

**Wound healing of polyglycolic acid sheets with fibrin glue (MCFP method) on the vulnerary process of wounds after partial resection of the rabbit margo linguae: a preliminary study**

**Short title: Wound healing of PGA and fibrin on rabbit tongue wounds**

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## ABSTRACT

Aim of this study is to examine the effectiveness of covering tongue wounds with a polyglycolic acid sheet and fibrin glue. Eighteen mature male Japanese white rabbits underwent unilateral glossectomy involving an area of 10x10x2 mm. The glossectomized tongues were covered with 8x8-mm PGA sheets and fibrin glue (Mucosal defect Covered with Fibrin glue and Polyglycolic acid sheet: MCFP method) 1 week after the operation (group A-1 [n =3]), after 2 weeks (group A-2[n =3]), and after 4 weeks (group A-3 [n =3]). In control groups, after 1, 2, and 4 weeks (groups B-1[n =3], B-2[n =3] and B-3[n =3], respectively), the partially resected tongues were closed with absorbable surgical sutures. One week (groups A-1 and B-1), 2 weeks (groups A-2 and B-2) and 4weeks (groups A-3 and B-3) after the operation, the tongues were harvested and stained for microscopic observation.

Histological examination showed that the covered wound surface had not epithelialized and the basal layer had yet to form in group A-1, but had formed in group A-2. On the other hand, in group B-1, epithelialization of the sutured wound was observed. Immunohistochemical examination revealed that, in group A-1, the non-uniform epithelial layer of the covered wound surface expressed cytokeratin AE1/AE3, and the epithelial and connective tissue layers were strongly positive for FGF-2. Similar results were obtained in group A-2, whereas, in group A-3, FGF-2 was expressed only in the connective tissue layer, and epithelialization was complete. On the other hand, in group B-1, AE1/AE3 was expressed in the epithelial layer, and FGF was expressed in the connective tissue layer beneath the basal layer. In groups B-2 and B-3, AE1/AE3 and FGF-2 were expressed in patterns similar to those in groups A-2 and A-3,

respectively.

According to the results, we suggested that this method is a useful and the operation was simple. However, further basic examination of the method is needed and many clinical cases should be performed to be established.

## **KEY WORDS**

Polyglycolic acid sheet, Fibrin glue, Rabbit, Tongue, Wound healing

## INTRODUCTION

To repair the wound surface after partial glossectomy for early-stage (I and II) tongue cancer or a precancerous lesion, primary suture closure, the tie-over method, or split-thickness skin graft (STSG) is generally used<sup>1, 2</sup>. However, when primary suture closure is not feasible, the tie-over method or STSG is used, which involves complex surgical procedures, making it difficult to maintain good postoperative oral hygiene. Therefore, in this study, we conducted an animal experiment to examine the effect of wound surface coverage on healing using the Mucosal defect Covered with Fibrin glue and Polyglycolic acid (PGA) sheet method (MCFP method).

MCFP method has been reported in the field of respiratory surgery<sup>3</sup> and other fields<sup>4</sup>, and basic experiments within these fields<sup>5-7</sup> have also been reported. This method is simpler than primary suture closure and the tie-over method, and causes no damage during the healing process (Fig. 1). The healing process of the method in oral cavity follows that the wound surface begins to epithelialize and the PGA sheet also starts to exfoliate. After that, the dressing material completely exfoliates and the wound surface newly epithelializes. However, to date, no studies have reported its application to lesions in the oral cavity.

Cytokeratin AE1/AE3 and the FGF-2 play important roles in the healing of the wounded surface and the process of newly epithelialization as markers. The squamous epithelium was strongly positive for AE1/AE3<sup>8</sup>. On the other hand, FGF2 promotes fibroblast proliferation and angiogenesis<sup>9</sup>. Therefore active fibrous tissue or granulation tissue was strongly positive for FGF-2. We paid attention to these characteristics to confirm epithelialization and fibroblast proliferation of wound healing.

In this study, we performed partial glossectomy in rabbits, covered the wound

surface with the MCFP method, and evaluated morphological changes in H-E- or immunohistochemically stained sections of the wound bed 1, 2, and 4 weeks after surgery in comparison with those in rabbits that had undergone partial glossectomy followed by primary wound closure.

