

RESEARCH ARTICLES

EFFECT OF EPIDERMAL GROWTH FACTOR ON TITANIUM IMPLANTED ORAL MUCOSAL WOUND HEALING IN THE RABBIT

EPİDERMAL BÜYÜME FAKTÖRÜNÜN TAVŞANDA TİTANYUM YERLEŞTİRİLMİŞ AĞIZ MUKOZAL YARALARININ İYİLEŞMESİNE ETKİSİ

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ABSTRACT

Purpose: To investigate the effect of epidermal growth factor (EGF) dosage form prepared in polyethylene glycol 4000 (PEG) on titanium implanted oral wounds. **Methods:** Five-month-old New Zealand rabbits were divided into three groups (n=10): in the first group a titanium ring was implanted in the oral mucosa by incision, in the second group a titanium (Ti) ring was implanted together with EGF, and in the third group a titanium ring was implanted with EGF (40 ng) dosage form in PEG. All groups' incision wounds were closed with two stitches. At the end of the fifth day wound healing was examined histologically and by means of wound tear strengths, and the hydroxyproline content of wound edges. **Results:** The wound tissue tear strength value of the PEG+EGF + Ti group was higher than both that of the PEG+ Ti group ($P<0.05$) and that of the Ti group ($P<0.01$). The hydroxyproline level of wound tissue in the PEG+EGF+ Ti group was higher than that in the other groups. A histologic examination of the wounds revealed that epithelial growth differed between groups. One layer of epithelium was observed in the PEG+ Ti groups' wound tissues. It was noteworthy that the epithelia were multilayer and smooth in the PEG+EGF+ Ti groups' wounds. **Conclusion:** The results suggest that EGF application in polyethylene glycol to dental titanium implants augments oral mucosal incision wound healing.

Key Words: EGF, Ti, PEG, Wound Healing, Rabbit, Tear Strength.

INTRODUCTION

Titanium (Ti) has received a great deal of attention from dental researchers and clinicians. Apart from in the mouth, Ti implants have also been used in several other parts of the body for many years (1). It is well known that growth

ÖZET

Amaç: Bu çalışma epidermal büyüme faktörü (EGF)nün polietilen glikol (PEG) 4000 içinde hazırlanan formunun titanyum yerleştirilmiş- ağız yaralarındaki etkisini saptamak üzere planlanmıştır. **Metod:** 5 aylık Yeni Zelanda tavşanları 10 ar deneklik 3 gruba bölündü: ilk grupta ağız kesi yarasına titanyum halka yerleştirildi, ikinci grupta titanyum halka polietilen glikol ile , üçüncü grupta titanyum halka içinde polietilen glikol ile hazırlanan 40 ng.lık epidermal büyüme faktörü yerleştirildi. Tüm kesi yaraları iki dikiş ile kapatıldı. Beş gün sonunda yara iyileşmesi histolojik olarak ve yara yırtılma kuvveti ve hidroksiprolin içeriği bakımından kontrol edildi. **Sonuç:** üçüncü grubun yara yırtılma kuvvetine ait değerler ikinci ve birinci gruptan yüksekti (sırasıyla $p<0.05$ ve $p<0.01$). Üçüncü grubun hidroksiprolin değerleri de diğer gruplardan yüksekti. Histolojik inceleme özellikle epitel büyümesinin gruplar arasında farklı olduğunu gösterdi. İkinci grupta tek katlı epitel tabakası izlendi. Üçüncü gruba ait epitelin çok katlı ve düzgün olması dikkat çekiciydi. Sonuçlar propilen glikol içinde epidermal büyüme faktörünün dental titanyum implantlara uygulamasının ağız mukozal kesi yaralarının iyileşmesini arttırdığını düşündürmektedir.

