

Enhancing wound healing in cattle through the application of vitreous humour fluid and platelet-rich plasma

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Abstract: The aim of this work: to examine the impact of applying vitreous humour fluid as well as platelet-rich plasma (PRP) on cattle's wound healing. Twelve dairy cows, randomly divided into three groups, constituted the subjects for this research. Those cows consumed standard quantities—grains; alfalfa; straw—with *ad libitum* clean water. We collected Vitreous humour fluid from slaughtered cows, storing it at 4°C. Through withdrawing blood from the same cows and separating PRP from plasma, we prepared Autologous PRP. Furthermore, we prepared a mixture of PRP with vitreous humour fluid which has been subsequently applied to wound site. At 7, 14, and 21 weeks, we performed biopsies on the cows for histopathological examination. On day 7, the histopathological examination revealed improved tissue reorganization and proliferation in wounds treated with PRP and vitreous humour fluid. Furthermore, in comparison with the control group—at this same time point—collagen organization has been notably superior. Moving onto day 14: a complete or partial union of wound margins manifested in the two groups treated with PRP and vitreous humour fluid; also, better granulation tissue formation stood out significantly—an indicator that healing progress had been robustly effective. By day 21; complete epithelialization—alongside dense collagen bundles filling the wound site—signified successful healing of wounds treated specifically with the use of vitreous humor fluid. The findings of the presented work indicate that the utilization of vitreous humour fluid and PRP could facilitate the process of wound healing in cattle. Improvements in tissue reorganization, proliferation, and collagen organization were all found. In the process of developing wound healing therapies for cattle, such findings offer useful insights that can be further developed.

Keywords: Cattle Dairy cows, Platelet-rich plasma, Vitreous humour fluid, Wound healing.

1. Introduction

Wound healing is a complex biological process that involves a series of activities aimed at restoring the integrity and function of damaged tissue the importance of wound healing in veterinary medicine is remarkable because it immediately affects the fitness and welfare of animals There are These lesions can cause significant financial losses to farm animal producers and further damage the animal's overall health and productivity. (1-3)

Over the years, several strategies were used to improve wound healing in farm animals. These include the use of various dressings, antibiotics, surfactants and herbal remedies. However, powerful and innovative strategies may still be needed that can accelerate the wound healing process, reduce complications, and improve overall outcomes (4,5).

The value of vitreous humor fluid and platelet proliferation (PRP) presents a significant theory for wound regeneration studies (6,7). Comprising water, collagen, hyaluronic acid—a gel-like substance found in the eyes of various animals including humans—vitreous humor also harbors: remodeling enhancing element-beta or TGF- β ; platelet-derived growth factor PDGF - along with other growth factors (8). On

the contrary; PRP—a stable plasma brimming with numerous platelets—is capable of producing an abundance: it secretes multiple cytokines and growth factors (9-15).

Vitreous humor fluid's unique composition and therapeutic potential have garnered attention for its application in wound healing. Studies demonstrate that crystal humor possesses anti-inflammatory, antibacterial, and anticoagulant properties crucial to the process of wound healing (16-18). In addition to that; with high wound growth factor concentrations— it can be stimulating the proliferation of cells: synthesis of collagen; extracellular matrix deposition—all necessary components for the success of tissue re-generation (19-21).

Appearing as one of the highly promising therapeutic strategies in the healing of wounds is platelet-containing plasma utilization. The stem cells, through the release of the cytokines and the growth factors (22-24) lead to the initiation of inflammatory responses and the stimulation of the cell migration and proliferation; which is an indication of their vital role in the processes of the preliminary wound healing(25).

Whole blood is centrifuged, which results in dense platelet float: which simplifies PRP acquisition. The application of those concentrated platelets to wound site leads to delivering plentiful cytokines and growth factors – which is an accelerated process of healing (26). Vitreous humor fluid as well as the PRP present important advantages in wound recovery of the farm animal; notably, those therapies are originated from the respective animal's body – thereby reducing risks that are related to the negative reactions or rejections (18).

Concerning the health of livestock in particular, this holds quite a high level of importance; taking under consideration them being frequently exposed to harsh conditions of the environmental, they could have a higher level of vulnerability to the infections (27). Secondly, it is possible to readily access PRP as well as vitreous humour fluid through minimally invasive methods; such level of accessibility results in them being cost-effective and pragmatic options for the restoration of wounds in the livestock (10,28,29).

In addition to their healing characteristics, vitreous humour fluid and PRP can also conquer numerous challenges that are associated with the restoration of wounds in the livestock (30). For example, the bio-films—communities of bacteria nestled within protective matrix—often result in impeding the recovery of the chronic wounds that are found in the animals (31, 32).

Researchers have discovered that Vitreous Humor Fluid and Platelet-Rich Plasma (PRP) can surmount the obstacles of wound healing in livestock, such as chronic wounds with biofilms. They demonstrated improved efficacy over synovial fluid (33, 34) for improving rabbit model's wound healing; moreover - a gel-like substance found within the eye- vitreous humor was shown to enhance this process significantly. Further studies revealed an acceleration in cow eyeball vitreous gel on the overall speed of wound closure, an augmentation of fibroblast population and a reduction – indeed, attenuation is more accurate – inflammation within models representing chronic wounds (35). The findings posit a potential therapeutic role for vitreous humor and vitreous gel in enhancing wound healing among livestock.

Biofilms significantly impede the wound healing process and foster chronic infections' development. Research reveals that vitreous humour fluid holds potent antimicrobial properties, proficiently curbing bacteria growth, including those within biofilms. Likewise, PRP's growth factors promote wound debridement and aid in removing necrotic tissue—a commonly challenging task with persistent wounds (36,37). The research underscores: Vitreous humor fluid harbors antimicrobial properties that effectively inhibit bacterial growth—even of biofilm-associated strains. Moreover, PRP contains growth factors; typically, these pose a challenge to removal from chronic wounds(38) . Yet here lies their benefit—they don't just aid in wound debridement but also promote necrotic tissue elimination (18, 39).

2. Methods

The College of Veterinary Sciences Committee provided instructions: in compliance with these guidelines, we executed this work using specific materials and techniques. Our research focused on evaluating the effects of a platelet proliferator (PRP) within vitreous humor fluid found in dairy cows.

2.1. Selected Topics

To accomplish our goal, we randomly divided twelve such bovines into three groups—each group composed of four members. The cows exhibited an average weight of 370 ± 30 kg; their ages averaged at 42 ± 1.5 months. We ensured the cows had ample quantities of grain, alfalfa and hay - all supplemented with water that met necessary quality standards.

2.2. Collection of vitreous humor fluid

Vitreous humor fluid was collected from cattle slaughtered in the eye room of the slaughterhouse of Al-Dewaniya district, approximately 30 ml of vitreous humor fluid was collected from each cow and stored at 4 °C until use will be used again.

2.3. Autologous platelet-rich plasma (PRP) collection:

During surgery, 20 milliliters of blood was sterilely drawn from the dorsal vein of each cow. The blood was divided into two 10-ml tubes, each containing 0.5 ml of 3.2% sodium citrate. The tubes were then centrifuged at 1500 rpm for 10 minutes. Three layers were obtained after centrifugation. The superficial layer, which consisted of negative platelets (PPP), was removed. The remaining portion of the blood in the tube represented PRP, with a volume of approximately 2 mL. For separation of PRP and PPP, both tubes were pipetted and centrifuged again at 1500 rpm for 15 min quantity measured. Two drops of 10% calcium chloride were added to activate the isolated PRP.

