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Original Article

Investigation on the Influence of Synovial Fluid and Vitreous Humour on Avulsion Wounds Healing in Rabbits

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Abstract

In the present study, we investigated the efficacy of vitreous humour and synovial fluid on avulsion wound healing in a rabbit's model. The vitreous humour is a fluid that resembles gel consisting of approximately 98–99% water, little hyaluronic acid, glucose, collagen, anions, cations and ions. It is in the posterior eye chamber for the comparison with synovial fluid that consisted of hyaluronin, lubricin, proteinase, prostaglandins and collagenase. In this study, both synovial fluid and vitreous humour were collected from rabbits by aspiration of vitreous humour from the eye (postmortem) and arthrocentesis procedure was applied for collection of synovial fluid. Twelve adult rabbits were used in this study, they divided into three groups each group consisted of four animals wounded experimentally (an avulsion wound). Our results showed the influence of vitreous humour (group B) on healing of the wound is better than the synovial fluid (group C) in the clinical evaluation of shrinkage of the wound. The histo-pathologically changes also revealed that in the vitreous humour treated group (group B), the wound healing process proceeded better than other groups (control and synovial fluid groups). In conclusion, the histopathological and clinical observations demonstrated that application of vitreous humour on wound might be pivotal in improving the healing of avulsion wounds and establish a new tissue in rabbits.

Keywords: avulsion wounds healing, rabbits, synovial fluid, vitreous humour

1. Introduction

Wound is cellular and anatomical disruption of superficial body tissues in which is critical for continuity of function of the living tissue. Physical, microbial, thermal, chemical, or immunological insult to these tissues produce the injuries leading to wound formation. Torn, punctured or cut, in the skin tissue usually leads to open wounds. If the blunt force trauma results in contusion, it develops a closed wound. In addition, the avulsion wounds are result of accidental trauma or experiments (1, 2). Vitreous fluid is a gel that is similar to fluid filling the eye. In addition, it contains so many small fibers attached to retina (the tissue light-sensitive layer at the eye back). It is in the eye globe, between the retina and the lens (Figure 1), this fluid is viscous, cellular and colorless. It is normally clear and consisted of (99%) water and glucose, ascorbic and hyaluronic acid, inorganic salts and collagen fibers (type II) (3).

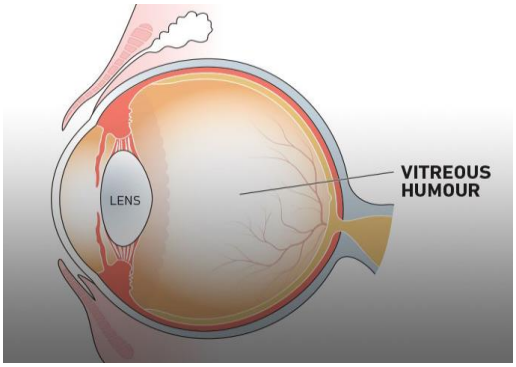

Figure 1. Globe with vitreous humour. Available at: https://visioneyeinstitute.com.au/eyematters/the-vitreous-humour/

The vitreous is important in protecting the eye. Most significantly, it helps to hold eyeball in its 'spherical' shape. It also touches the retina, so the pressure of vitreous humour assist to keep the retina in its place (4).

Synovial fluid, found within the articulation of bones, is a thick covering layer functioning as a lubricant and improves the joints mobility. It has small quantities within the articular space, where synovial membranes produce and secret it. It cushions bone ends to minimize the friction when the joints move the interconnected bones (5).

Synovial fluid is consisted of hyaluronic acid, collagenases, proteinases and lubricin and normal synovial fluid also contain hyaluronan (hyaluronic acid) secreted by the synovial membrane released into the joint cavity for the increase of the viscosity and elasticity of articular cartilages. It also operates as lubricant of the surfaces between the cartilage and synovium. It is a polymer of disaccharides of D-N-acetyl glucosamine and D-glucuronic acid, linked by thebeta-1,3 glycosidic bonds and alternating beta-1,4.