## **MATERIAL AND METHODS**

### **Animals and tongue resection**

Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University with the approval of the Institutional Animal Care and Use Committee. Eighteen specific-pathogen-free bred and maintained mature male Japanese white rabbits were purchased from KBT Oriental Co., Ltd. (Saga, Japan). The body weights ranged from 3.0 to 3.5 kg at the beginning of the experiment<sup>10</sup>. Under sedation by the intramuscular injection of xylazine hydrochloride (3-3.5 mg/kg), unilateral resections of the margo linguae, involving an area of 10x10x2 mm, were performed with a scalpel and electric knife for coagulation.

### **Procedure of MCFP method**

The rabbits were assigned to two groups. Group A consisted of nine rabbits in which the wound was covered with 8x8-mm bioabsorbable fabric (Neoveil®, Gunze Co., Ltd., Tokyo, Japan) of polyglycolic acid and 0.1 ml of fibrin glue (Bolheal®, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan), which consists of lyophilized powder of fibrinogen, lyophilized powder of thrombin, an aprotinin solution for the reconstitution of fibrinogen, and a calcium chloride solution for the reconstitution of thrombin<sup>11</sup> on the wound. The procedure of MCFP method is that the

wound was covered with bioabsorbable fabric of polyglycolic acid and fibrin glue. At first, the solution of fibrinogen was applied on the wound, and the wound was covered with PGA sheet that was slightly smaller than the extent of resection. Finally, the wound was atomized with a mixture of the solution of fibrinogen and the thrombin, and the mixture stiffened approximately three minutes later. Group B consisted of nine rabbits in which the wound was closed by absorbable surgical sutures (Vicryl, Johnson & Johnson PTY., Ltd., NSW, U.S.) in layers.

In groups A and B, three rabbits were sacrificed and the tongues were harvested 1 week after the operation (groups A-1 and B-1, respectively), three were sacrificed 2 weeks after the operation (groups A-2 and B-2, respectively), and the remaining three were sacrificed 4 weeks after the operation (groups A-3 and B-3, respectively). All animals were examined in a clean and aseptic manipulation room and did not suffer during this study.

### **Histological and immunohistochemical examination**

The tongues were cut into the operative side halves. The harvested tongues were fixed in formalin, decalcified, and embedded in paraffin. The sections were stained with hematoxylin and eosin. Furthermore, the sections were also observed for immunomorphological changes by immunohistochemical examination. The sections were incubated with primary antibodies against cytokeratin (AE1/AE3 (mouse monoclonal IgG, 1:100 dilution; Life Span Biosciences Inc., Seattle, USA) and FGF-2 (rabbit polyclonal IgG, 1:2,500 dilution; Trans Genic Inc., Kobe, JAPAN). The visualization System was Envision™, Rabbit/HRP (K4003, Dako, Tokyo, Japan).

## **RESULTS**

### **Histological and immunohistochemical examination**

In the MCFP groups, wound-covering materials became detached within 1 week. H-E staining showed that, in group A-1, no basal layer formed on the covered wound surface, and epithelialization was incomplete, and a part of blood clotting along the shape of PGA sheet and blood clotting were also observed (Fig. 2). The basal layer was formed in group A-2 and blood clotting was still partially observed, and epithelialization was complete in group A-3 (Fig. 3). On the other hand, epithelialization of the sutured wound was already observed in group B-1 (Fig. 4), partly uniform epithelialization was noted in group B-2, and complete uniform epithelialization was achieved in group B-3.

Immunohistochemical staining revealed that, in group A-1, the entire thickness of the irregular epithelium, including the basal cell layer, was strongly positive for AE1/AE3 (Fig. 5), and the epithelial and connective tissue layers were strongly positive for FGF-2 (Fig. 6). In group A-2, the entire epithelial layer displayed a somewhat uniform morphology, and was positive for AE1/AE3, but the epithelial and immediately underlying connective tissue layers showed slightly decreased FGF-2 expression. In group A-3, tissue repair in the wound area had progressed further, and only the epithelial and connective tissue layers were positive for AE1/AE3 and slightly positive for FGF-2, respectively, suggesting that the wound had epithelialized normally. Similarly, in group B-1, the epithelial layer was strongly positive for AE1/AE3 1 week after surgery, and the connective tissue layer was positive for FGF-2. In group B-2, the epithelial layer showed AE1/AE3 expression and decreased FGF-2 expression. In group B-3, AE1/AE3 was expressed only in the epithelial layer, and FGF-2 was slightly

expressed only in the connective tissue layer, which was similar to the histological morphology in group A-3.