2.4. Preparation of Mixed PRP and Vitreous Humorous Fluid

For the experimental technique, 1 ml of PRP become introduced to at least one ml of vitreous humor fluid to create an aggregate fluid. The take a seat region become then dealt with this 1 ml combination fluid.

2.5. Sedation and Incision

All the experimental dairy cows have been sedated with xylazine at a dose of zero.05 mg/lb.B.W. The superficial gluteal muscle of the cows become exposed by making a 5mm incision inside the excessive dorsal area the usage of a scalpel.

2.6. Biopsy and Histopathological Evaluation

Biopsies have been taken from each business at 7, 14, and 21 weeks. The accumulated biopsies had been processed for histopathological examination. Histo-pathological stains had been carried out to the biopsy samples, and they have been tested under a mild microscope (Olympus Incorporation, Japan).

2.7. Ethical Considerations

The study become performed in adherence to ethical hints, and the experimental tactics had been permitted by using the College of Veterinary Medicine committee. The cows have been treated humanely during the have a look at, and efforts had been made to reduce any discomfort or pain experienced by way of the animals.

The experimental procedures concerned the collection of vitreous humor fluid and autologous PRP, their preparation and mixture, sedation and incision, and histopathological assessment of the biopsies. Statistical analysis turned into completed to investigate the acquired records. The carried out have a look at adhered to ethical recommendations and prioritized animal welfare.

3. Results

In the experiment, histological studies were performed on lesions of dairy cows. The aim was to compare pathological changes in muscle remodeling, proliferation and inflammation in samples obtained at 7, 14 and 21 days post-injury

Histopathological examination at day 7 revealed that the epithelialization was more pronounced in the different groups. Lesions treated with PRP and vitreous humor fluid remained uncovered by epithelial tissue similar to control lesions. However, there were marked differences in collagen structure, with wounds treated with crystal humoral fluid exhibiting a denser and more organized collagen compared with other wounds

By day 14, significant changes in wound healing were observed. Wounds treated with PRP showed partial fusion of the wound margin, whereas control wounds did not show fusion unlike wounds treated with vitreous humor fluid showed complete fusion. Wounds treated with PRP and vitreous humor fluid also showed granulation tissue compared to the control group.

On day 21, a new collagen pocket was inserted that resulted in a wound heavily filled with vitreous humor fluid composed of fully mobilized tissue, demonstrating the efficacy of the treatment. In contrast, the control group showed incomplete healing. This was reflected in the histopathology images, which showed more collagen pockets in vitreous humor fluidized lesions compared to the control group.

Histopathology results showed that vitreous humor fluid therapy resulted in better wound healing compared to PRP treatment and control group. They provided rapid and complete epithelialization, complete wound margin fusion, granular tissue formation and collagen structure were enhanced in lesions with vitreous humor fluid.

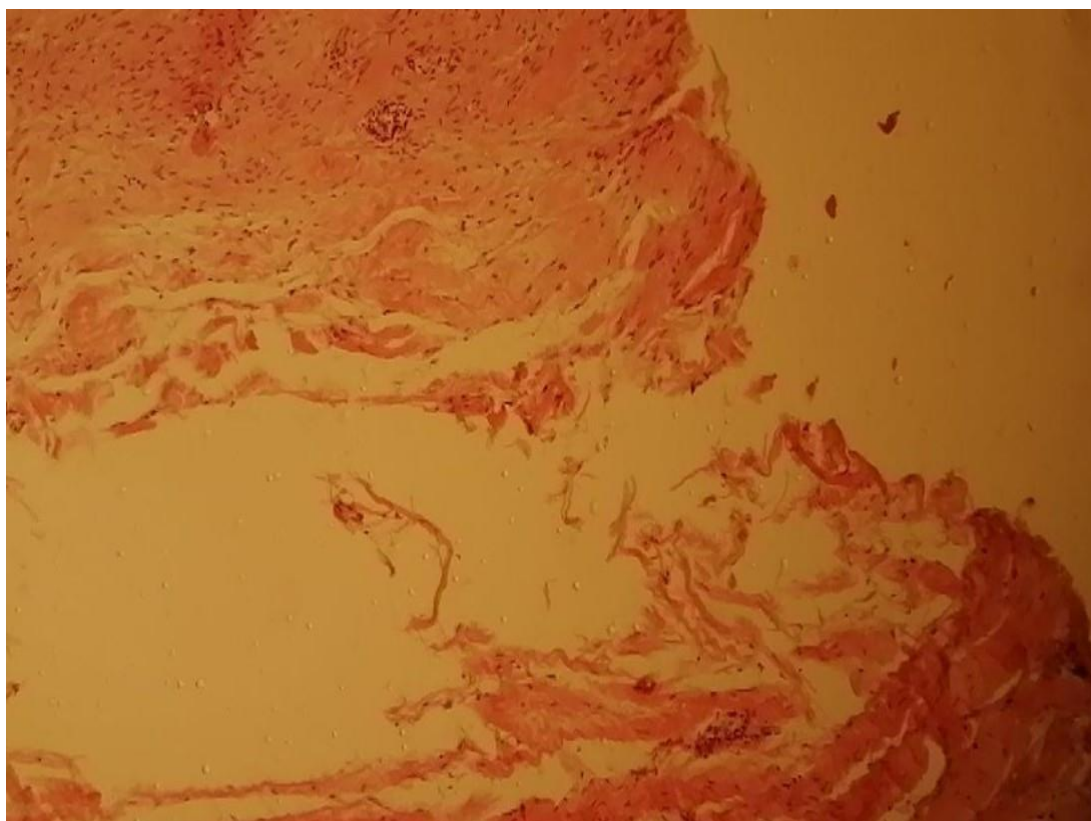


Figure 1.

A section of skin showed open skin wound. The wound entirely uncovered with accumulation of few numbers of macrophages and fibroblast as well as accumulation of collagen fibers. H&E, X100.