The synovial fluid is made of lubricin (also known as PRG4), as a minor lubricating element, released by synovial fibroblasts. In particular, it reduces the friction between opposing cartilage surfaces and also includes phagocytic cells (6, 7). Based on some similarities between the synovial fluid and vitreous in some functions and ingredients, in the current study we endeavored to evaluate the efficacy of vitreous humour and synovial fluid on avulsion wound healing in rabbit's model.

2. Materials and Methods

In this comparative study, we used 12 healthy rabbits, weighted between 2-2.5kg. The animals were put in cages with the same environment in animal house in Veterinary Medicine College, University of Basrah. Mixed local breed rabbits were housed under controlled environmental conditions (20±2°C,14:10h light: dark cycle) and allowed ad libitum access to food and water. Rabbits spent two weeks in the cages for acclimatization. Then the animals were divided randomly into three equal groups (n = 4), the control group (group A), the vitreous humour treated group (group B) and the last treated group was synovial group (group C). pre-operation, the animals were anesthetized by an injection of 0.25 ml of ketamine (15 mg/kg b.w.): xylazine (10 mg/kg b.w.) mixture into the marginal ear vein (8-10).

2.1. Avulsion Wound

Avulsion wounds were surgically created in all rabbits in the dorsal surface of the back (Figure 2) and the size of wounds were measured to be evaluated during the study period (7 and 14 days). The collected treated material was applied (Figure 3) on the wounded skins two times/day during the experiment period (days 7 and 14).

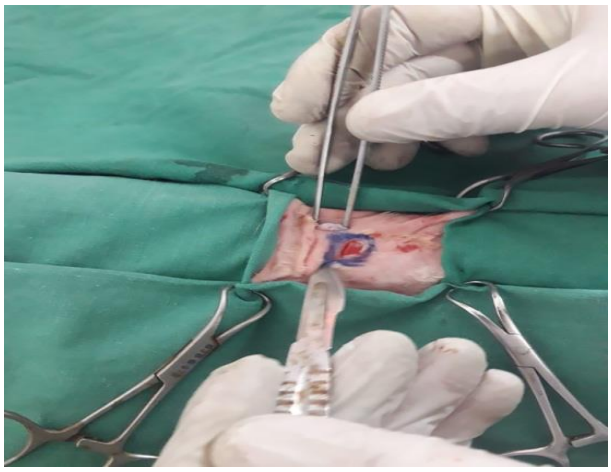

Figure 2. Avulsion wound

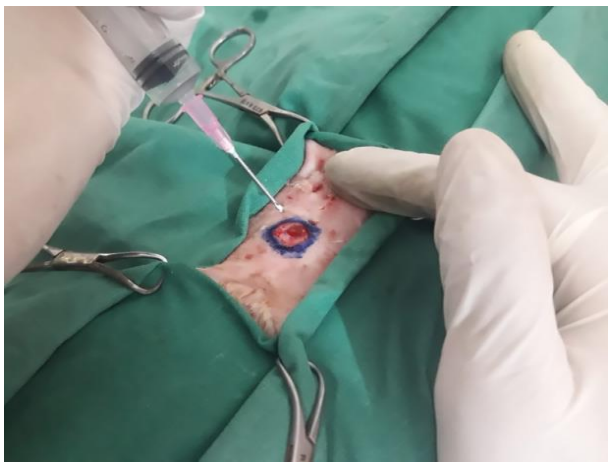

Figure 3. Applied Treated Substance

2.2. Vitreous Humour Aspiration

The vitreous humour fluid obtained from eye of a slaughtered sheep was aspirated after enucleation of the eye by fine needle aspiration technique (FNA) (Figures 4 and 5).