## **DISCUSSION**

In general, in the field of oral surgery, after the resection of stage I and II tongue cancer or a precancerous lesion, primary suture closure<sup>12</sup> is performed, and, if the wound surface is large, skin grafting or the tie-over method employing artificial dermis products<sup>13, 14</sup> is used. However, when the wound surface is too large to perform primary closure, or when scar contracture due to primary closure results in articulation disorder or oral function disturbance, the tie-over method is used, which involves complex surgical procedures, making it difficult to maintain good postoperative oral hygiene. Therefore, we conducted an animal experiment using rabbits to test the MCFP method (used, for example, to treat<sup>15</sup> pneumothorax) as an alternative wound-covering method.

Bolheal®, a fibrin glue derived from human blood, is characterized by being a product utilizing the marked adhesiveness resulting from the reaction of fibrin and thrombin. There have been reports of the tissue adhesion<sup>16</sup> and wound-healing effects of fibrin products<sup>17</sup>, as well as clinical reports of their use<sup>18</sup>. On the other hand, Neoveil®, a wound surface-covering agent made of PGA, has been clinically<sup>19, 20</sup> and experimentally<sup>21</sup> reported to be effective for wound healing. In this study, we evaluated the wound-healing effects of the combined application of these products on the wound surface.

In the present study, specific-pathogen-free bred and maintained mature male rabbits were used to exclude the effects on the wound surface of various pathogens and

to observe only the vulnerary process by equalizing experiment environment as pathogen-free as in a similar experimental study using the pathogen-free animal models<sup>22</sup>. Even if it is far from clinical situation, it is important to equalize an experiment environment due to observe only the healing progress.

We performed H-E and immunohistochemical staining of the wound surface 1, 2, and 4 weeks after wound coverage, using anti-cytokeratin AE1/AE3 and anti-FGF-2 as the primary antibodies, to comparatively examine the morphological changes in the wound surface treated employing the MCFP method and those in the wound closed with simple suturing. Since cells of the upper basal layer express AE1/AE3 in the epithelialization phase of wound healing, AE1/AE3 was used as a marker for evaluating epithelialization<sup>8</sup>. FGF-2 was used as a marker for evaluating the degree of granulation and wound healing by fibroblast proliferation<sup>23, 24</sup>.

H-E staining showed that, in group A, epithelialization of the wound surface had not completed at 1-2 weeks after surgery: in one-week postoperative specimens, irregular granulation tissue looked to be sitting on the concave resected surface, the basal cell layer was irregular depending on the site of AE1/AE3 expression on the wound surface, and FGF-2 was strongly expressed not only in the connective tissue layer, but also in the epithelial layer. These phenomena were not observed in group B. These observations suggest that blood clotting, fibroblast proliferation, and active granulation tissue formation take place due to the action of fibrin products in the MCFP method at 1 week after surgery, and that epithelialization is not completed until 2 weeks after surgery. Similarly, in group B, basal cells on the wound surface were arranged irregularly until 2 weeks after surgery, but the keratinized squamous epithelial layer did not morphologically differ from that around the wound surface, suggesting that primary

closure and the MCFP method result in different morphological changes until 2 weeks after surgery. However, there was no significant difference in morphological changes at 4 weeks after surgery between the two methods of wound coverage, suggesting that the MCFP method results in epithelialization by 4 weeks after surgery, and that the time-course of wound healing after the MCFP method is similar to that after primary closure. Thus, the absence of factors delaying the progress of healing after wound coverage using the MCFP method, coupled with its operational simplicity, suggests that the MCFP method is useful and unique for the healing of postresection wound surfaces and showed the efficacy. However, the results of the presented study are preliminary in that they observed the process of healing in an open glossectomy wound. Therefore, and further studies of the MCFP method are required using comparison experiments with an open wound and the tie over method in a larger number of samples to confirm the advantage of this method.

In the future, it will be necessary to investigate the influence of the extent of resection on wound healing when using the MCFP method, and the difference in healing depending on the location of resection in the oral cavity (the tongue, buccal mucosa, or floor of the mouth), and to compare the results of the MCFP method, tie-over method using other artificial covering materials, and methods with no wound-covering materials (i.e., a simple open wound). Mogford reported that fibrin sealant with fibroblast and platelet-derived growth factor (PDGF) of rabbit can enhance cutaneous wound healing<sup>25</sup>. It will be also necessary to investigate and apply for clinical practice the effect of those or other growth factors with PGA sheet with fibrin glue to activate wound healing and regeneration. However, at present, the MCFP method is simpler in operation and less time-consuming than the tie-over method, skin grafting, and methods using other

artificial covering materials. This suggests that the MCFP method may replace the tie-over method and skin grafting to repair wounds (which are difficult to close by primary suture) and prevent cicatrization of wounds after resection of early-stage (I and II) gingival cancer or a precancerous lesion and further clinical trials are required.