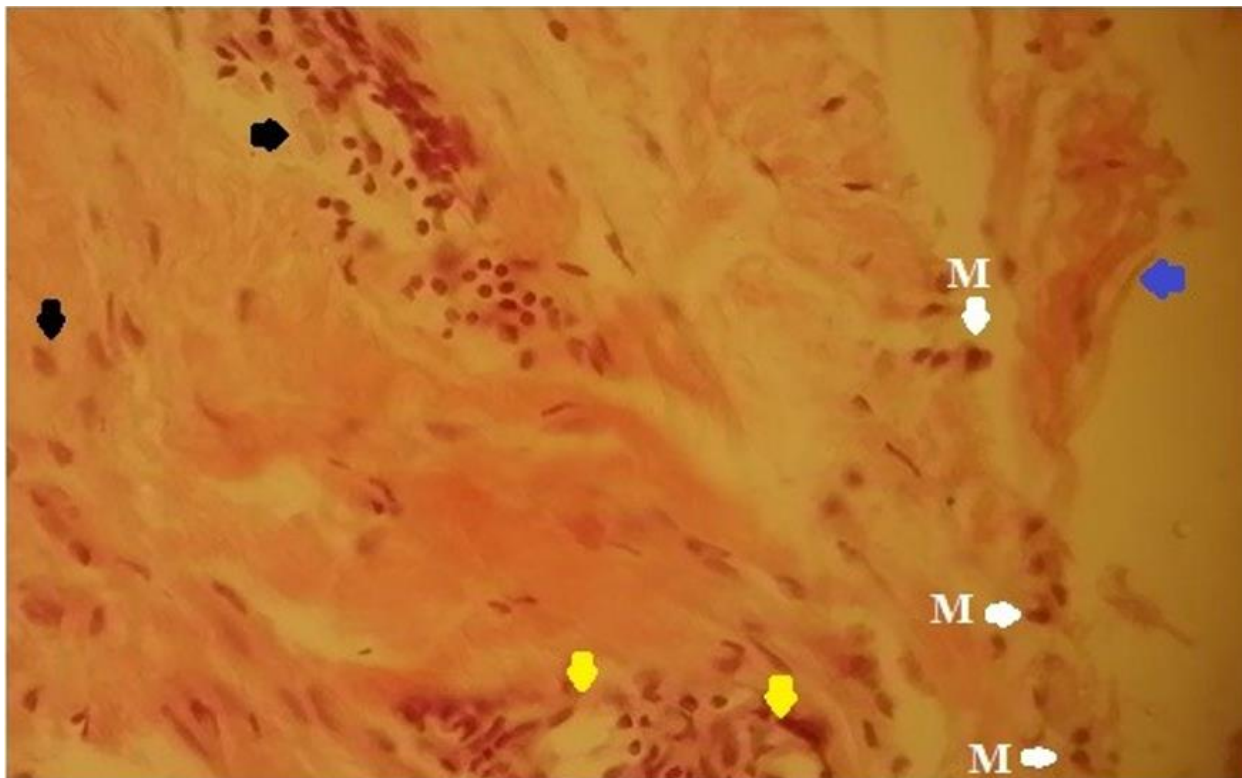


Figure 2.

A section of skin showed open skin wound. The edge of the wound is entirely uncovered with accumulation of few numbers of macrophages (M) and fibroblast (black arrows) as well as newly formed fibrin network (blue arrow) and presence of newly formed blood vessels (Yellow arrows). H&E, X400.

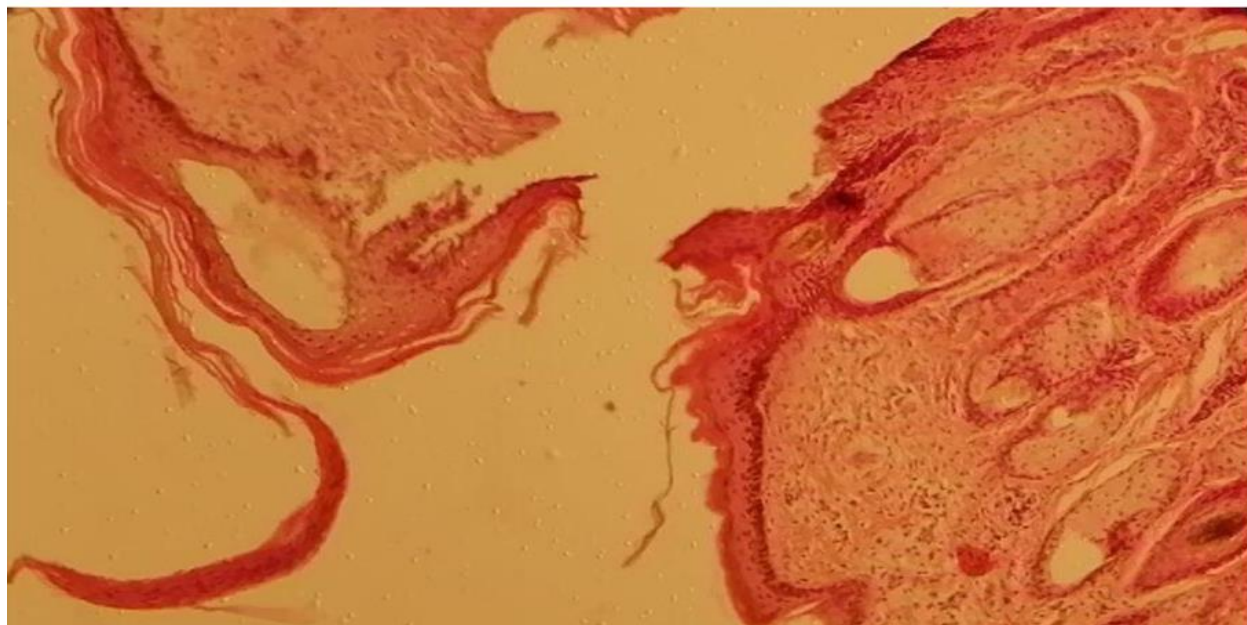


Figure 3.

A Section of skin showed open skin wound. The wound entirely uncovered with accumulation of collagen in the edge of the wound. H&E, X10.

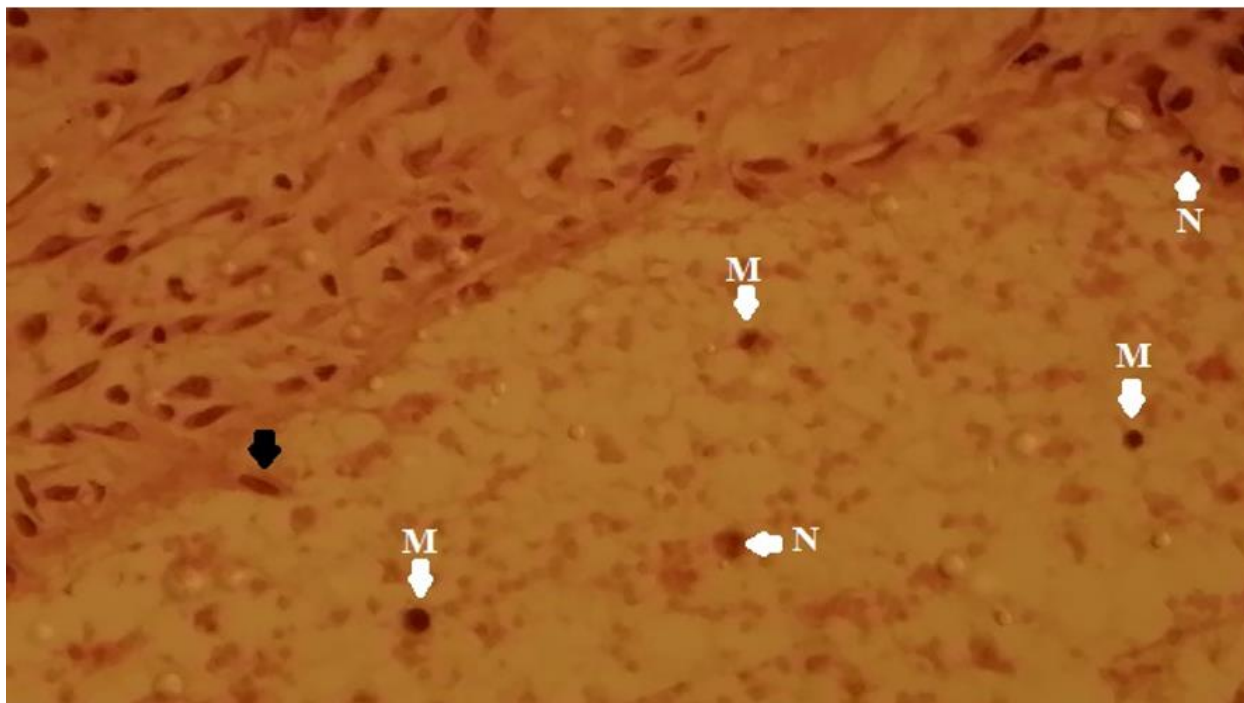


Figure 4.

A Section of skin showed open skin wound. The wound entirely uncovered with accumulation of inflammatory cells (Neutrophil=N; Macrophage=M), fibroblast (black arrow) and newly formed fibrin network. H&E, X40.

