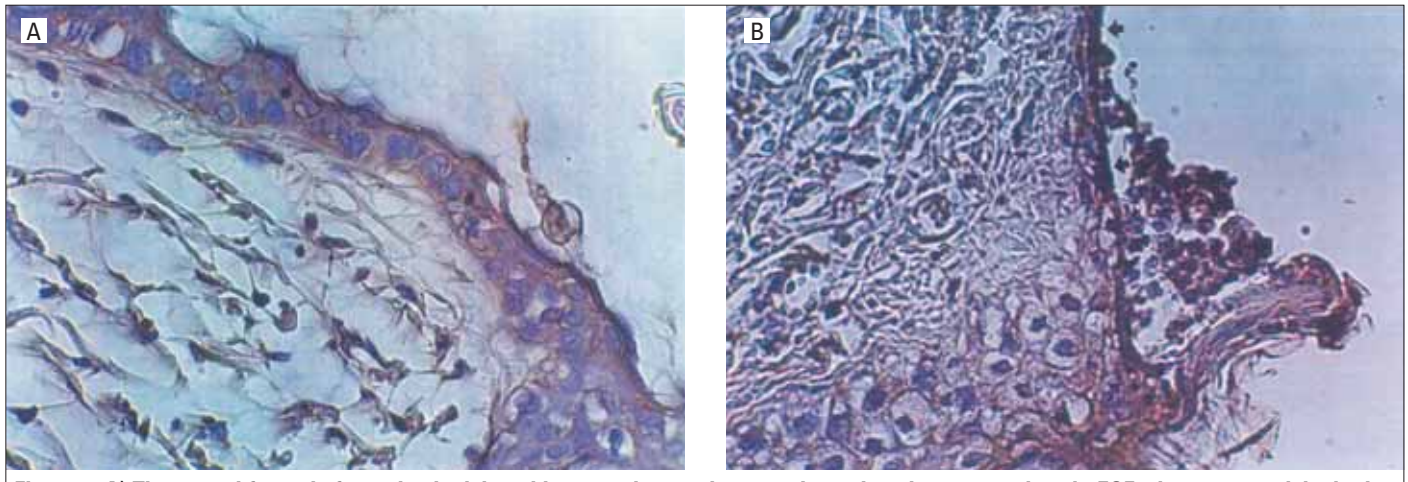

Figure 2. A) The wound formed after 1 day incision chitosan gel treated group, showed moderate cytoplasmic EGF-r immunoreactivity in the epithelium surround the wound area (*). The fibroblast in dermis showed variable immunoreactivity (↑).
B) The wound formed after 1 day incision taurin-chitosan gel treated group, showed EGF-r immunoreactivity at the epithelization area (⇒). In the adjacent epithelial tissue strongly observed membranous immunoreactivity (↑↑) from the basal to apical area of the cells, and variable cytoplasmic immunoreactivity (*) at the apical region. In dermis collagen fibers were irregular, abundant of blood vessels (↑), and moderate immunoreactivity observed in the endothelium of blood vessels (>>)