## **CONCLUSION**

The MCFP method did not have an effect of delaying wound healing as compared with surgical suture, and this method was useful from the aspects that the form of the wound was favorable and the operation was simpler and easier.

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## **CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest associated with the present study.

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platelet-derived growth factor enhance wound healing in excisional wounds. *Wound Repair and Regeneration*. 2009;17:405-10.

## FIGURE LEGENDS



Fig. 1. Clinical photograph of the MCFP method. With respect to the wound surface, a PGA sheet and fibrin paste were coated.

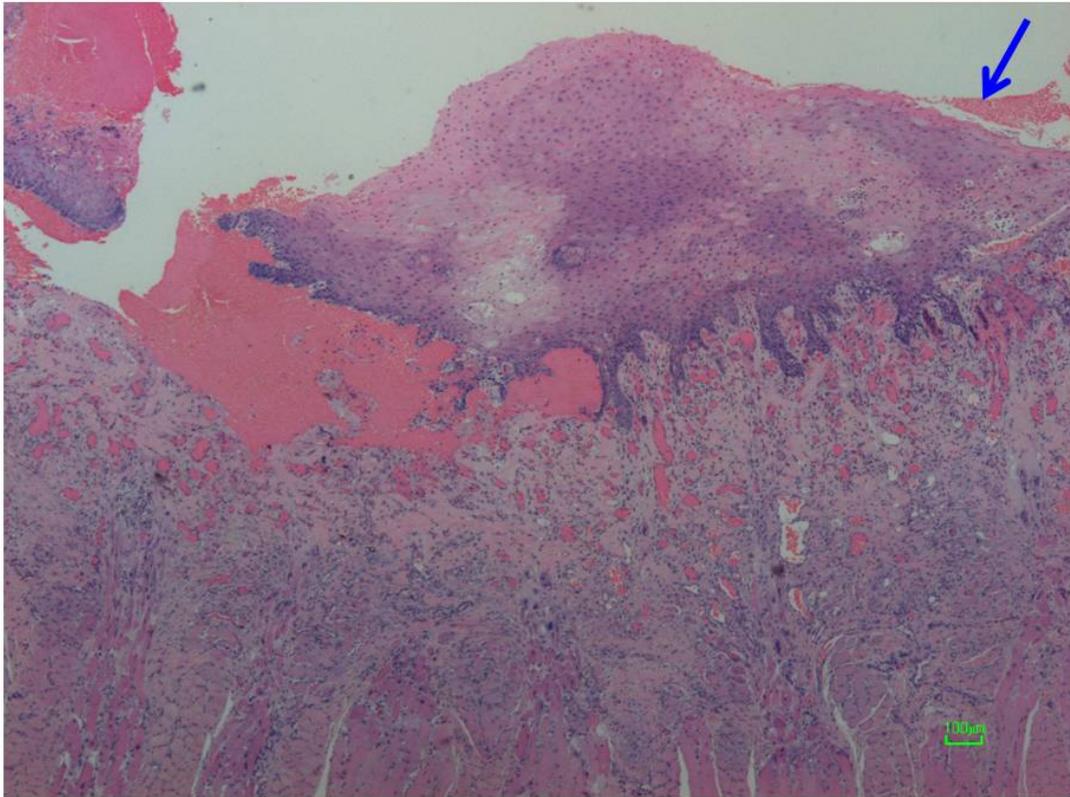


Fig. 2. Photomicrographs of specimens from group A-1.

An irregular epithelial layer was observed, but no basal layer had formed. A part of blood clotting along the shape of PGA sheet (blue arrow) and Blood clotting were also observed. Hematoxylin and eosin. Scale = 100 micro meters.