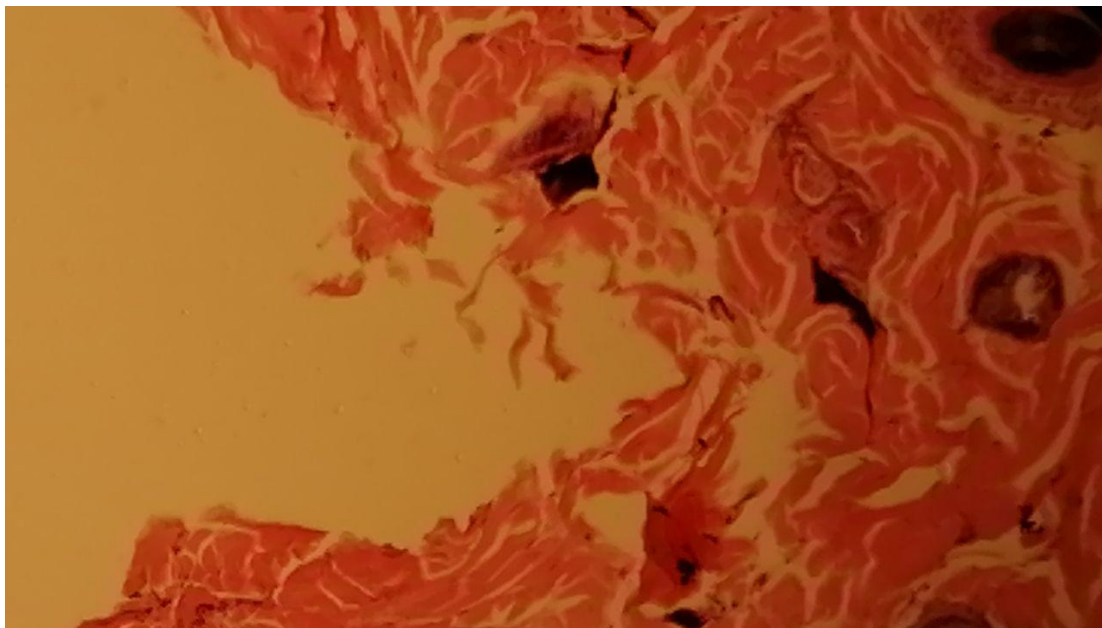


Figure 5.

A Section of skin showed open skin wound. The wound entirely uncovered with accumulation of collagen fiber in the edge of the wound. H&E, X10.

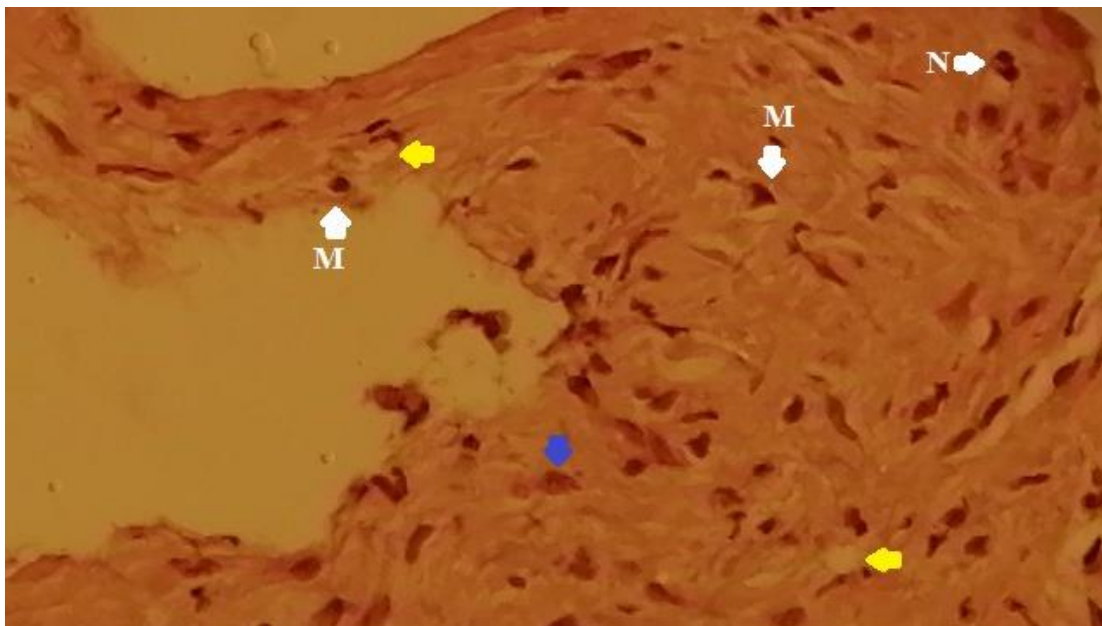


Figure 6.
A section of skin showed the edge of the open wound. H&E, X10.

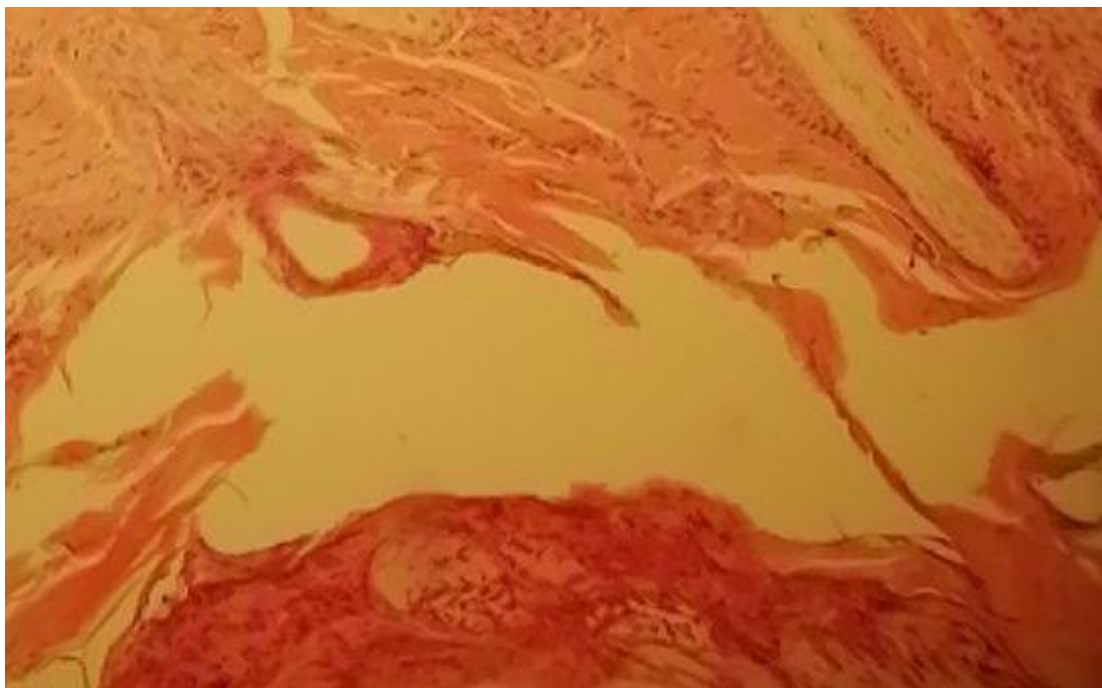


Figure 7.
A section of skin showed the regeneration process in the incision line . The re-epithelialization is still slow and incomplete and the surface of the wound is open, as well as accumulation of few numbers of macrophages, and few fibroblasts. H&E, X10.