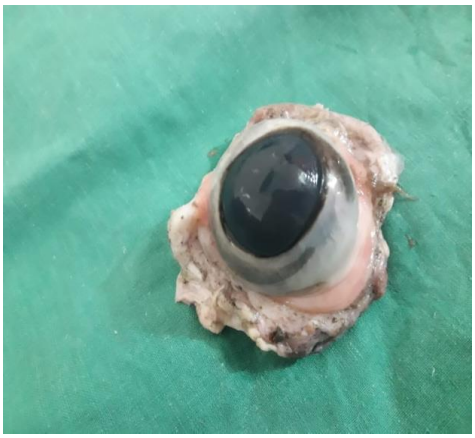

Figure 4. Enucleated Eye

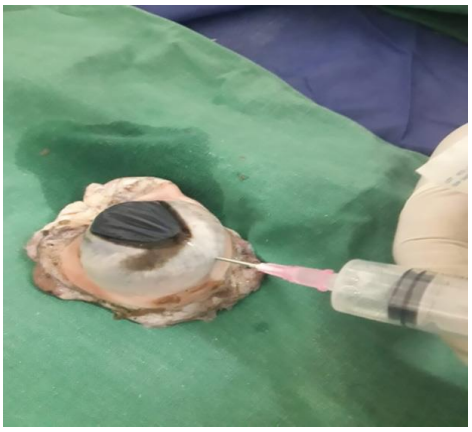

Figure 5. Aspiration Technique

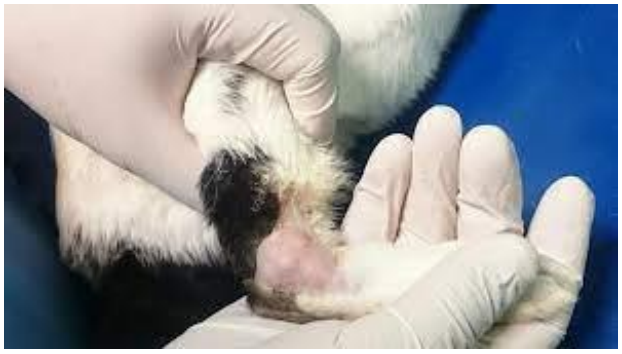

Figure 6. Stifle joint preparation

Figure 7. Synovial fluid aspiration

2.3. Synovial Fluid Aspiration

The synovial fluid was collected from a rabbit stifle joint by single-needle arthrocentesis (Figure 6 and 7). In the process of arthrocentesis, the synovial fluid collection was performed by penetrating in the joint space by a 20 G needle.

3. Results

3.1. Macroscopically Finding

In the present study we assessed the wound healing according to the wound dimensions (narrowing) in three different groups within the different period of our study.

3.1.1. Wound Status Evaluations after Seven Days

3.1.1.1. Control Group

The initial dimension of wound in the control group was 17.5 mm in average, after one day from the operation, the wound size, with the mean size of 17.1mm, apparently tended to narrowing as a result of wound shrinkage (Figure 8). In the last day of the first week, the narrowing of the wound continued and their sizes reduced to mean of 16.5 mm by scar tissue formation (Figure 9).

3.1.1.2. Vitreous Humour Group

The initial dimension of wound in the vitreous group was 17.5 mm, a day after the operation apparent narrowing the wound size was observed (13.8 mm) as a result of wound shrinkage with exudates formation

(Figure 10). In the last day of the first week, the continuity in the narrowing of wound was obvious and its mean decreased to the size of 4.8mm with scar tissue formation (Figure 11).

3.1.1.3. Synovial Group

The mean of dimension of wound in the synovial group at the first day was 17.5 mm. After one day from the beginning of the present study (wound extraction) it was obviously detectable that the wound apparently tended to narrowing. The avulsion wound sizes as a result of wound shrinkage with exudates formation reached to 15.1mm. (Figure 12). In the last day of the first week the results showed the continuity in the narrowing of wound decreasing to the size of 13.7 mm with scar tissue formation (Figure 13).