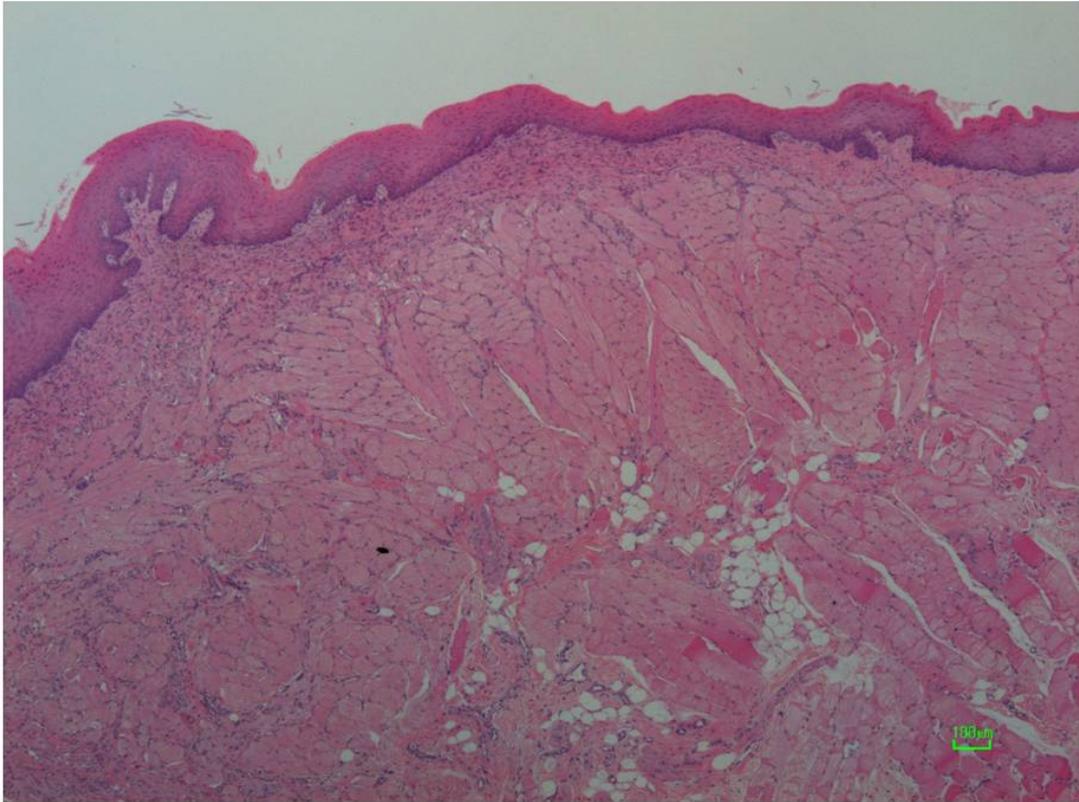


Fig. 3. Photomicrographs of specimens from group A-3.  
Complete epithelialization was noted. Hematoxylin and eosin.  
Scale = 100 micro meters.