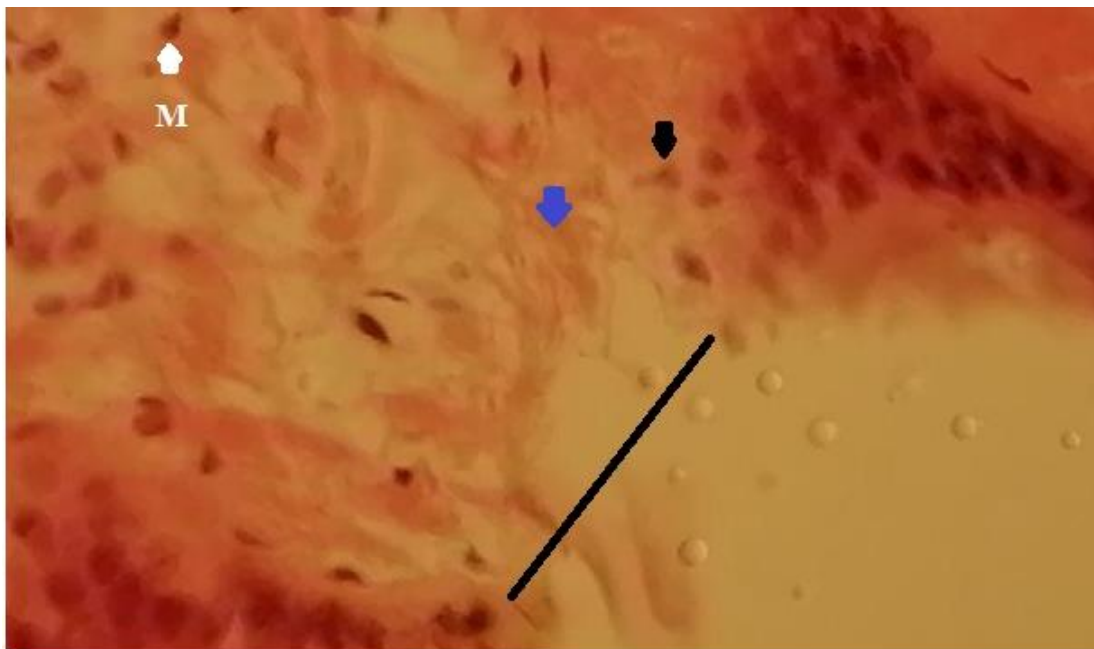


Figure 8.

A section of skin showed the regeneration process in the incision line (White arrows). The reepithelialization is still slow and incomplete and the surface of the wound is open (black line), as well as accumulation of few number of macrophages (M), and few fibroblasts (black arrow). H&E, X10.

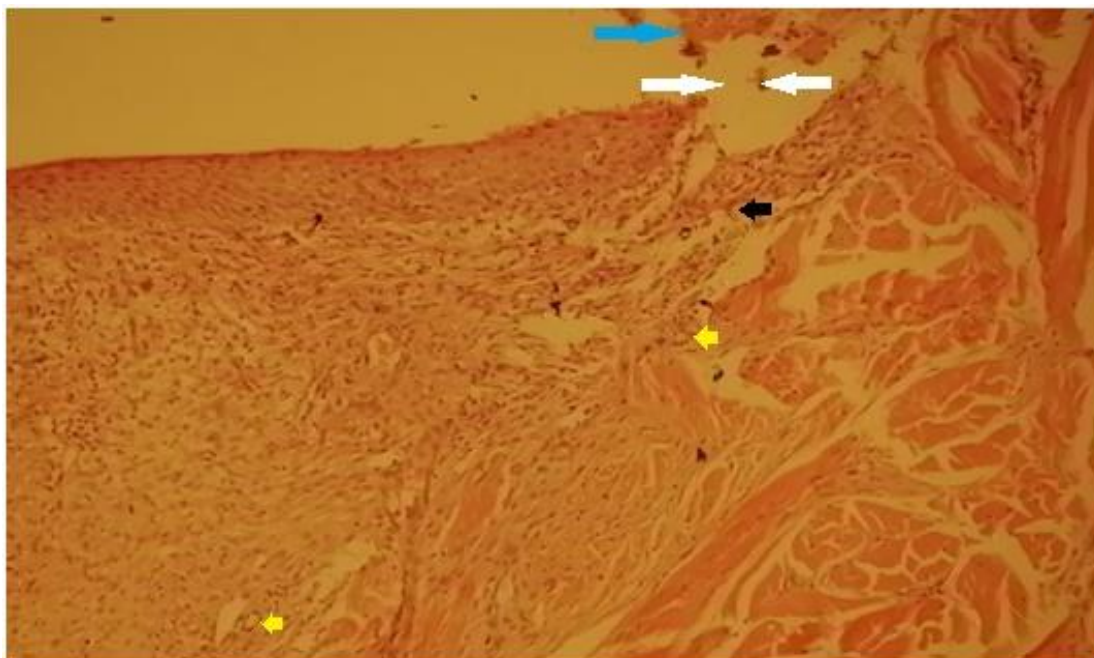


Figure 9.

A section of skin showed the regeneration process in the incision line (White arrows). There are a degree of reepithelialization (blue arrow), formation of newly blood vessels (yellow arrows), as well as, accumulation of inflammatory cell, fibroblasts and newly formed fibrin network. H&E, X10.

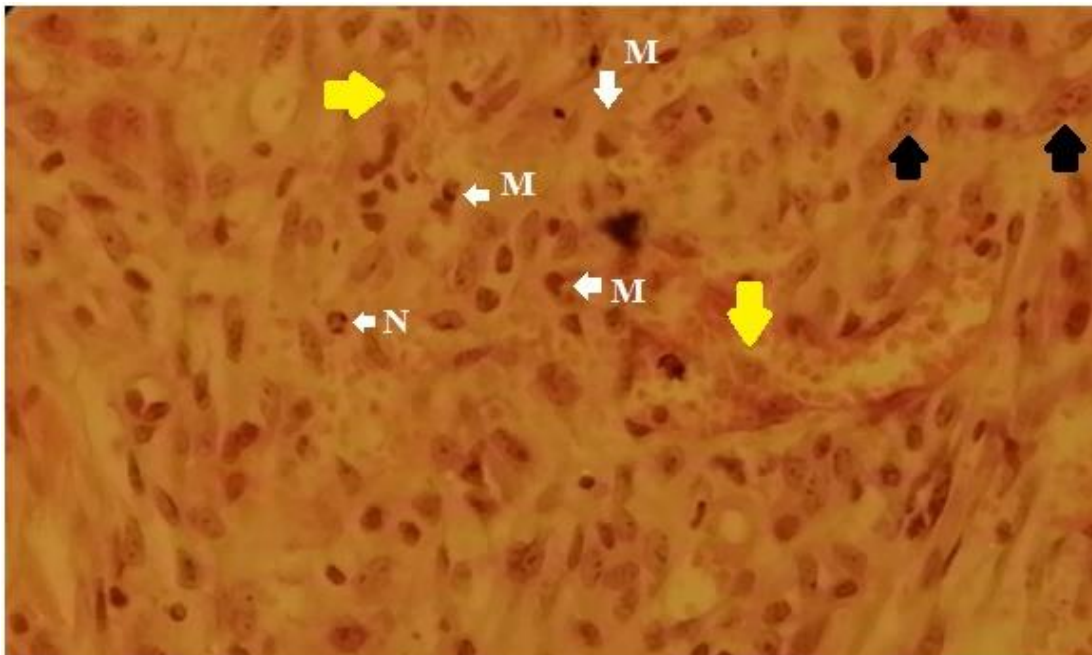


Figure 10.