Figure 8. Control group after the first day

Figure 9. Control group after 7 days

Figure 10. Vitreous group after 1 Day

Figure 11. Vitreous group after 7 Day

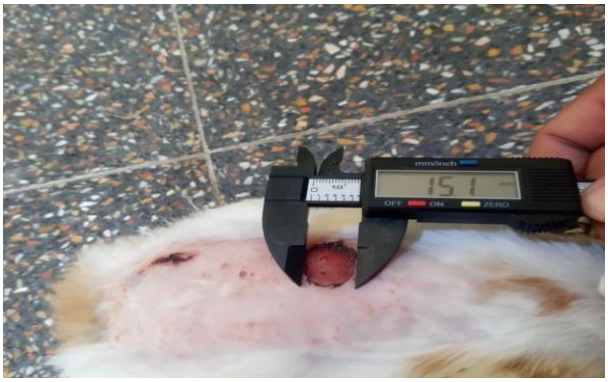

Figure 12. Synovial group after first day

Figure 13. Synovial group after 7 Days

3.1.2. Wound Status Evaluations after 14 Days

3.1.2.1. Control Group

In the control group after a period of 14 days from the initiation of the experiment, the recorded data showed the continuous reduction in the dimension of the wound in the control group. It reached to mean of 8.3 mm with scar formation (Figure14).

3.1.2.2. Vitreous Humour Group

In the vitreous group after a period of 14 days from the initiation of the experiment, the results demonstrated that the wound area decreased to 2.9 mm which was closer to the size of wounded skin in the last day of study (Figure 15).

3.1.2.3. Synovial Group

In the synovial group the dimension of wound after a period of 14 days from beginning of the study showed a significant reduction in the avulsion wound size of 6.6 mm as a result of wound shrinkage (Figure 16).

Figure 14. Control group

Figure 15. Vitreous group

Figure 16. Synovial group

3.2.1. Wound Status Evaluations after 7 Days

3.2.1.1. Control Group

After seven days from beginning of the current experiment, the microscopically alterations in the section of wounded skin revealed in the site of wound with surrounding inflammation and hemorrhage (Figure 17).

3.2.1.2. Vitreous Humour Group

In the vitreous humour fluid treated group after a period of seven days from the initiation of the experiment the microscopic observations in the section of wounded skin showed re-epithelization of the epidermal layer, also a clear deposition and arrangement of the collagen fibers in the dermal layer was obvious (Figure 18).

3.2.1.3. Synovial Group

Synovial fluid treated group showed a clear delay in re-epithelization of the epidermal layer after seven days with deposition of keratinized structures. Besides, a deposition and arrangement of collagen fibers in the dermal layer was recorded (Figure19).

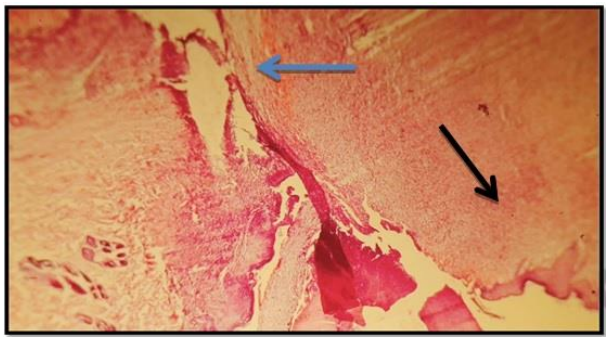

Figure 17. Section of wounded skin revealed inflammation (green arrow) and hemorrhage (blue arrow head) around the wound H&E 40×