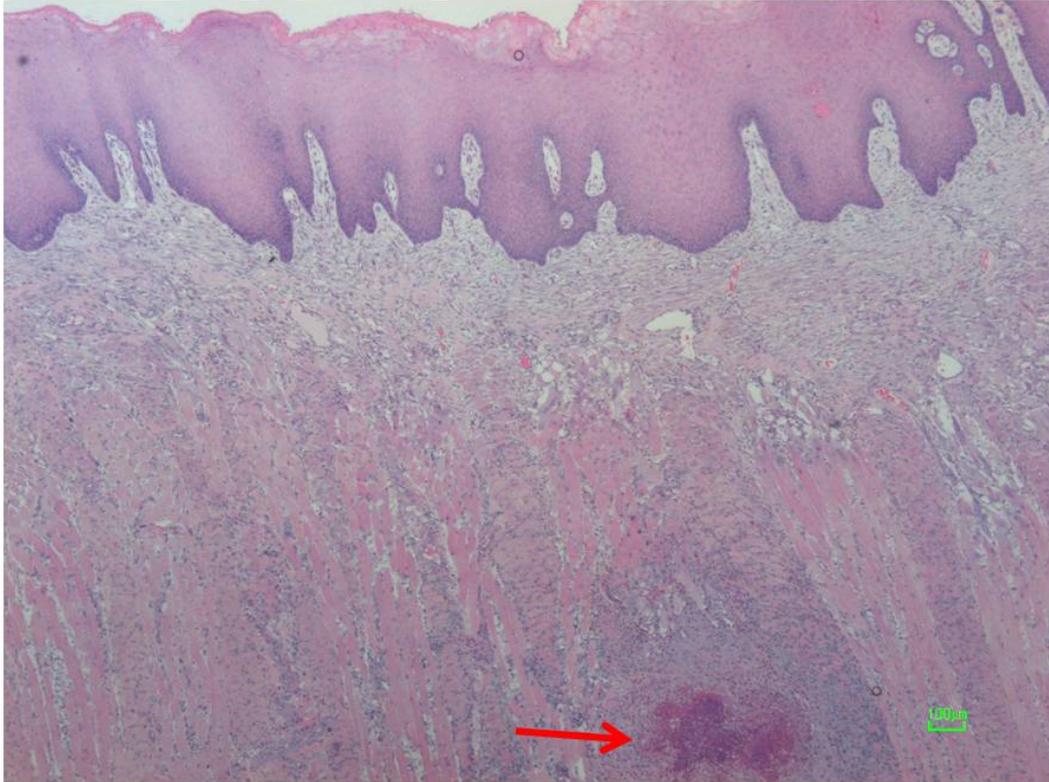


Fig. 4. Photomicrographs of specimens from group B-1. Epithelialization of the sutured wound surface was already observable along with absorbable sutures (red arrow). Hematoxylin and eosin. Scale = 100 micro meters.



Fig. 5. Photomicrographs of specimens from group A-1.

The entire thickness of the irregular epithelium, including the basal layer, was strongly positive for AE1/AE3. Scale = 100 micro meters.

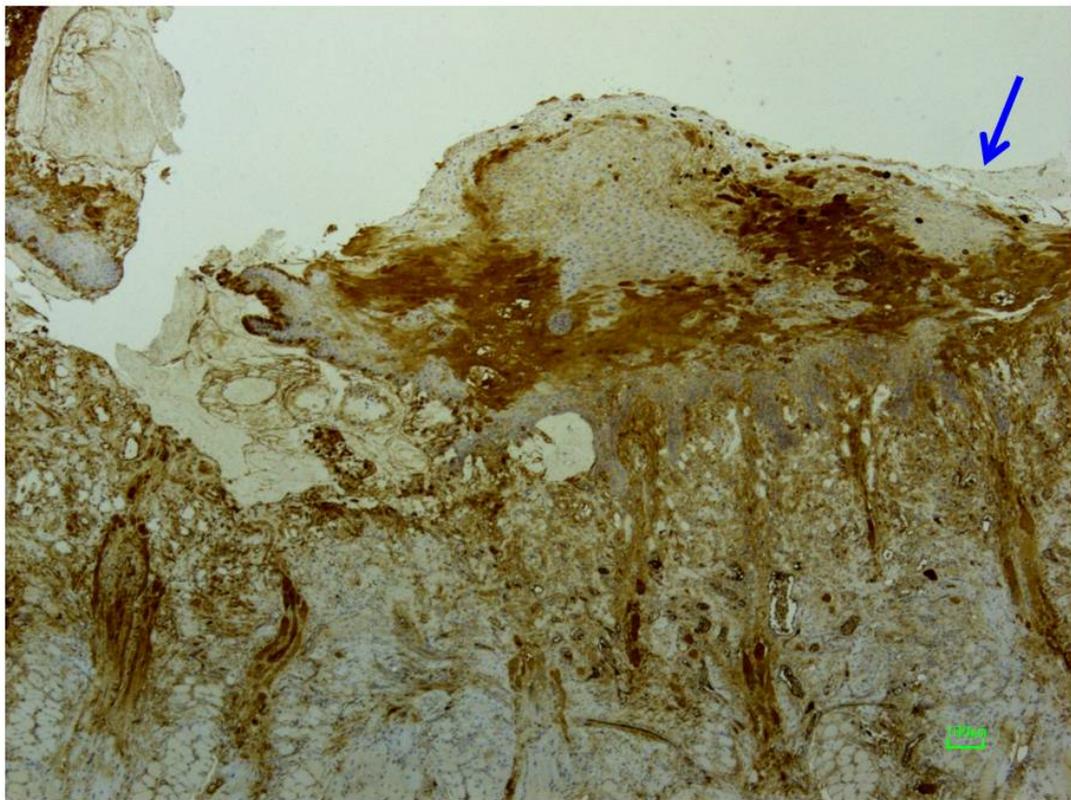


Fig. 6. Photomicrographs of specimens from group A-1.

The epithelial and connective tissue layers were strongly positive for FGF-2.

Scale = 100 micro meters.