A section of skin showed the regeneration process, there are accumulation of inflammatory cells (Neutrophil =N; Macrophage=M), and fibroblast (blue arrows), as well as newly formed blood vessels (black arrows). H&E, X40.

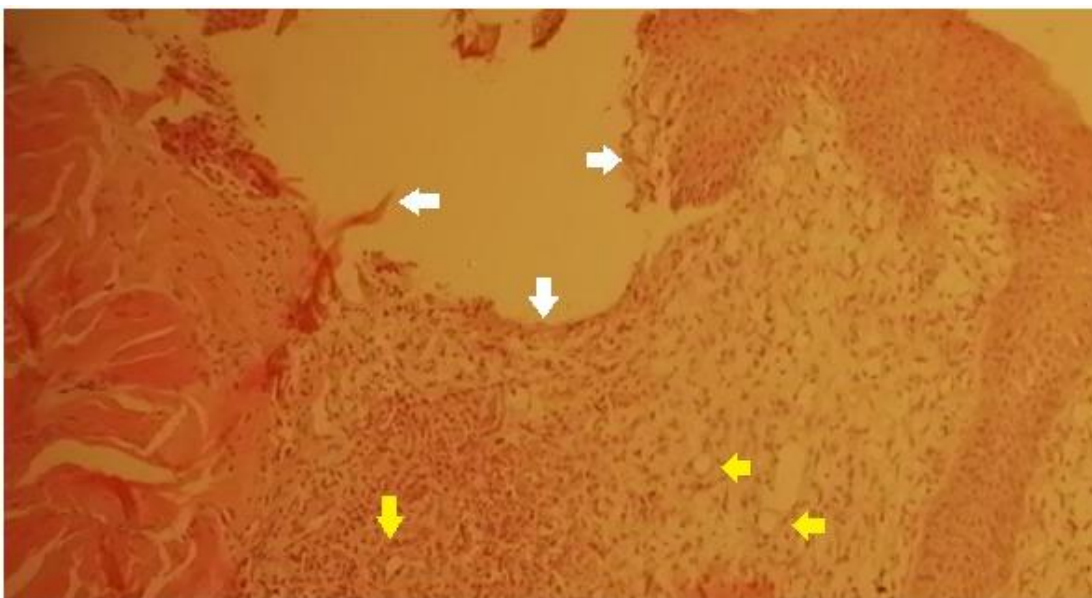


Figure 11.

A Section of skin shows the regeneration process in the incision line. The process of reepithelialization is shown and the wound is partially covered by epithelial tissue (white arrows), as well as, presence of newly formed blood vessels (yellow arrows) and accumulation of inflammatory cells and fibroblast. H&E, X10.

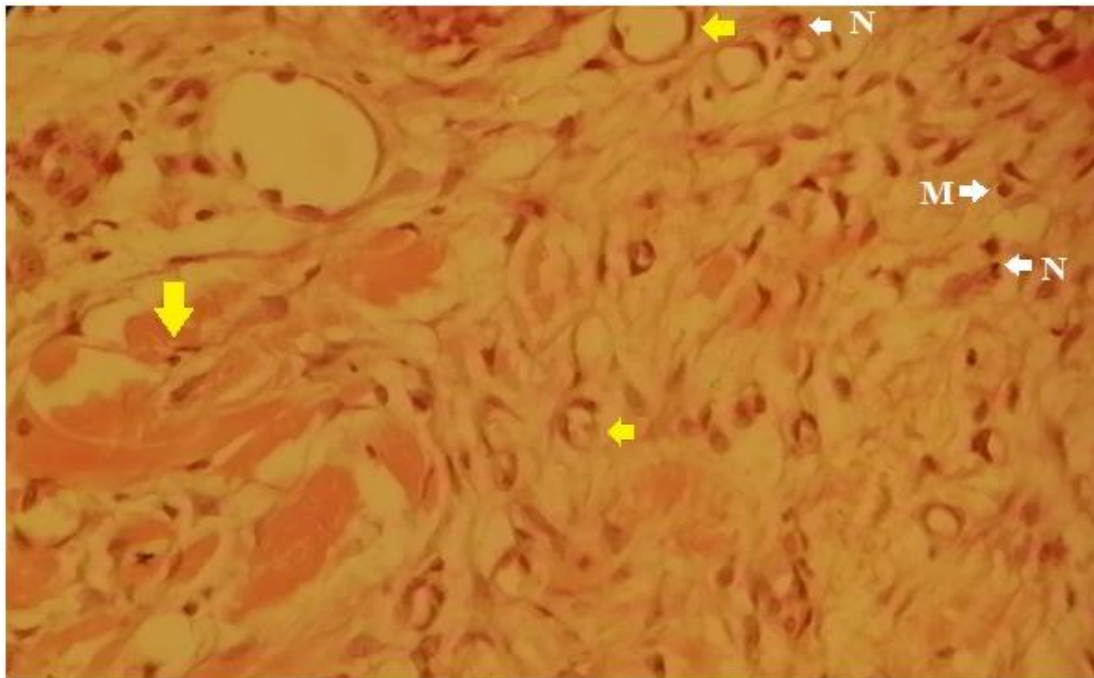


Figure 12.

A Section of skin: regeneration process in the incision line showed accumulation of inflammatory cells (Neutrophils=N; Macrophage=M) and fibroblasts, as well as newly formed blood vessels (yellow arrows). H&E, X40.

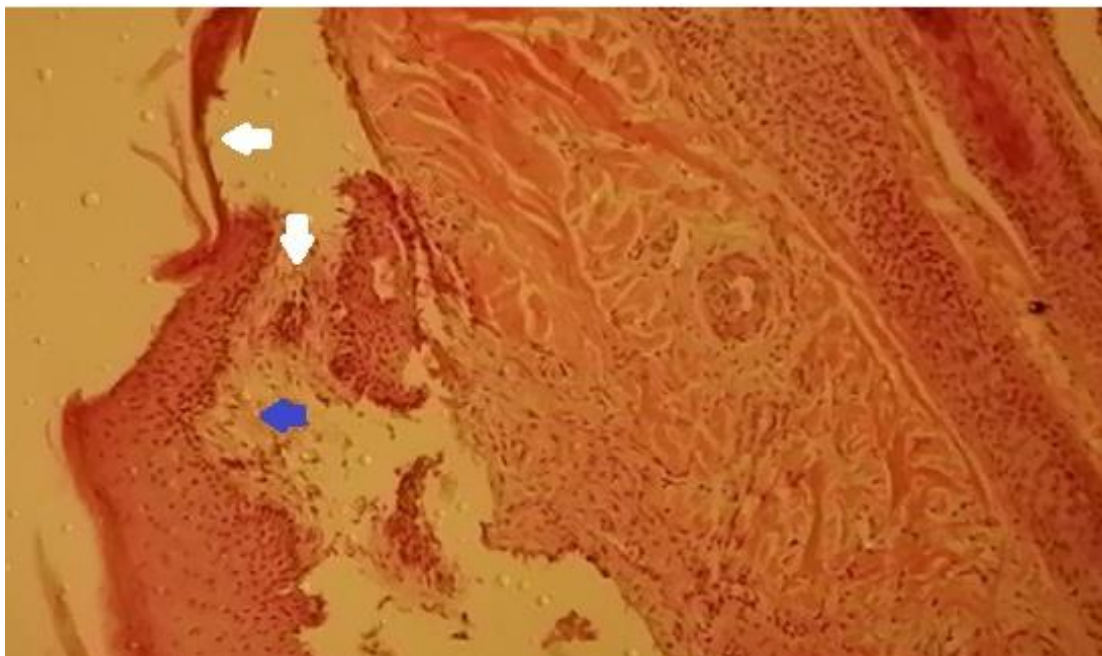


Figure 13.

