



# Growth Factor-Collagen Relationship in Wound Healing

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## ABSTRACT

Various growth factors such as epidermal growth factor (EGF) are effective in wound healing. For this reason, it was targeted in our study to observe the impact of systematically given EGF on collagen and protein synthesis in hepatic tissue. Rats to which dorsolateral excisional wound was made were divided into 2 equal groups: 1-Untreated control group, 2-Systemic EGF applied group. A daily dose of EGF (10 ng/mL) was given to the animals intraperitoneally (ip) in the application group. Rats were sacrificed in 1st, 5th, 7th and 14th days of the study. Collagen and protein concentrations in hepatic tissues were measured spectrophotometrically. In consideration of the phases of systemic EGF application and its comparison with the controls, it was determined that EGF stimulates the synthesis of collagen up to 7 days and protein up to 5 days in liver and this stimulation stops in the later days and EGF begins to make pressure on collagen and protein synthesis.

**Keywords:** *Epidermal growth factor, wound healing, collagen, protein synthesis*

## 1. INTRODUCTION

Wound is the degeneration of soft tissues normal anatomical structure and function [1]. Wound healing is a cellular and biochemical events process starting with trauma and resulting with formation of new tissue [2].

Growth factors are effective proteins in wound healing [3]. EGF is mitogenic for many mesodermal and ectodermal origin cell properties. Ion, glycolysis, RNA and protein synthesis cause increase in DNA synthesis of cells [4]. EGF which is a mitogenic polypeptide begins effecting the wound healing at the end of inflammation phase and it is known that it induces formation of fibroblastic and granulation tissues and stimulation of epithelialization [5,6,7].

Connective tissue provides the shape of cells by filling the spaces between that and it prevents the tissue losses in organism [8]. Extracellular macromolecular intermediate collagen forming the connective tissue is consistent of collagen, proteoglycan, elastine and reticuline [2]. Collagen provides the stabilization of

most of the organ in the body. Collagen fibrils have different arrangements in each species according to the different tissues [9,10]. Type I and type III collagen plays the role in wound healing [11]. Collagen synthesis reaches to its highest levels in 5th and 7th days and the increase continues for 3-4 weeks in thickest wounds. After four weeks, it gradually decreases and eventually descends to a level that balances the destruction caused by collagen [12].

They also make the collagen synthesis in the structure of glycoprotein with the synthesis of temporary main substance components such as fibroblasts reaching the area of the wound, elastin, fibronectin and glycosaminoglycan having importance in wound's formation and proteases such as collagenase synthesis [13,14,15].

Fibroblasts are seen in the 3rd - 4th days after injury [14,16-20] and they reach to the highest level in the 7th days [16,21] and they remain active in the wound until the 15th - 21st days [14]. Fibroblasts are the main elements of healing process and they are responsible of

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the production of structural proteins used in reproduction of the tissues [16].

In accordance with all of this information, the changes occurring in collagen and protein levels synthesized from the hepatic tissue due to EGF applied systemically during physiologic wound healing process.

## 2. EXPERIMENTALS

### 2.1. Animals and treatment

In experiments, totally 48 male rats (Wistar Albino) which are 200-250 g in weigh and provided by Gazi University Laboratory Animal Rearing and Experimental Research Center (GUDAM) were used. Experimental animals were divided into two groups as Control and Systemic EGF applied group and each group was divided into 4 sub-groups as 1, 5, 7 and 14 days. For providing the postoperative analgesia, pharmacological agent paracetamol was applied as 2 mg/mL into the fresh water. Rats were fed with free feed and water during the experiment. A daily dose of EGF (10 ng/mL) was given ip to the animals in the application group. The rats were sacrificed by taking blood from their hearts under anesthesia in 1st, 5th, 7th and 14th days of the experiment.

## 3. RESULTS

The study's collagen and protein levels are given in Table 1.

Table1: Hepatic tissue collagen and protein levels

		Collagen (mg/g tissue)	Protein (mg/g tissue)
1 day wound	Control group	44.900 ± 2.869	26.400 ± 4.889
	Systemic EGF applied group	54.766 ± 4.182 <sup>a</sup>	36.166 ± 4.628 <sup>a</sup>
5 day wound	Control group	50.833 ± 2.687	24.267 ± 5.001
	Systemic EGF applied group	58.917 ± 3.143 <sup>c</sup>	33.183 ± 3.000 <sup>c</sup>
7 day wound	Control group	50.267 ± 2.959	28.917 ± 2.502
	Systemic EGF applied group	58.800 ± 4.292 <sup>e</sup>	23.267 ± 2.248 <sup>b,d</sup>
14 day wound	Control group	65.450 ± 3.399 <sup>a,c,e</sup>	29.000 ± 1.553
	Systemic EGF applied group	56.466 ± 2.602 <sup>g</sup>	24.383 ± 1.696 <sup>b,d</sup>

<sup>a</sup>P< 0,05; when compared with the same values of 1-day incision wound group

### 2.2. Creation of the wound model

As general anesthesia, ketamine and xylazine were injected to the animals. Dorsolateral excisional wounds of 2 cm were made on both sides of the animal's dorsal medulla spinal. The wound lips were adapted with 2 sutures after excisional wound. The EGF serum to be applied following the injury was given to the animals ip as 1 dose in one day (10 ng/mL).