Anahtar Kelimeler: EGF,Ti, PEG, Yara İyileşmesi, Tavşan, Yırtılma Kuvveti.

factors have powerful effects on wound healing (2,3). These factors control the growth, differentiation and metabolism of cells (4). The submandibular gland has been shown to produce and secrete many biologically active compounds, including epidermal growth factor (EGF), nerve

growth factor, kallikrein and renin. Local daily application of EGF enhances epithelial tissue growth (2,5) and enhances the accumulation of granulation tissue cells, collagen and glycosaminoglycans in experimental rat wounds (6). Repeated or prolonged administration of drugs is necessary for effective therapy (7). Studies are reported on the use of liposomes, lipid-surfactant micelles, oil vehicles, emulsions, microemulsions etc. for this purpose (8). In a recent study we examined the effects of EGF in Carbopol 940 gel on skin full-thickness incision wound healing and observed a negative relation between the tearing strength and zinc level of the wound (9). In another study we examined the effect of EGF in a different volume and a mixture of Carbopol 940 preparations on corneal wound healing and observed that carriers act in different ways on the physiological tissue condition, such as oxidant status (10,11). There are many studies on EGF's augmenting effect on wound healing (12,13); however, there is not enough information regarding the interaction of EGF with Ti on wound healing. Using Ti together with EGF may shorten the adaptation time of implants.

PEG polymers are hydrogels that are used for tissue adhesion. PEG is bioabsorbable, and its degradation takes approximately 3 months. One of the general uses of tissue adhesives is local delivery of exogenous substances. The delivery systems proposed recently include medications, growth factors and cell lines (14).

This study was planned to investigate the effect of EGF dosage form prepared in PEG 4000 on Ti implanted oral wound healing.

MATERIALS AND METHODS

Thirty adult New Zealand rabbits weighing 2.8 ± 0.3 kg were used and were fed pellets and tap water ad libitum. The mean weight of the Ti ring was 0.138 g; its thickness was 2 mm and radius 5 mm. Four flacons of EGF (0.1 mg, Sigma E7755, from mouse submaxillary gland) were dissolved in distilled water and mixed with molten PEG 4000 to give 40 ng of EGF per subject (15-17). The Ti rings were filled with EGF+PEG 4000 mixture to form bead shape dosage forms (0.02 g) and kept in closed ampules until assayed. The dosage forms were prepared under aseptic conditions.

Research Protocol: The rabbits were weighed before the operation. All animal

experimentation was carried out according to the ethical rules of "Statements on the Use of Animals in Biomedical Research and Education" (Adopted and revised 1996 by The American Physiological Society). Oral incisions were made on both sides of the mandibular mucosa between the incisor and molar regions of all rabbits under Xylazine (5 mg/kg) + Ketamine (35 mg/kg) anesthesia. Following the incisions, Ti rings were localized on both side of the periosteum. Ti rings were applied to the rabbits in the first group (n=10, 20 wounds), PEG 4000 within the Ti ring to the second group (n=10, 20 wounds), and EGF (40 ng) in PEG 4000 within the Ti ring to the third group (n=10, 20 wounds). All the wounds were closed with two silk stitches. Following the operation all rabbits received a single dose of 400,000 IU procaine penicillin. During the experimental period the rabbits were fed pellet chow and tap water ad libitum. On the 4th postoperative day the rabbits were weighed and sacrificed by an overdose of IV sodium pentothal. Then 3 mm thick and 8 mm wide tissue strips were excised to measure wound tissue tear strength. Tissue samples were taken for hydroxyproline measurement and histologic examination.

Measurement of Wound Tear Strength: Thicknesses of the tissue samples were measured by millimetric dental probe before measuring tissue tear strength. In each group ten tissue samples approximately 3 mm thick and 8 mm wide were used to measure wound tear strength. Tissue strips were attached to the ends of a tissue tear strength apparatus (Fig. 1). The electric motor of the apparatus applies gradually increasing power and this power is recorded on polygraph paper synchronously by a force displacement transducer (Grass Model 7 polygraph, Grass force displacement transducer FT 03) (18). When the wound tissue is separated into two pieces, a peak is recorded by polygraph (19,20). Then another peak is recorded by polygraph using one gram standard. Standard and sample peaks are compared and the results are divided by tissue area and expressed as g/mm^2 .