A Section of skin showed formation of complete thin layers of epithelial tissue (white arrows), but edema in the dermis layer edema still present (blue arrows), and a newly formed fibrin network in dermis is seen. H&E, x10.

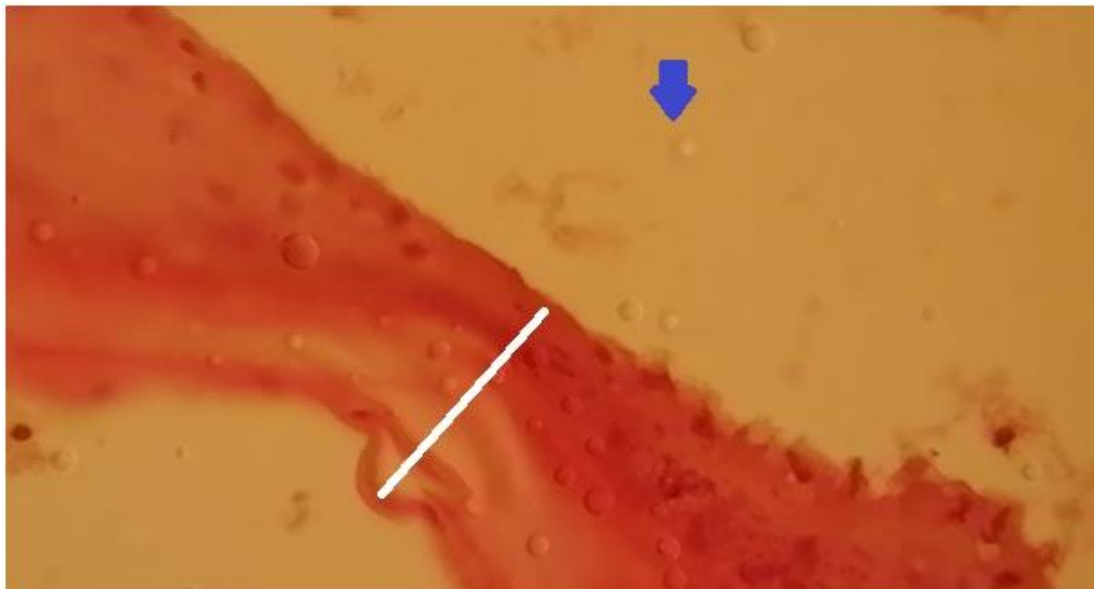


Figure 14. A Section of skin showed formation of complete thin layers of epithelial tissue (white arrows), but edema in the dermis layer edema still present (blue arrows). H&E, X40.

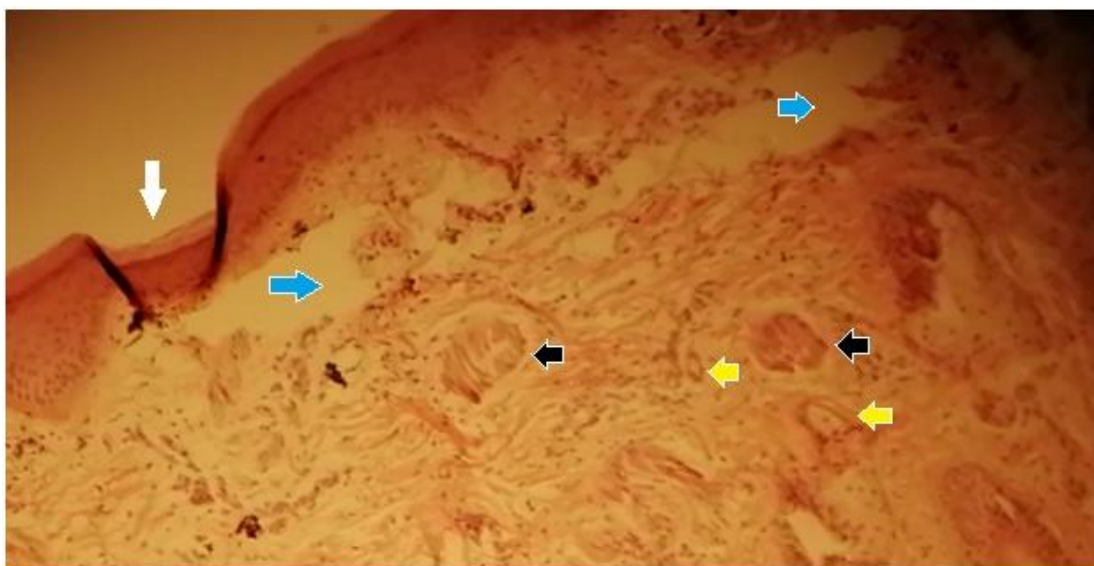


Figure 15. A Section of skin showed formation of complete thin layer epithelial tissue (white arrow), but edema in the dermis layer edema still present (blue arrows), as well as, newly formed blood vessels, newly fibrin network and hair follicles is obviously seen. H&E, x10.

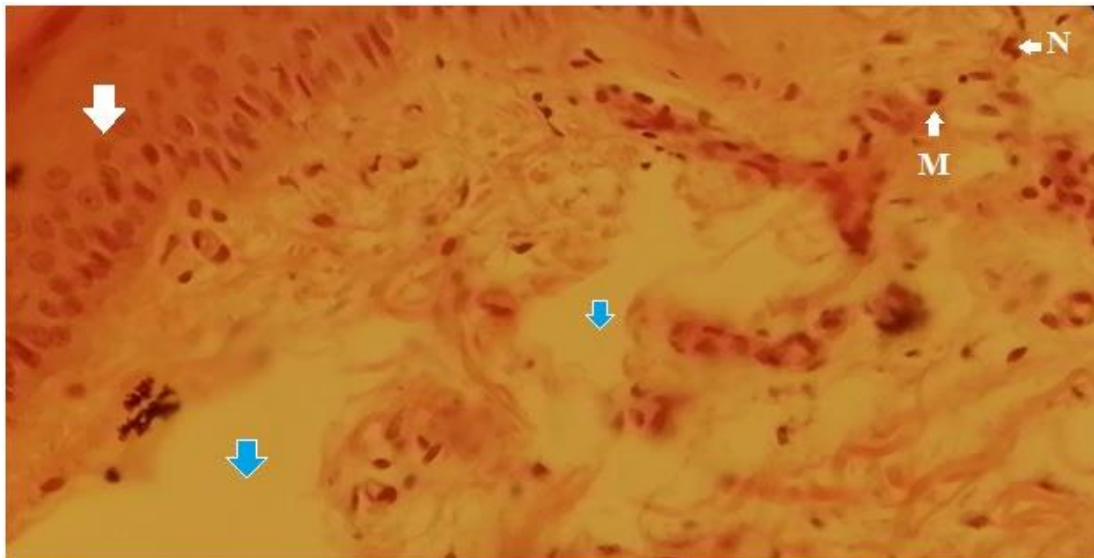


Figure 16.

A Section of skin showed formation of complete thin layers of epithelial tissue, but edema in the dermis layer edema still present (blue arrows) in the dermis layer, also, inflammatory cells (Neutrophil=N; Macrophage=M), newly formed blood vessels are seen. H&E, X40.