### 2.3. Determination of proteins in the tissue

The protein concentration in tissues was determined with Lowry method [22].

### 2.4. Determination of collagen in the tissue

The concentration of collagen in tissues was determined according to the modified Lowry method [23].

### 2.5 Statistical analysis

Mann-Whitney U test was used in evaluating the findings. All values were given with arithmetic mean ± standard deviation and p <0.05 value was considered statistically significant.

<sup>b</sup>P< 0,05; when compared with the same values of 1-day incision + EGF applied group

<sup>c</sup>P< 0,05; when compared with the same values of 5-day incision wound group

<sup>d</sup>P< 0,05; when compared with the same values of 5-day incision + EGF applied group

<sup>e</sup>P< 0,05; when compared with the same values of 7-day incision wound group

<sup>f</sup>P< 0,05; when compared with the same values of 7-day incision + EGF applied group

<sup>g</sup>P< 0,05; when compared with the same values of 14-day incision wound group

When Control 1 day wound (Control 1) and Systemic EGF applied 1 day wound (IP 1) were compared; systemically applied EGF caused an increase in collagen and protein values from the first day. When Control 1 and Control 14 groups were compared; it can be said that the healing of wound was completed in the 14th day. When Control 14 group was compared with Control 5 and Control 7 groups; it was seen that synthesis of collagen increased in Control 14. When Control 5 and IP 5 groups were compared; it was seen that the amount of collagen increased in IP 5 and in parallel, the amount of protein increased. When Control 7 and IP 7 groups were compared; it was seen that the value of collagen in IP 7 increased when the value of protein decreased. When Control 14 and IP 14 groups were compared; it was seen that the amount of collagen decreased in IP 14 and in parallel, the amount of protein decreased. When IP 1 and IP 7 groups were compared; it was seen that the value of protein decreased in IP 7. When IP 1 and IP 14 groups were compared; it was seen that the value of protein decreased in IP 14.

#### 4. DISCUSSION

Recently, in the studies made both in the general medical field and in oral surgery, it has been shown that the EGF has a positive impact on wound healing [24,25,26,27].

In our study, the role of liver tissue in the process of wound healing was shown and the collagen synthesis increased in 1st, 5th and 7th days in groups applied with systemic EGF.

In compliance with our findings, Tettamanti and his colleagues, recorded that the activity of fibroblasts were controlled by EGF and the collagen synthesized by fibroblasts had the main role in formation of the new blood vessels during wound healing [28]. Laato and colleagues showed that the wound healing and collagen production stimulated by EGF was bound to the fibroblast proliferation [29]. As result of the studies made by Babul and his colleagues, it was recorded that EGF accelerates wound healing [30]. In the studies made by Erbas and his colleagues to show the accelerant effects of EGF in wound healing, it was shown that EGF increases the serum zinc level [31]. Celebi and colleagues mentioned that EGF has positive effect in wound healing with the studies that made in mice wound [32]. This information seem to support our studies.

As result of the fact that EGF has mitogenic activity on corneal endothelial cells, fibroblasts, cells that support the nervous system tissue [33,34], its inhibiting effect on gastric acid secretion [33,35] and its effects on the development of embryo and the creation of new blood vessels, intensive studies were made on wound healing. It is also known that it increases wound tension force in animal models [36]. In the studies made for implementation of EGF as in systemic way which is similar to our study, it was reported that EGF accelerates the wound healing by stimulating the new vessel formation and the creation of epithelisation and granulation [8,37-39]. Turkyilmaz and colleagues have reported that the topic application of EGF accelerates the healing of wound [40]. Cellini and colleagues applied a commercial preparation containing the EGF to the patients with herpetic corneal ulcer and it was seen that EGF accelerates the recovery of this disease [41]. Most of the studies for the development of the face were made on rats and mice. The results taken from these studies showed that EGF stimulates the synthesis of DNA increasing the number of cells with phenotypes in stomach organ epithelium [42,43,44].

As a result of the studies, it was seen that EGF is effective on the healing of wounds in ocular and dermal gastrointestinal areas and reduces the duration of recovery [41]. Amberger mentioned that EGF is effective on angiogenesis [45,46]. This information seem to support our studies.

The protein synthesis increased in 1st and 5th days in groups applied with systemic EGF.

EGF is mitogenic for many mesodermal and ectodermal origin cell properties and it is known that it causes increase in ion, glycolysis, RNA and protein synthesis, DNA synthesis of cells [4].

In rats which are applied with systemic EGF, it was seen that collagen reduced after 7 days and decrease in the amount of protein was seen after 5 days. EGF increases collagen and protein synthesis in liver in low concentrations while it made pressure on the aforementioned synthesis in high concentrations. Kim and colleagues reported that EGF suppresses the inflammation reaction, reduces the expiration of TGF-beta and increases collagen formation to reduce injuries of the skin [47].

As a result, in consideration of the phases of systemic EGF application and its comparison with the controls, it was determined that EGF stimulates the synthesis of collagen up to 7 days and protein up to 5 days in liver and this stimulation stops in the later days and EGF begins to make pressure on collagen and protein synthesis.

#### CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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