Measurement of Tissue Hydroxyproline Level: Hydroxyproline levels of seven wound tissues were measured in each group and the value was assessed by the Modified Woessner method (21). The principle of this method is to measure the optical density of the mixture, which

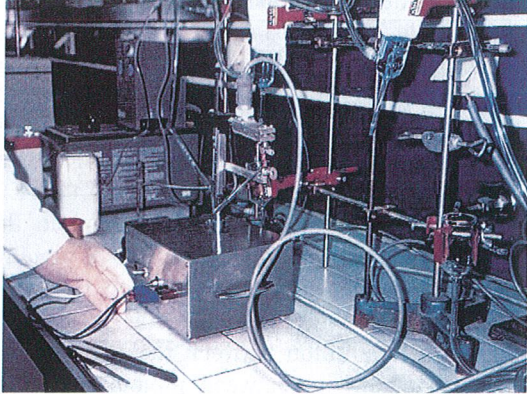


Fig. 1: Photograph of the apparatus used in tissue tear strength measurement.

is composed of hydrolyzed wound tissue, chloramine T, perchloric acid and p-dimethyl amino benzoaldehyde at 557 nm. A Shimadzu UV-1208 spectrophotometer was used for the hydroxyproline assessment.

Histological Examination: After staining with hematoxylin-eosin, all samples (3 random samples for each group) were examined under a BH2 Olympus light microscope.

Data are presented as the mean \pm S.D. ANOVA and Mann-Whitney U tests were used in the statistical analysis of the tear strength and hydroxyproline results.

RESULTS

Wound tissue tear strengths and hydroxyproline levels are shown in Table 1. According to the results, the tissue tear strength of the Ti ring implanted group was 0.995 ± 0.170 g/mm², that of the PEG 4000 treatment in the Ti ring group was 1.202 ± 0.350 g/mm² and that of the EGF+PEG treatment in Ti ring group was 1.723 ± 0.290 g/mm². We found that tissue tear strength in the third group was higher than both that in the second group ($P < 0.05$) and that in the first group ($P < 0.01$). The mean hydroxyproline level was 2.318 ± 0.610 mg/g in the Ti ring implanted group, 2.555 ± 0.480 mg/g in the PEG treatment in the Ti ring group and 3.174 ± 0.560 mg/g in the EGF+PEG treatment in the Ti ring group.

Although we could not observe any

statistically significant difference between the Ti implanted and PEG treatment in the Ti ring groups' hydroxyproline levels, the difference between the EGF+PEG treatment in the Ti ring group and only Ti ring implanted group's hydroxyproline level was statistically significant ($P < 0.05$). A histological examination revealed that wound healing differed between the groups. We observed that epithelization was completed, the epithelial lamina proprial junction was returned to its normal structure, collagen fibrils had no bundle formation and were irregular in the deep and superficial lamina propria incision area and there was neutrophilic infiltration in the EGF+PEG treatment in the Ti ring group (Fig. 2a). Epithelization was incomplete in the incision area, and neovascularization was observed in superficial lamina propria in the PEG treatment in the Ti ring group (Fig. 2b). The epithelial-lamina proprial junction was incomplete, the basal membrane did not complete its linear form in the incision area and there was irregularity of the collagenous structure and neutrophilic infiltration in deep and superficial lamina propria in the Ti ring implanted group (Fig. 2c x200).

It was noteworthy that the epithelium was similar to unwounded tissue in the EGF+PEG treatment in the Ti ring group (Fig. 2a).

DISCUSSION

Titanium is the most stable surface coating. Absolute contraindications to placing implants include abnormal wound healing that would result in implant failure. The healing of soft tissue wounds can affect the ultimate success of the dental implant. The first postoperative observation is usually scheduled 7 to 10 days after surgery (22).

Despite the fact that the saliva bathed oral cavity is constantly exposed to significant amounts of EGF, the results of salivary gland hypofunction (SGH) tests on oral wound healing in experimental rats suggest that SGH patients undergoing oral surgery may have prolonged wound healing (23). In animals, salivary EGF has been shown to affect wound closure, mediated through licking. Orally administered EGF is absorbed and delivered to various tissues (stomach and intestinal lining, lung, liver and skin) within 30 min of ingestion. Salivary EGF levels were elevated in patients after oral and juxtaoral surgery (24).