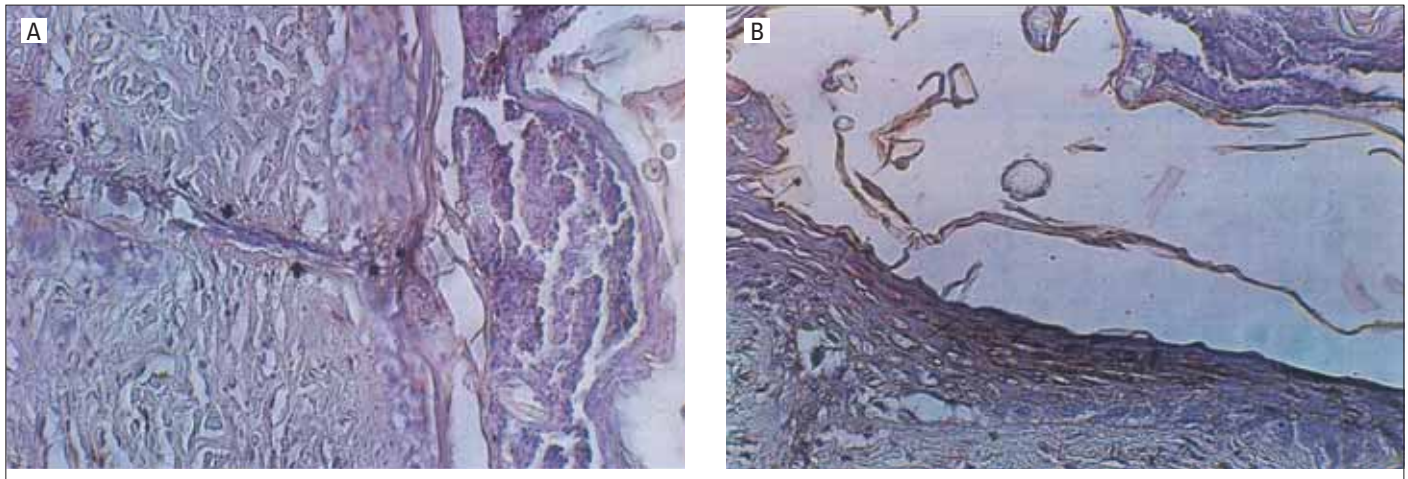

Figure 3. A) The wound formed after 3 day incision chitosan gel treated group, wound lips merged (⇒), in compare with surrounding epithelial tissue EGF-r immunoreactivity was strong in this epithelization area (*). The irregular collagen fibers and normal structured vascularity observed in the incision area of the dermis (↑↑). Weak immunoreactivity observed in endothelium of blood vessels (↑)
B) The wound formed after 3 day incision taurin-chitosan gel treated group, when compared with chitosan treated grup, epithelization formed and strongly cytoplasmic EGF-r immunoreactivity commonly observe towards to the apical area of the cells (↑). Generally cytoplasmic immunoreactivity observed to the apical side (*). The collagen fibers distributed irregularly (>>) and moderate to strong immunoreactivity of fibroblast observed in dermis (↑↑)

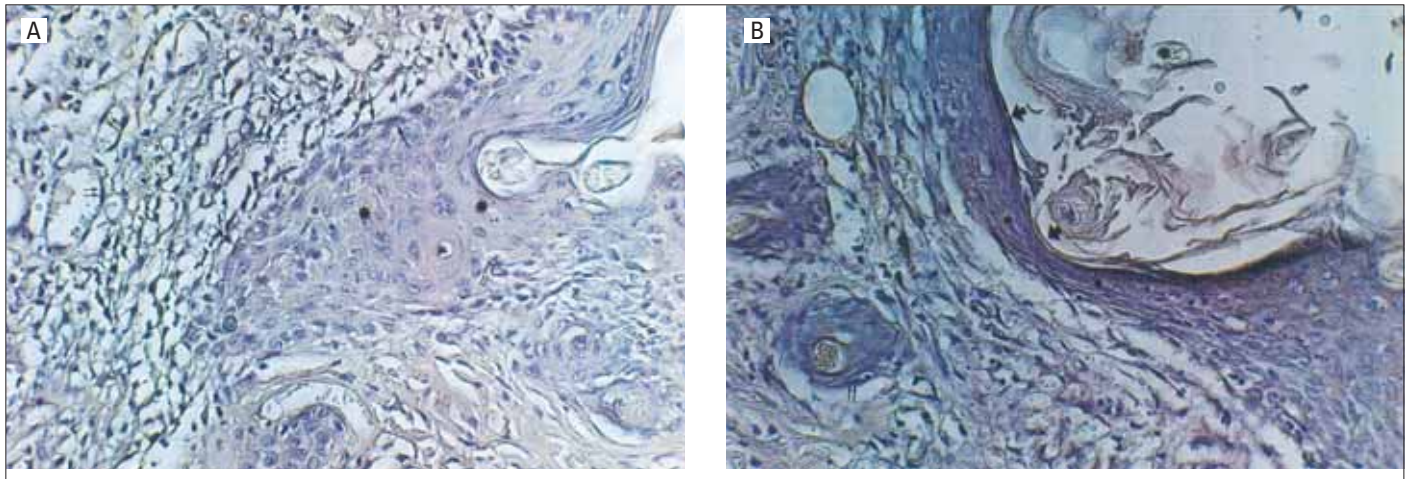

Figure 4. A) The wound formed after 5 day incision chitosan gel treated group the epithelium regeneration and moderate to weak EGF-r immunoreactivity was detected (*), but somewhat moderate immunoreactivity observed in the basal cells (↑). The moderate immunoreactivity observed in endothelium of blood vessels in the dermis (↑↑)
B) The wound formed after 5 day incision taurin-chitosan gel treated group epithelization completed and the weak EGF-r immunoreactivity observed from basal to the apical area of the cell (*). But ceratin shows moderate immunoreactivity (⇒). The cells at the base of the hear follicule have a little bit stronger EGF-r immunoreactivity (↑↑)

moderate amount of immunostaining for EGF-r was detected in the fibroblasts and vascular endothelial cells on day 7. However, in the taurine-chitosan group stronger immunoreactivitiy was observed than in the other group on same day (Figure 5 A, B).

Fibronectin

Within 6 hours after incision, both the chitosan and taurine-chitosan groups highlighted a strong immunoreactivity for fibronectin in the dermis, basement membranes of epithelial layer and skin appendages. The organization of collagen bundle was random and stained strongly (Figure 6 A, B).

Fibronectin was easily detected throughout the wounded area on day 1. Fibronectin was expressed in a somewhat diffuse pattern at the dermal-epidermal junction in each group. In the taurine-chitosan group especially, very strong fibronectin immunostaining was observed (Figure 7 A, B).