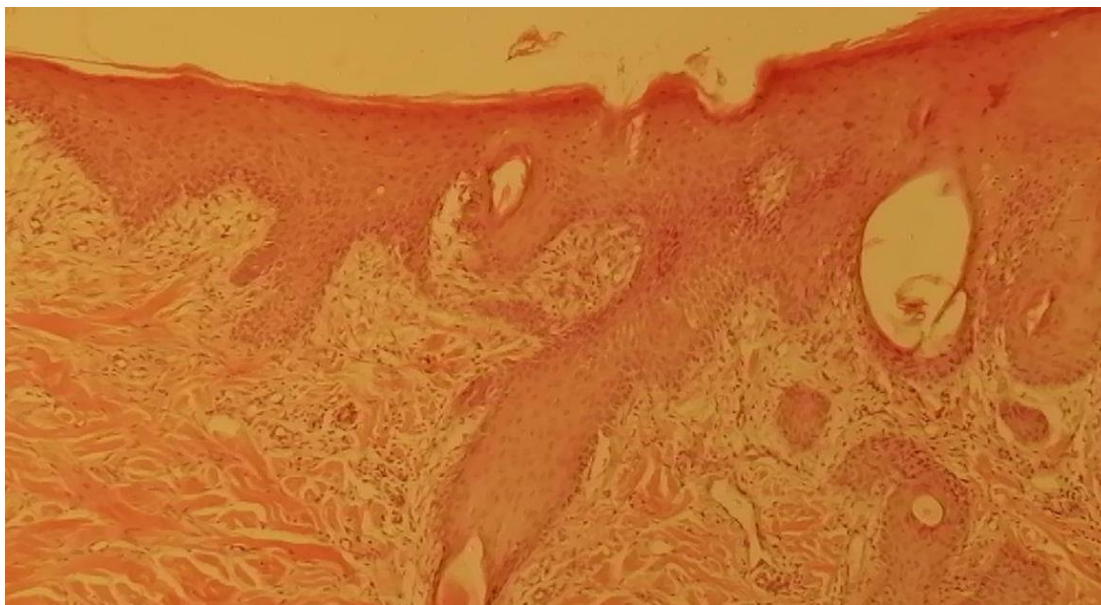


Figure 17.

A Section of skin showed complete healing of open skin wounds and formation of complete thick and condensed layers of epithelial tissue. All structures of the epidermis and dermis layers are present. H&E, X10.

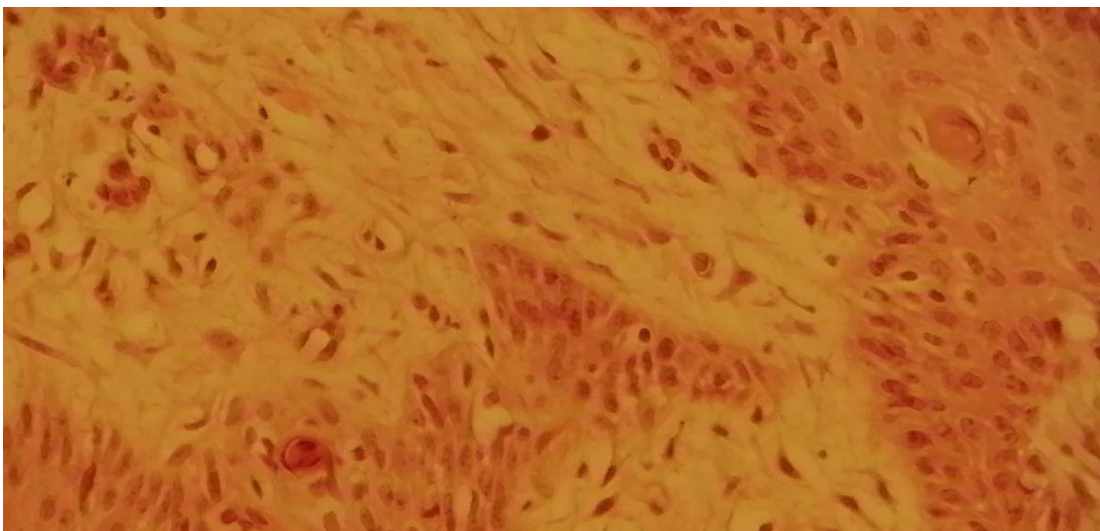


Figure 18. A Section of skin showed showed complete healing of open skin wound formation of complete layers of skin: epidermis and dermis layer. All structures of dermis layer are present. H&E, X40.

4. Discussion

The purpose of this contribution is to investigate the therapeutic potential of a combination of platelet-rich plasma (PRP) and vitreous humor fluid in the context of enhancing the recovery of cutaneous wounds in farm animals. The histological examination of the wound lesions at three separate time points—seven, fourteen-, and twenty-one-days post-harm—provided invaluable insights into the tissue reorganization, proliferation, and irritation that occurred in the experimental groups (40,41).

At day 7, the histopathological examination found out a positive accentuation of the degree of epithelialization in all organizations. However, the wound side surfaces within the PRP- and vitreous humor fluid-dealt with companies remained exposed with the aid of epithelial tissue, similar to the control wounds (18,42). This suggests that at this early stage of wound recuperation, neither PRP nor vitreous humor fluid by myself become capable of promote epithelialization (43-45). Interestingly, the collagen employer appeared to be denser and more prepared within the wounds treated with vitreous humor fluid in comparison to the opposite wounds (46). This locating suggests that vitreous humor fluid can also have a superb effect on collagen synthesis and company during the early tiers of wound recuperation (47).

At day 14, a cardinal alternate in wound restoration was located. Partial union of the wound margins turned into discovered within the PRP-dealt with group, even as entire union turned into located in the vitreous humor fluid-dealt with organization (48,49). In evaluation, the manage institution showed no tremendous improvement in wound healing. Furthermore, the wounds treated with PRP and vitreous humor fluid exhibited nearly whole epithelialization and higher granulation tissue compared to the control group(50,51). These findings indicate that the aggregate of PRP and vitreous humor fluid may additionally have a synergistic impact on wound closure and tissue regeneration throughout the mid-degree of wound healing (52, 53).

At day 21, whole epithelialization and healing have been observed in the wounds treated with vitreous humor fluid, with completely joined tissues. The new collagen bundles were densely crammed at the site of the wound in the dealt with companies, whereas the manipulate organization confirmed confined collagen deposition. This shows that the mixture therapy of PRP and vitreous humor fluid can also sell collagen synthesis and deposition, leading to stepped forward wound recuperation results in cattle (44, 54-55).

Overall, the results of this study demonstrate the potential therapeutic benefit of combining PRP with vitreous humor fluid to enhance cutaneous wound healing in animals. Histopathology examination

revealed faster wound closure, improved tissue regeneration, increased collagen synthesis, and decreased inflammation in the treated groups compared to the control group. Thus these data support the use of PRP and vitreous humor fluid as a promising treatment for wound healing in animals (44,56-60).

Acknowledging the limitations of this study, we must first consider its relatively small sample size; such a factor potentially restricts the generalizability of our findings. To confirm these results: future studies—with significantly larger samples—are not only suggested, but indeed necessary. This study also did not investigate the underlying mechanisms that drive the observed clinical interactions between PRP and vitreous humor fluid. We need further studies to elucidate both molecular and cellular pathways involved in wound healing, as well as potential roles of PRP and vitreous humor in integrating these pathways.

Concluding, we observed promising results in animal cutaneous wound healing when combining PRP with vitreous humor fluid. Our histopathology examination disclosed faster wound closure, enhanced tissue remodeling, escalated collagen synthesis and diminished inflammation within the treated groups compared to their respective control counterparts; these findings illuminate the potential therapeutic application of both PRP and crystal humoral fluids within veterinary medicine. Larger scale studies should further investigate this warranting research. Therefore, further investigation into the potential benefits of incorporating vitreous humor fluid and PRP for enhancing animal wound healing is necessary.

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