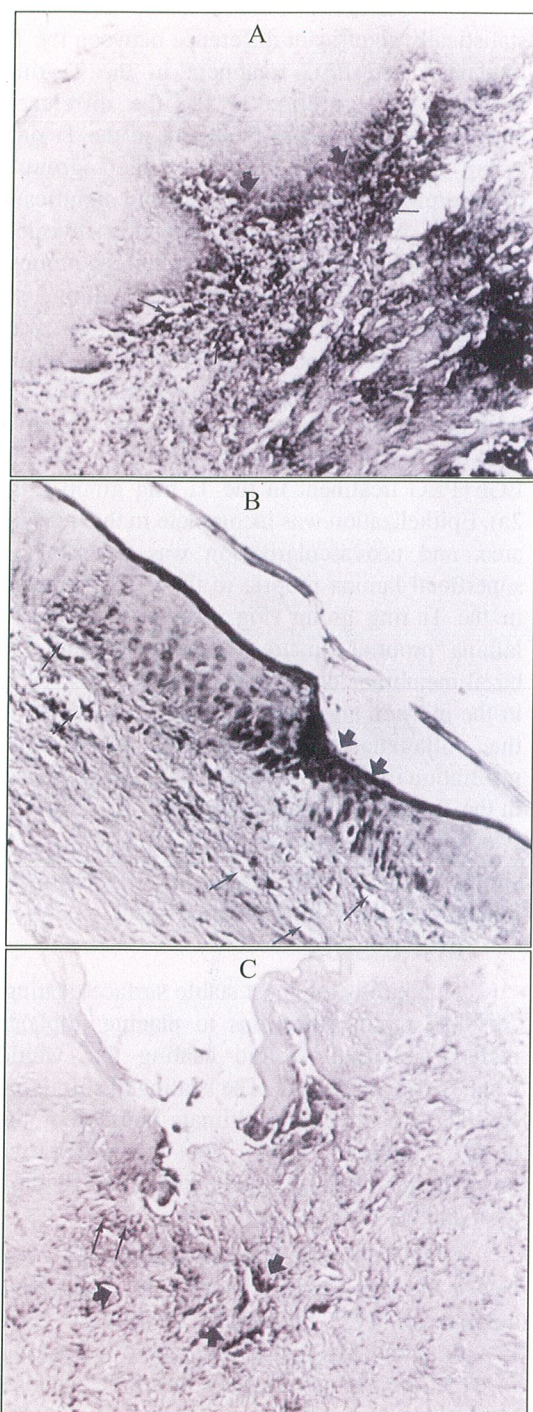


Fig. 2: Histologic results from oral mucosal wounds: Epithelial-lamina propria junction (thick arrows), neutrophilic infiltration in incision area (thin arrows) in the EGF+PEG treatment in the Ti ring group (x200) (A). Incomplete epithelization (thick arrows), neovascularization in the incision area (thin arrows) in the PEG treatment in the Ti ring group (x200) (B). Epithelial-lamina propria junction was not complete, neutrophilic infiltration (thin arrows), irregular collagenous structure (thick arrows) in the Ti ring implanted group (x200) (C).

PEG is a polymer that finds widespread use in medical and pharmaceutical applications. PEG treated animals showed reestablishment of the mesothelial layer and tissue morphology similar to anormal unoperated tissues. An inherent property of PEG is that cells do not attach to the material surface (25). It was demonstrated that proteins could be released from the hydrogel, both in vitro and in vivo. As expected, small proteins (relative to the permeability of hydrogel, as determined by the molecular mass of the PEG in the gel) were released by diffusion in the absence of degradation, whereas larger proteins were released by diffusion exclusively following degradation. With this molecular mass PEG chain, the transition between the two regimes occurs somewhere between protein molecular masses of 60,000 and 150,000 Da. Within the regime of release independently of degradation (i.e. molecular mass of 60,000 Da or less), the rate of permeation of the protein through the gel is inversely and linearly related to the molecular mass of the protein. EGF has a molecular mass of 6045, and thus can be released bioactively without statistically significant loss (26).