Moderate to strong fibronectin immunoreactivity was seen in the dermis as well as underneath the newly formed epidermis in the chitosan group by day 3. Fibronectin in the form of strongly reacting strings with an initial formation of network-like structures was detected in the taurine-chitosan group (Figure 8 A, B).

By the 5th day after incision, the expression of interstitial fibronectin markedly decreased, moderate staining in the epidermis and strong staining of the dermal-epidermal junction persisted in the chitosan group. Myofibroblasts defined intense immunoreactivity in the dermis. Unlike the chitosan group, weak fibronectin reactiv-

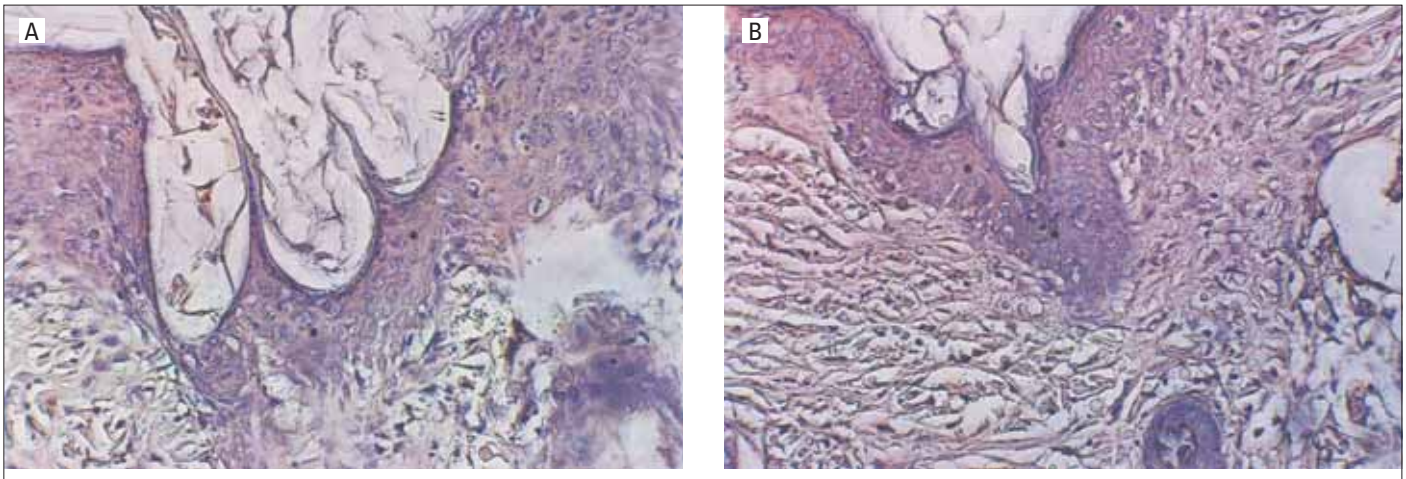

Figure 5. A) The wound formed after 7 day incision chitosan gel treated group, the common moderate EGF-r immunoreactivity observed in the epithelium (*). Strong membranous immunoreactivity detected in the apical cells (↑↑)
B) The wound formed after 7 day incision taurin-chitosan gel treated group, all structures became normal and moderate EGF-r immunoreactivity observed in the epithelium (*), also strong immunoreactivity detected in fibroblast (↑↑) and blood vessels (↑) of dermis