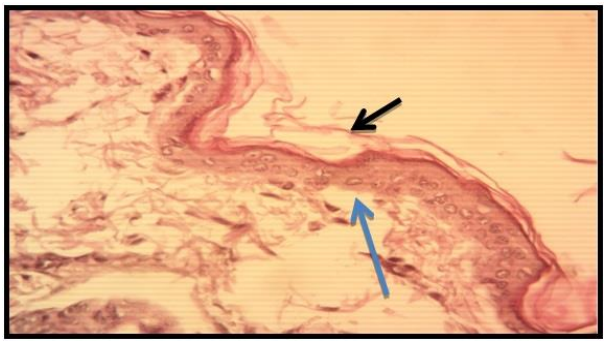

Figure 18. Vitreous humour fluid treated group showed re-epithelization of the epidermal layer (black arrow), also a clear deposition and arrangement of collagen fibers in the dermal layer (blue arrow). H&E stain. 40×

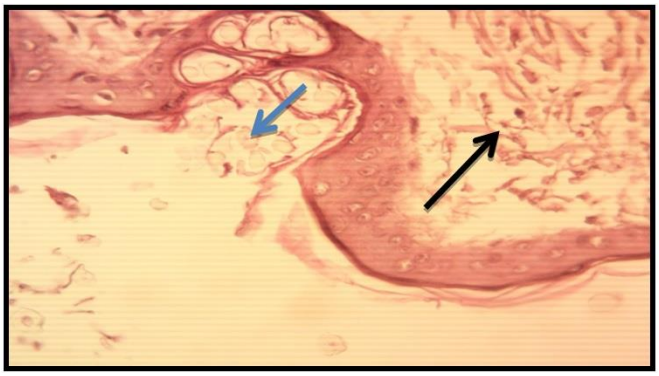

Figure 19. Synovial fluid treated group, delay in re-epithelization of the epidermal layer, deposition of keratinized structures (black arrow) and deposition with arrangement of collagen fibers in the dermal layer are demonstrated (blue arrow). H&E stain. 40×

3.2.2. Wound Status Evaluations after 14 Days

3.2.2.1. Control Group

The results of the microscopic evaluations after a period of 14 days in the section of the wounded skin in the control group showed some degrees of inflammation in the wound and scale formation with delayed re-epithelization (Figure 20).

3.2.2.2. Vitreous Humour Group

In the vitreous humour fluid treated group after 14 days from the beginning of the study, the microscopically observations in the section of wounded skin demonstrated the continuity in the epithelization of the epidermal layer; and also revealed a complete deposition and arrangement of collagen fibers in the dermal layer with new vascularization (Figure 21).

3.2.2.3. Synovial Treated Group

Synovial fluid treated group showed clear delay in re-epithelization of the epidermal layer with scale formation and continuous deposition of keratinized structures and with arrangement of collagen fibers in the dermal layer after 14 days (Figure 22).

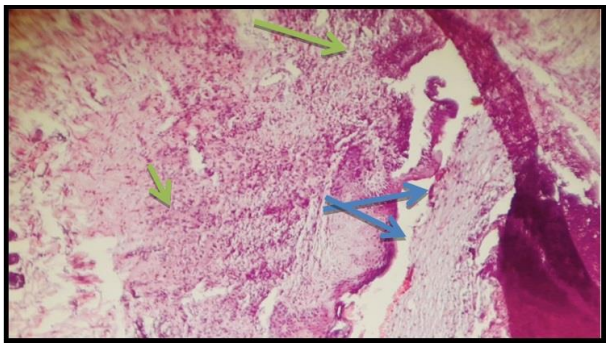

Figure 20. Control group after 14 days, inflammation in the site of wound and (green arrow head) re-epithelialization of epidermal layer (blue arrow head) H&E 40X

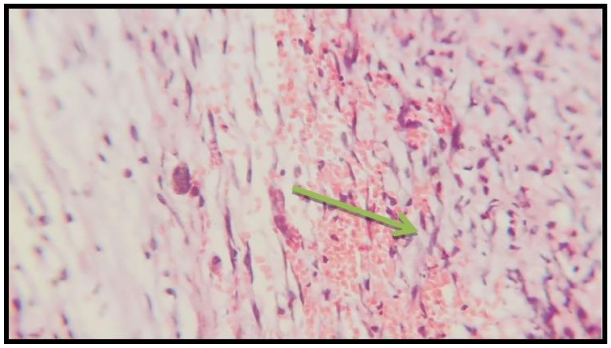

Figure 21. Vitreous group after 14 days, hemorrhage of the wound, arrangement and deposition of collagens with new vascularization (green arrow head). H&E 500X