In this study we planned to use EGF in submucosal beads in doses higher than in saliva in the Ti ring and thus tried to schedule the first postoperative visit in a shorter period. We used wound tear strength and wound tissue hydroxyproline level and a histological examination of wound edges to observe the tissue regeneration phase of wound healing. The unstimulated salivary flow rate in rats has been measured as 0.41 ml/h, which does not include the amount stimulated by eating. Thus, in a 24 h period, a minimum of 10 ml of saliva is expected (21). With the use of these data, the EGF dose applied to animals in this study was somewhat greater than the physiological level and five times in the first day (40 ng/bead), but in total (five days) the same amount as in other research studies (3,8-10). According to the results, wound healing was better in the third group, in which EGF+PEG 4000 was applied to titanium rings. Tear strength was increased by EGF treatment compared to the control groups (Table 1). Tear strength is an important factor that shows vigorous wound healing. Incised oral wound healing usually takes a week. In this study tissue HP levels and tear strengths were measured on the 5th day of injury. In this research protocol the

Table-1: The effect of EGF with polyethylene glycol carrier in the Ti ring on the tear strength and hydroxyproline level of the rabbit oral mucosal wound.

Group	Wound tear strength (g/mm ²) n=10	Hydroxyproline level (mg/g) n=7
Ti ring implanted	2.318 ± 0.610 a	0.995 ± 0.170 a
PEG in Ti ring implanted	2.555 ± 0.480 b	1.202 ± 0.350 b
EGF+PEG in Ti ring implanted	3.174 ± 0.560 c	1.723 ± 0.290 c

Results are expressed as the mean±SE

a-b p> 0.05, a-c P<0.01, b-c P<0.05 with Mann-Whitney U test

increased tear strength in a shorter period of healing (5 days) is related to EGF+PEG application in the Ti ring. Hydroxyproline levels in this group were also significantly higher than those in the other groups. Collagen contains large amounts of proline and hydroxyproline. Hydroxyproline stabilizes the collagen triple helix to digestion by proteases, and thus a higher hydroxyproline level in wound tissue treated with EGF in PEG 4000 beads is important for increasing wound strength. Nagelschmidt and coworkers reported that PEG decreased adhesion formation on the 3rd, 7th and 21st days and collagen deposition into the adhesion strands on the 7th and 21st days (27). In this study tear strengths and hydroxyproline levels of wound tissues were measured on the 4th postoperative day. There was no difference in the tear strength and hydroxyproline levels of PEG 4000 in the Ti ring group compared to the controls. However, EGF in PEG 4000 in the Ti ring group's tear strength and hydroxyproline levels of wound tissues were significantly higher than those of the other groups. This result is supported by a histologic examination of wound tissue at the same period as multilayer, squamous epithelium in the same EGF+PEG 4000 treated in the Ti ring group. PEG 4000 seems also to be effective in wound healing and in supporting EGF effects of wound healing as also described in Subrahmanyam's report (28). In addition, PEG 4000 may prolong the exposure of wound tissues to EGF.

In conclusion, it is suggested that epidermal growth factor carried by polyethylene glycol pellets in dental titanium implants are effective in accelerating wound healing and in shortening the adaptation time of the patient to the implant.

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REFERENCES

1. Nakajima H, Okabe T. Titanium in dentistry development and research in the USA. *Dent Mater* 1996; 15: 77-90.
2. Gönül B, Koz M, Ersöz G, Kaplan B. Effect of EGF on the corneal wound healing of alloxan diabetic mice. *Exp Eye Res* 1992; 54: 519-524.
3. Gönül B, Erdoğan D, Özoğlu C, Koz M, Babül A, Çelebi N. Effect of EGF dosage forms on alkali burned corneal wound healing of mice. *Burns* 1995; 21: 7-10.
4. Steed DL. Modifying the wound healing response with exogenous growth factors. *Clin Plast Surg* 1998; 25: 397-405.