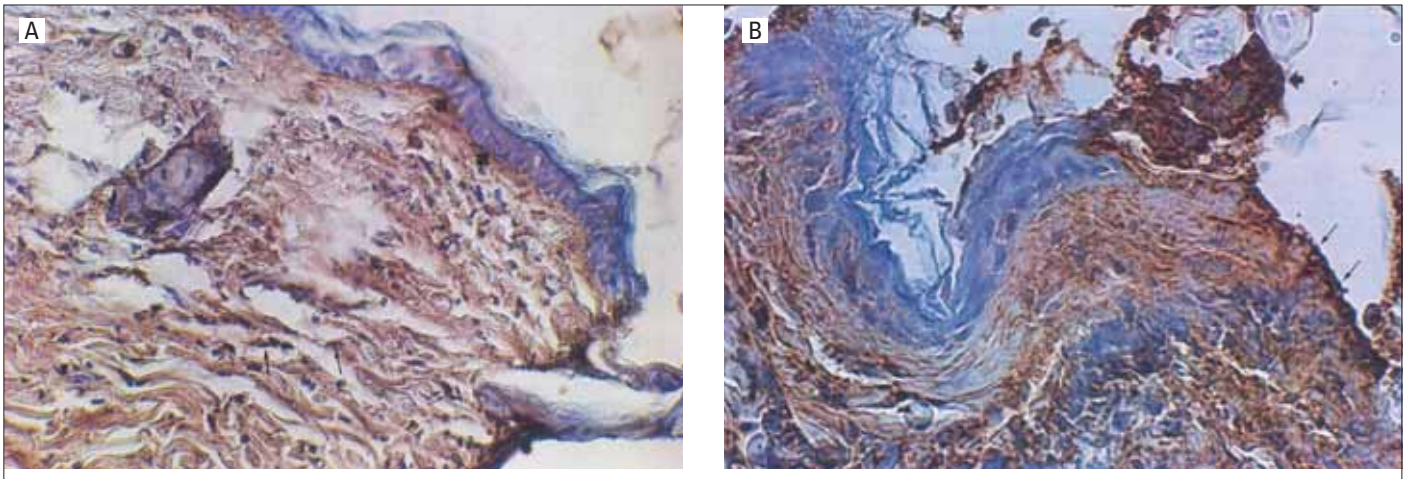

Figure 6. A) The wound formed after 6 hours incision chitosan gel treated group, basal membrane (⇒) of epithelium tissue surrounding wound in this region where epithelization didn't started (*), and strong fibronectin immunoreactivity was remarkable. The irregular collagen fibers and strong immunoreactivity was observed (>>). The fibroblast immunoreactivity (↑) and also in this fibroblast like cells detected in the wound area (↑↑)
B) The wound formed after 6 hours incision taurin-chitosan gel treated group, strong fibronectin immunoreactivity (⇒) observed in fibrin plug. In the wound zone where epithelization has not started (↑) and in the collagen fibers of wound zone (↑↑), the strong fibronectin immunoreactivity was detected

ity was seen in the taurine-chitosan group. However, organization of the collagen bundles displayed a more regular structure and stained more strongly than the other group (Figure 9 A, B).

After day 7, the area of incision was prominent in the group treated with chitosan gel. Fibronectin staining of randomized collagen bundles was neither strong nor weak. In the taurine-chitosan group, fibronectin staining returned to baseline patterns, but still presented very weak immunoreactivity of the basement membrane and collagen fibers (Figure 10 A, B).

DISCUSSION

The quantity and pattern of EGF, PDGF, basic fibroblast growth factor (bFGF) and TGFβ expression in the early skin wound healing appears to follow the acute inflammatory process. The early source of these growth factors appears to be the cells from the peripheral circulation (5). Nevertheless, EGF mRNA and EGF-r mRNA also increase in the epithelium following a wound (25). The major effect of EGF seems to show itself on growth and differentiation of normal epithelium (26) and plays an active role in epidermal wound repair (27). The stimulating migration of EGF is more important than the stimulating cell proliferation in wound healing (28, 29). EGF-r is available on the extracellular surface of epithelial cells. Binding activity of EGF is increased in the basal keratinocytes, the healing wound, the hypertrophic epithelium and in the dermal appendages such as the hair follicles, sweat glands and sebaceous glands (1).

Parallel to the literature, we showed that the strength of the EGF-r immunoreactivity is parallel to reepithelization in wound healing. The staining of EGF-r is intense in the early wounds. On postoperative days 5 and 7, EGF-r showed weak staining in the dermis and epidermis.

Fibronectin is important in the process of epidermal keratinocyte migration (30). Fibronectin activity is located primarily in the graft-