Figure 22. Synovial group after 14 days, collagen deposition in the wound with scale formation and re-epithelization (green arrow). H&E 40X

4. Discussion

Avulsion wounds with entrance or wounds with pockets tend to heal superficially and encapsulate infection. The consequence is that the wound breaks open again after a short time, possibly also with manifestations in other places on the body surface. The aim of the current study was to understand that a wound actually granulates from bottom to top or from inside to outside. For this purpose, the wound care must always be extended to the wound bed. Such treatments are often very painful due to increased friction occurs when tamponing and removing the wound care material, which can also injure the granulating tissue (11). In our study usage of the liquid materials (vitreous and synovial) by drops application technique was evaluated.

The use of synovial fluid and vitreous humour lead to easy access to the damaged tissue. In addition, it helps to resist against microbial contamination and, most significantly, as a sources of collagen and hyaluronic acid. It also assists their influences on wound healing.

Wound contraction, kept by contractile elements in the scar tissues leads to the closure of "open wounds". Likewise, it might help to have appropriate avulsion wound incision closure specially when there is lack of contraction in the wound due to differences in the scarring patterns (12, 13).

Macroscopic evaluation in the current study at day 7th from the beginning of the experiment indicated that there were significant effects on the vitreous and synovial treated groups on wounds compared with the control group. On the 14th days, both vitreous and synovial substances significantly reduced the diameter of the wounds compared with the controls, these results are in agreement with other studies are done by Miller, Miller (14) and Mohamadi, Lari (15), but such effect was not observed on the fourteenth day at the control group.

Microscopic observations on piece of wounds sections, in all three groups, demonstrated inflammation within 7 days post-operation. In the vitreous group, the vitreous gel had cells of various sources that are capable of transforming into cells similar to fibroblast or myofibroblast. In addition, they work like fibroblasts or myofibroblasts and increase wound healing and scaring in the wound cavity in the comparison to the other two groups (synovial and control). In this group, this manifestations was evident seven days post-operation agreeing with Binder (16).

After 14 days, to evaluate the histopathology of the cells, the tissue was removed and examined. The re-epithelization with new vascularization of the wounded tissue indicated the benefits of pattern manner of healing, and in the present study these changes were very clear in one treated group (vitreous) after 14 days compared with control group. Moreover, in the synovial group when delay in re-epithelization with

scale formation was observed, this finding is discussed and supported by Pastar, Stojadinovic (17).

Vitreous is unique fluid in the body. It has a different structure, anatomic place, and is isolated from other fluids of the body. The synthesis of the citreous represents the serum concentrations of several components in the next mortem period. It can be easily retrieved and analyzed. Also, there are several identified exogenous chemicals and molecules in the vitreous (17).

In conclusion the results of this work revealed that improvements in wound healing is expected by topical synovial and vitreous fluid formulation. This formulation requires additional analyses to identify the underlying molecular mechanisms.

Authors' Contribution

Study concept and design: L. A. N. and Z. B. A. K.
Jabbar, Z. A1
Acquisition of data: L. A. N. and Z. B. A. K.
Analysis and interpretation of data: M. M. J.
Drafting of the manuscript: A. A. K.
Critical revision of the manuscript for important intellectual content: L. A. N. and Z. B. A. K.
Statistical analysis: Z. A. J.
Administrative, technical, and material support: L. A. N. and Z. B. A. K.

Ethics

The present study was approved by the Ethics Committee of the Veterinary Medicine College, University of Basrah.

Conflict of Interest

There are no conflicts of interest to disclose. The authors of this study were not financially or otherwise associated with organizations that would bias the outcome of the present study.

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