5. Podolsky DK. Healing the epithelium: solving the problem from two sides. *Gastroenterology* 1997; 32: 122-126.
6. Laato M, Niinikoshi J, Gerdin B, Lebel L. Stimulation of Wound Healing by EGF. *Ann Surg* 1986; 203: 379-381.
7. Sheardown H, Wedge C, Chou L, Apel R, Rootman DS, Cheng YL. Continuous epidermal growth factor delivery in corneal epithelial wound healing. *Invest Ophthalmol Vis Sci* 1993; 34: 3593-3600.
8. Çelebi N, Türkyılmaz A, Gönül B, Özoğul C. Effects of epidermal growth factor microemulsion formulation on the healing of stress-induced gastric ulcers in rats. *J Control Release* 2002; 83: 197-210.
9. Gönül B, Söylemezoglu T, Babül A, Çelebi N. Effects of epidermal growth dosage forms on mice full-thickness skin wound zinc levels and relation to wound strength. *J Pharm Pharmacol* 1998; 50: 641-644.
10. Gönül B, Kaplan B, Bilgihan K, Budak MT. Effects of EGF in artificial tears on vitamin C levels of corneal wounded eye tissues. *Eye* 2001; 15: 213-216.
11. Akbulut KG, Gönül B, Türkyılmaz A, Çelebi N. The role of epidermal growth factor formulation on the stress ulcer healing of the gastric mucosa. *Surg. Today* 2002; 32: 880-883.
12. Greenhalgh DG. The role of growth factors in wound healing. *J Trauma* 1996; 41: 159-167.
13. Zelles T, Purushotham KR, Macauley SP, Oxlord GR, Humphreys-Beher MG. Saliva and growth factors. The fountain of youth resides in us all. *J Dent Res* 1995; 74: 1826-1832.
14. Reece TB, Maxey TC, Kron IL. A prospectus on tissue adhesives. *Am J Surg* 2001; 182: 40S-44S.
15. Yang J, Tyler LW, Donoff BR, Song B, Tario AJ, Gallagher GT, Tsuji T, Elovic A, McBride J, Yung CM, Gall SJ, Weller PF, Wong DTW. Salivary EGF regulates eosinophil derived TGF-alpha expression in hamster oral wounds. *Am J Physiol* 1996; 270: 191-202.
16. Boylan JC, Cooper J, Chawhan ZT, Land W, Wade A, Weir RF, Yates BJ. *Handbook of pharmaceutical excipients*, Washington, The Pharmaceutical Press, 1983.
17. Swinyar EA, Lowenthal W. *Remington Pharmaceutical Sciences*, Pennsylvania, Mack Printing Comp, 1985.
18. Hayt RC. The effects of orientation and location on the strength of dorsal rat skin and low speed tensile failure experiments. *J Biomed Eng* 1989; 111: 136-140.
19. Brown M, Gogia P, Smacore DR, Menton DN. High-voltage galvanic stimulation and wound healing in guinea-pigs: long term effect. *Arch Phys Med Rehab* 1985; 6: 1134-1137.
20. Okada M. The influence of intestinal flora on wound healing in mice. *Surg Today* 1994; 24: 347-355.
21. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportion of this amino acid. *Arch Biochem Biophysics* 1961; 93: 440-447.
22. Dolce C, Anguita J, Brinkley L, Karnam P, Humphreys-Beher M, Makagawa Y, Keeling S, King G. Effects of sialoadenectomy and exogenous EGF on molar drift and orthodontic tooth movement in rats. *Am J Physiol* 1994; 266: E731-E738.
23. Dayan D, Bodner L, Horowitz I. Effect of salivary gland hypofunction on the healing of extraction wounds: A histomorphometric study in rats. *J Oral Maxil Surg* 1992; 50: 354-358.
24. Oxford GE, Johnsson R, Olofsson J, Zelles T, Humphreys-Beher MG. Elevated levels of human salivary epidermal growth factor after oral and juxtaoral surgery. *J Oral Maxil Surg* 1999; 57: 154-158.
25. Hendrix M, Mees U, Hill AC, Egbert B, Coker GT, Estridge TD. Evaluation of a novel synthetic sealant for inhibition of cardiac adhesions and clinical experience in cardiac surgery procedures. *Heart Surg Forum* 2001; 4: 204-209.
26. Hubbell JA. Hydrogel systems for barriers and local drug delivery in the control of wound healing. *J Control Release* 1996; 39: 305-313.
27. Nagelsmith M, Minor T, Saad S. Polyethyleneglycol 4000 attenuates adhesion formation in rats by suppression of peritoneal inflammation and collagen incorporation. *Am J Surg* 1998; 176: 76-80.
28. Subrahmanyam N. Addition of antioxidants and polyethylene glycol 4000 enhances the healing property of honey in burns. *Ann Burns Fire Disaster* 1996; 9: 93-96.