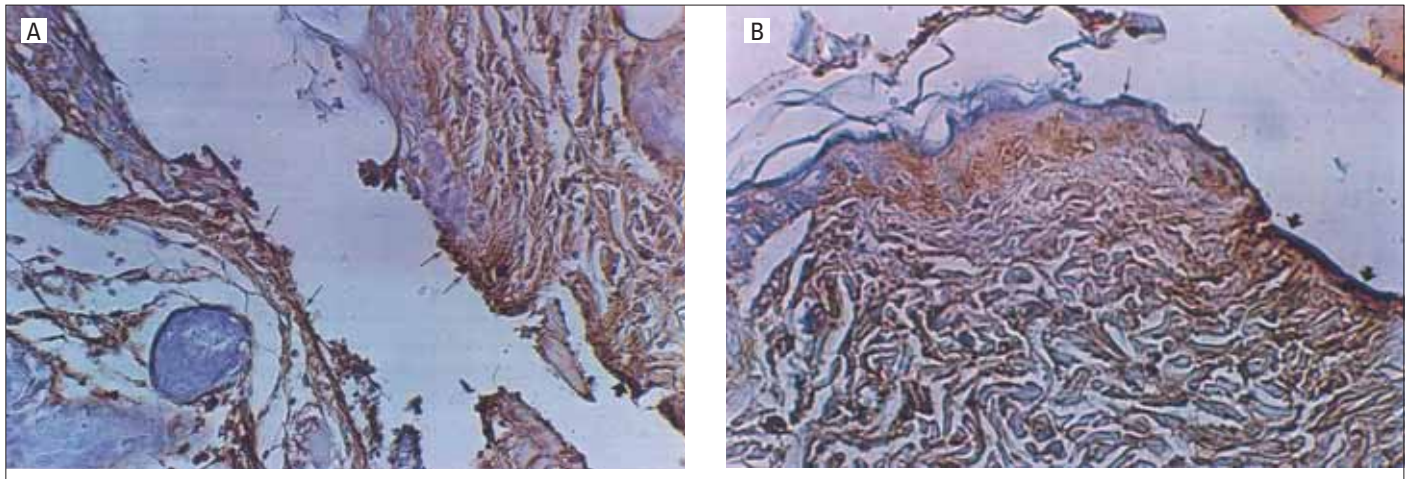

Figure 7. A) The wound formed after 1 day incision chitosan gel treated group, at the wound lip area regeneration didn't occur (↑↑). But in this area increase of collagen fibers with strong fibronectin immunoreactivity was detected (⇒)
B) The wound formed after 1 day incision taurin-chitosan gel treated group, simple epithelium started to form (↑), strong fibronectin immunoreactivity remarkable at dermis collagen fibers (↑↑) and the wound zone where epithelium started to form (⇒)

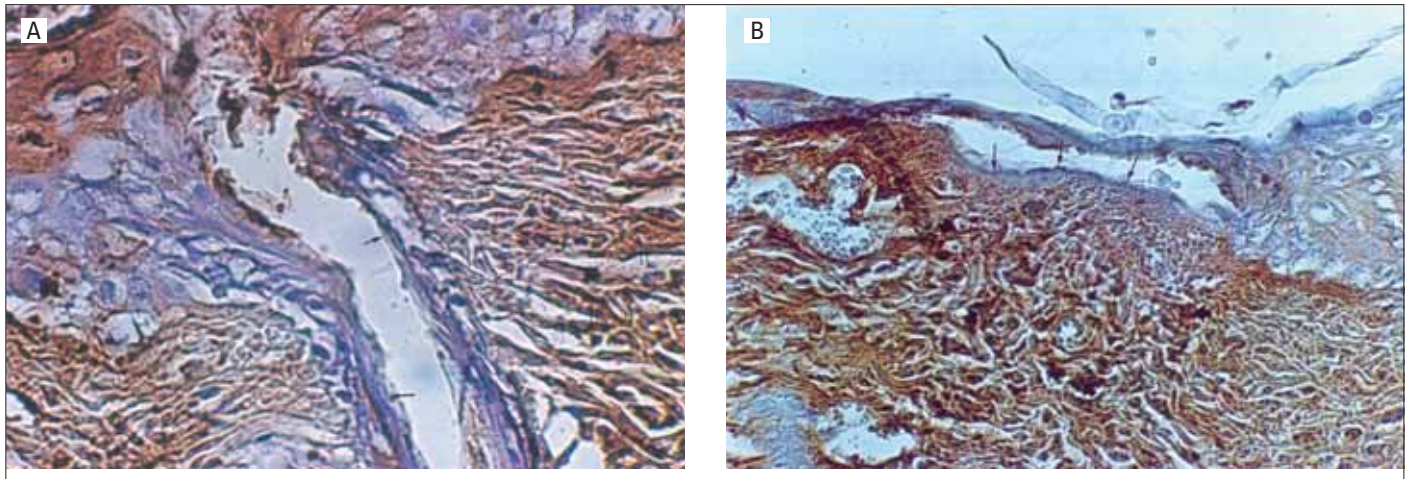

Figure 8. A) The wound formed after 3 day incision chitosan gel treated group, there is a remarkable epithelization zone (↑), the strong fibronectin immunoreactivity in the basal membrane (⇒) and the collagen fibers of dermis (↑↑) was observed
B) The wound formed after 3 day incision taurin-chitosan gel treated group, the properly epithelium structure was remarked (↑). And also the newly formed collagen fiber showed strong fibronectin immunoreactivity in the dermis (⇒)

wound bed interface and secondarily along the wound margins. There is a relationship between fibronectin and the appearance of myofibroblasts. Furthermore, myofibroblasts themselves may produce fibronectin (31). Fibronectin induces faster maturation of the granulation tissue and dermal regeneration in wound healing (10). Fibronectin shows the only known reliable histological parameter indicating the vitality of skin wounds after short survival times (32). The immunohistochemical identification of fibronectin is useful for classifying skin lesions (7).

The immunostaining of fibronectin is observed in the basement membrane of the epidermis and collagen bundles in the dermis. The staining of fibronectin is strongest at the 6th hour and on days 1 and 3. On postoperative day 5, a weak diffuse staining for fibronectin is observed in the dermis and a strong reactivity is found near the basement membrane of the epidermis. A weak selective reactivity is seen in the basement membrane of the epidermis and the collagen fibers in the dermis.

Reepithelization tends to be greater in chitin and chitosan (33). The heparin in combination with chitosan stimulates reepithelization in an *in vitro* model of human wound healing (34). A pure chitosan material gives the best results in connection with cell-attachment and cell-proliferation in wound healing (14).

The wounds treated with chitosan gel are observed to have intense immunoreactivity for EGF-r staining in early wounds and show moderate immunoreactivity for EGF-r staining on days 5 and 7. The immunostaining techniques reveal positive areas of fibronectin staining in early wounds. The positive areas are basement membrane of the epidermis and collagen bundle and fibroblast in the dermis. Fibronectin staining decreases depending on healing.

Taurine prevents lipids peroxidation, probably via a calcium dependent mechanism (20, 35). The use of taurine in healing is effective in decreasing the oxidative stress of tissue during wound healing (21). The administration of taurine reduces the neutrophil activation (22). Mechanisms of the antioxidant action of taurine are attributed to the prevention of lactate accumulation in tissues in cell membrane structure disorders (19). Taurine significantly increases wound tensile strength by decreasing the malondialdehyde and

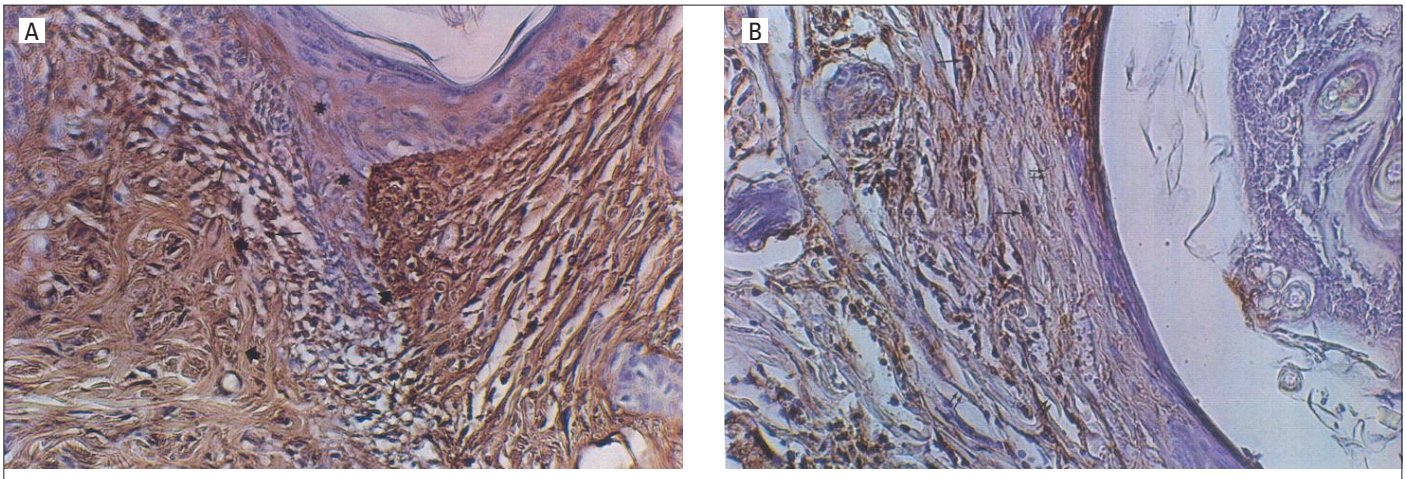

Figure 9. A) The wound formed after 5 day incision chitosan gel treated group, completed epithelization (*) clear wound zone and immunoreactivity of irregular collagen fibers was observed (⇒). At the wound zone group of fibroblast like cells, probably myofibroblast, showed strong fibronectin immunoreactivity (↑)
B) The wound formed after 5 day incision taurin-chitosan gel treated group repaired epithelium and dermis was observed. Fibronectin immunoreactivity detected in collagen fibers (↑↑) and myofibroblast (↑) at the dermis

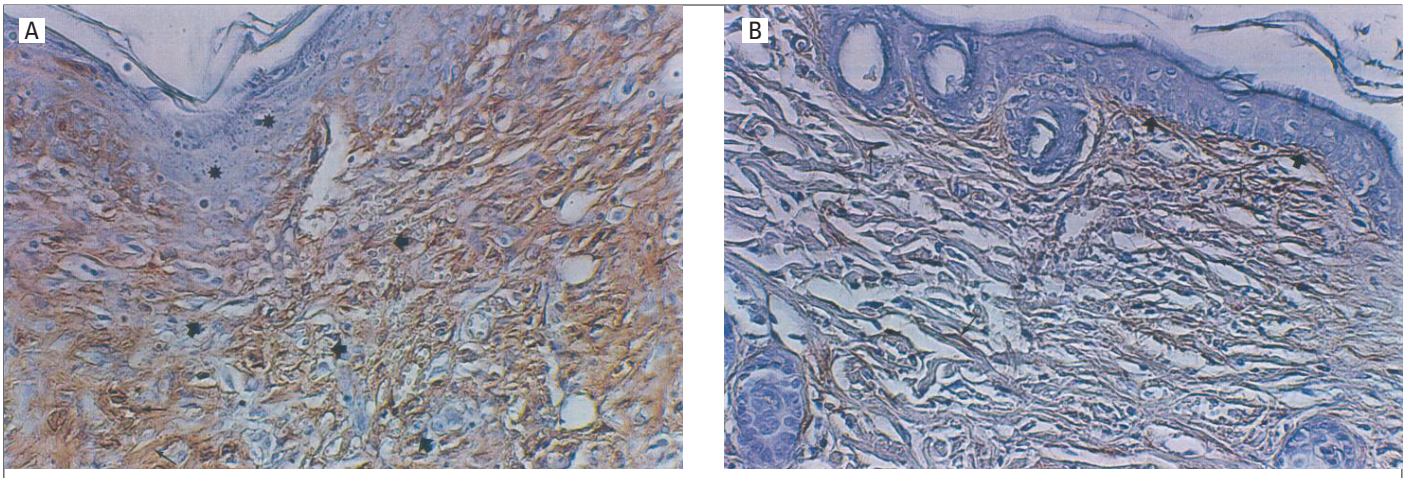

Figure 10. A) The wound formed after 7 day incision chitosan gel treated group, epithelium of the skin became normal (*), but there is also distinctive wound zone in the dermis (⇒). Collagen fibers were irregular and fibronectin immunoreactivity was detected in this newly formed fibers (↑)
B) The wound formed after 7 day incision taurin-chitosan gel treated group, all structures became normal and the weak fibronectin immunoreactivity was detected in basal membrane (⇒) and collagen fibers of dermis (↑)

histamine levels and prevents the degranulation of mast cells (16). Taurine is known to have a regulatory role on collagenogenesis and on prevention of abnormal collagen production such as fibrosis (17).

The group treated with taurine-chitosan gel has a faster reepithelization rate than the chitosan gel group. The immunostaining of EGF-r is stronger in early wounds. The positive staining decreases on late wound healing because both reepithelization and the repair of the dermis are processed during healing. The pattern of immunostaining and the tissue distribution of fibronectin reveal that the wound healing in the taurine-chitosan group is faster than in the other group.

These data suggest that the presence of the EGF receptor is a common denominator for the reepithelization process after an incision wound, and that the presence of fibronectin is an important sign for the dermal repairing process during wound healing. Taurine and chitosan; which play an important role in wound healing, can be a good combination for treating skin wounds.

Conflict of Interest
No conflict of interest was declared by the authors.

REFERENCES

1. Görgün M, Özoğul C, Erdoğan D. The effects of epidermal growth factor to newborn mice. Gazi Med J 1994; 5: 113-7.
2. Özoğul C, Karagöz E, Erdoğan D. Immunohistochemical localization of epidermal growth factor receptor in normal mouse tissues. Gazi Med J 1997; 8: 105-9.
3. Damjanow I, Mildner B, Knowles BB. Immunohistochemical localization of the epidermal growth factor reseptor in normal human tissues. Lab Invest 1986; 55: 588-92.
4. Das M. Epidermal growth factor: Mechanism of action. Int Review of Cytology 1982; 78: 233-46. [CrossRef]
5. Yu W, Naim JO, Lanzafame RJ. Expression of growth factors in early wound healing in rat skin. Lasers Surg Med 1994; 15: 281-9. [CrossRef]
6. Wenczak BA, Lynch JB, Nanney LB. Epidermal growth factor receptor distribution in burn wounds. Implications for growth factor-mediated repair. J Clin Invest 1992; 90: 2392-401. [CrossRef]

7. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W. The immunohistochemical analysis of fibronectin, kollagen type III, laminin, and cytokeratin 5 in putrified skin. Forensic Sci Int 1993; 61: 35-42. [CrossRef]
8. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, et al. The immunohistochemical localization of alpha1-antichymotrypsin and fibronectin and its meaning for the determination of the vitality of human skin wounds. International Int J Legal Med 1993; 105: 223-7. [CrossRef]
9. Cass DL, Bullard KM, Sylvester KG, Yang EY, Sheppard D, Herlyn M, et al. Epidemal integrin expression is upregulated rapidly in human fetal wound repair. J Pediatr Surg 1998; 33: 312-6. [CrossRef]
10. Lamme EN, de Vries HJ, van Veen H, Gabbiani G, Westerhof W, Middelkoop E. Extracellular matrix characterization during healing of full-thickness wounds treated with a collagen/ elastin dermal substitute shows improved skin regeneration in pigs. J Histochem Cytochem 1996; 44: 1311-22. [CrossRef]
11. Miyazaki S, Yamaguchi H, Yokouchi C, Takada M, Hou WM. Sustained release and intragastric- floating granules of indomethacin using chitosan in rabbits. Chem Pharm Bull (Tokyo) 1988; 36: 4033-8. [CrossRef]
12. Aiba S. Studies on chitosan: 2. Solution stability and reactivity of partially N-acetylated chitosan derivatives in aqueous media. Int J Biol Macromol 1989; 11: 249-52. [CrossRef]
13. Hsu SH, Huang GS, Lin SY, Feng F, Ho TT, Liao YC. Enhanced chondrogenic differentiation potential of human gingival fibroblasts by spheroid formation on chitosan membranes. Tissue Eng Part A. 2011 Jul 20. [Epub ahead of print]
14. Denuziere A, Ferrier D, Damour O, Domard A. Chitosan-condrotin sulfate and chitosan-hyaluronate polyelectrolyte complexes: biological properties. Biomaterials 1998; 19: 1275-85. [CrossRef]
15. Machida Y, Nagait, Abe M, Sannan T. Use of chitosan and hydroxypropylckitosan in drug formulations to effect sustained release. Drug Des Deliv 1986; 1: 119-30.
16. Dinçer S, Babül A, Erdoğan D, Özoğul C, Dinçer SL. Effect of Taurine on Wound healing. Amino Acids 1996; 10: 59-71. [CrossRef]
17. Wang O, Giri SN, Dallas MH, Nokashima JM. Effects of taurine on bleomycin-induced lungs fibrosis in hamsters. Proc Soc Exp Biol Med 1989; 190: 330-8.
18. Huxtable RJ. Physiological actions of taurine. Physiol Rev 1992; 72: 101-63.
19. Mankovska IM, Seredenko MM, Vavilova HL, Kharlamova OM, Bystriukov VO. The antioksidant action of taurine in acute hypoxic hypoxia. Fiziol Zh 1998; 44: 65-72.
20. Ha H, Yu MR, Kim KH. Melatonin and taurine reduce early glomerulopathy in diabetic rats. Free Radic Biol Med 1999; 26: 944-50. [CrossRef]
21. Konukoğlu D, İynem H, Ziylan E. Antioksidant status in experimental peritonitis: effects of alpha-tocopherol and taurolin. Pharmacol Res 1999; 39: 247-51. [CrossRef]
22. Son MW, Ko JI, Doh HM, Kim WB, Park TS, Shim MJ, et al. Protective effect of taurine on TNBS-induced inflammatory bowel disease in rats. Arch Pharm Res 1998; 21: 531-6. [CrossRef]
23. Değim Z, Celebi N, Sayan H, Babül A, Erdoğan D, Take G. An investigation on skin wound healing in mice with a taurine-chitosan gel formulation. Amino Acids. 2002; 22: 187-98. [CrossRef]
24. Fieguth A, Kleeman R, Wasielewski R, Werner M, Tröger HD. Influence of postmortem changes on immunohistochemical reaction in skin. Int J Legal Med 1997; 110: 18-21. [CrossRef]
25. Wilson SE, Chen L, Mohan RR, Liang Q, Liu J. Expression of HGF, KGF, EGF and receptor messenger RNAs following corneal epithelial wounding. Exp Eye Res 1999; 68: 377-97. [CrossRef]
26. Baskin LS, Sutherland RS, Thomson AA, Nguyen HT, Morgan DM, Hayward SW, et al. Growth factors in bladder wound healing. J Urol 1997; 157: 2388-95. [CrossRef]
27. Stoscheck CM, Nanney LB, King LE. Quantitative determination of EGF-R during epidermal wound healing. J Invest Dermatol 1992; 99: 645-9. [CrossRef]
28. Nakamura M, Nishida T. Differential effects of epidermal growth factor and interleukin 6 on corneal epithelial cells and vasculer endothelial cells. Cornea 1999; 18: 452-8. [CrossRef]
29. Kim JS, McKinnis VS, Nawrocki A, White SR. Stimulation of migration and wound repair of guinea-pig airway epithelial cells in response to epidermal growth factor. Am J Respir Cell Mol Biol 1998; 18: 66-74.
30. Istvan J, George FM, Horng-Chin Y, Meenhard H, Steven MA. Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. Am J Pathol 1993; 143: 1458-69.
31. Jery SVB, Ross R. Immunohistochemistry of fibronectin and actin in ungrafted wounds and wounds covered with full- and split-thickness skin grafts. Plast Reconstr Surg 1993; 91: 684-92. [CrossRef]
32. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, et al. Immunohistochemical localization of fibronectin as a tool for the age determination of human skin wounds. Int J Legal Med 1992; 105: 21-6. [CrossRef]
33. Okamato Y, Shibazaki K, Minami S, Matsuashi A, Tanioka S, Shigemasa Y. Evaluation of chitin and chitosan on open wound healing in dogs. J Vet Med Sci 1995; 57: 851-4. [CrossRef]
34. Kratz G, Back M, Arnander C, Larm O. Immobilised heparin accelerates the healing of human wounds in vivo. Scand J Plast Reconstr Surg Hand Surg 1998; 32: 381-5. [CrossRef]
35. Keys SA, Zimmerman WF. Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Rest 1999; 68: 693-702. [CrossRef]