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 humor and synovial fluid on avulsion wound healing in rabbit’s model. The vitreous humor 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 chambers for the comparison with synovial fluid that consist from hyaluronin, lubricin, proteinase, prostaglandins and collagenase. Both synovial fluid and vitreous humor were collected from rabbits by aspiration of vitreous humor from the eye (postmortem) and arthrocentesis procedure in the collection of synovial fluid. Twelve adult rabbits were used in this study divided into three groups each group consist of four animals wounded experimentally as avulsion wound, the results showed vitreous humor (group B) influence on the healing of the wound is better than the synovial fluid (group C) in the clinical evaluation of wound narrowing. The histo-pathologically changes also revealed that in the vitreous humor treated group (group B) wound healing process proceeded better than other groups (control and synovial fluid groups). In conclusion, the histopathological and clinical observations mention that application of vitreous humor on wound will be pivotal in improving the avulsion wound healing and establish a new tissue in rabbits.

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

1. Introduction

Wound is cellular and anatomical disruption which has a continuity of function of the living tissue. Physical, microbial, thermal, chemical, or immunological insult to the tissue produces the injuries leading to wound formation. Torn, punctured or cut, in the skin tissue is known as an open wound. If the blunt force trauma results in a contusion, it becomes a closed wound. In addition, the avulsion wounds result from accidental trauma and wire experimentally (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 with (99%) water and glucose, ascorbic and hyaluronic acid, inorganic salts and collagen fibers (type II) (3).

The vitreous is important to protect the eye. Most significantly, it helps to hold it in a ‘spherical’ shape. It also touches the retina. The vitreous humor pressure could assist in keeping the retina in its place (4).

Synovial fluid found within the articulation of bone’s, synovial fluid is thick functioning as lubricant and improving the body joint mobility. It has small quantities between the joints, where synovial membranes produce and surrounds it. It cushions bone ends reducing any friction when the joints move in the knees, feet, shoulders, hands and hips (5).

Synovial fluid consists of hyaluronic acid, collagenases, proteinases and lubricin while normal synovial fluid is made of hyaluronan (hyaluronic acid) 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 polymer of disaccharides of D-N-acetyl glucosamine and D-glucuronic acid linked by thebeta-1,3 glycosidic bonds and alternating beta-1,4. 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 includes phagocytic cells (6, 7). Based on some similarities between synovial fluid and vitreous in some functions and ingredients in the current study the efficacy of Vitreous humor and synovial fluid on avulsion wound healing in rabbit’s model were evaluated.
2. Materials and Methods

In this comparative study, we used 12 healthy rabbits, weighted between 2-2.5kg. The animals were put in cages and have 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. The animals were divided randomly into three equal groups (n = 4), the first group was control group (group A), and the second was vitreous humor treated group (group B) and the last treated group was synovial group (group C). Before surgery, 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

Make avulsion wound surgically for all rabbits in the dorsal surface of the back (Figure 2) and measured the size of wound to evaluate during the study period time (7 and 14 days). Applied the collected treated material (Figure 3) on the wounded skin 2 times/day during the experiment period (7 and 14 days).
2.2. Vitreous Humour Aspiration

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

2.3. Synovial fluid aspiration

The synovial fluid collected from a rabbit stifle joint by the syringe and needle aspiration technique called arthrocentesis (Figure 6 & 7). In the process of arthrocentesis, the synovial fluid collection happens through the penetration of 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 7 days

3.1.1.1. Control group:

The initial dimension of wound in the control group was 17.5 mm, after one day from operation the wound size (17.1 mm) apparently tend to narrowing the as a result of wound shrinkage (Figure 8), in the last day of the first week it shows continuity in the narrowing of wound decreasing to the size of 16.5 mm with 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, after one day of operation apparently tend to narrowing the wound size (13.8 mm) as a result of wound shrinkage with exudates formation (Figure10), in the last day of the first week it shows continuity in the narrowing of wound decreasing to the size of 4.8mm with scar tissue formation (Figure11).

Figure 8. Control group after 1 day
Figure 9. Control group after 7 days

Figure 10. Vitreous Group after 1 Day
Figure 11. Vitreous Group after 7 Day
3.1.1.3. Synovial group:

The initial dimension of wound in the synovial group was 17.5 mm, after one day from beginning of the present study (wound extraction) it was obviously detectable that the wound apparently tend to narrowing of the avulsion wound size (15.1mm) as a result of wound shrinkage with exudates formation (Figure12), in the last day of the first week results showed continuity in the narrowing of wound decreasing to the size of 13.7 mm with scar tissue formation (Figure13).

![Figure 12. Synovial Group after 1 Day](image1)
![Figure 13. Synovial Group after 7 Days](image2)

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, recorded data showed the continuous decrease in the dimension of wound in control group. It reached to 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 closure 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 present study showed significant decrease in the avulsion wound size of 6.6 mm as a result of wound shrinkage (Figure 16).

3.2. Microscopically observations of the wound status

3.2.1. Wound status evaluations after 7 Days

3.2.1.1. Control Group

After 7 days from beginning of the current experiment, the microscopical changes in the section of wounded skin reveal the site of wound with inflammation around the site of wound and hemorrhage (Figure 17).

3.2.1.2. Vitreous humour group

In the vitreous humour fluid treated group after a period of 7 days from the initiation of the experiment the microscopical observations in the section of wounded skin showed re-epithelization of the epidermal layer, also a clear deposition and arrangement of collagen fibers in the dermal layer was detectable (Figure 18).

3.2.1.3. Synovial group

Synovial fluid treated group showed clear delay in re-epithelization of the epidermal layer after 7 days with deposition of keratinized structures, also a deposition and arrangement of collagen fibers in the dermal layer was recorded (Figure 19).
Figure 17. Section of wounded skin reveal the site of wound inflammation around the site of wound (green arrow) hemorrhage (blue arrow head) 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×
3.2.2. Wound status evaluations after 14 days

3.2.2.1. Control group

The results of the microscopically 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 site of 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 of the beginning of the study, the microscopically observations in the section of wounded skin appear continuity in the epithelization of the epidermal layer; also reveal 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 in the deposition of keratinized structures and deposition with arrangement of collagen fiber in the dermal layer after 14 days (Figure 22).
Figure 20. Control group after 14 days reveal the inflammation in the site of wound (green arrow head), re-epithelialization of epidermal layer (blue arrow head) H&E 40X

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

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

Wound contraction, kept by contractile elements in the scar tissues shows the closure of "open wounds". Likewise, it could help the good experimental avulsion wound incision closures and the lack of contraction in the wound as the cause to their variety of the scarring patterns (12, 13).

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

Microscopical observations on piece of wounds sections appear in all three groups, inflammation appeared evidently within 7 days post-surgery, in the vitreous group the vitreous gel have cells of various sources that is able 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 for the comparison to the other two groups (synovial and control). In this group, they seem evidently 7 days post-surgery agreeing with Binder (16).

After 14 days, to change and evaluate the cell histopathologically, the tissue was removed and examined. The re-epithelization with new vascularization of wounded tissue indicate the benefits pattern manner of healing, in present our study appear this changes very clear in treated group (vitreous) after 14 days to compare with control group and also with synovial group when reveal delay in re-epithelization with scale formation, these result discuss clearly and agrees with 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 and in the vitreous.

In conclusion the results of this work revealed that appropriate wound healing affected by topical synovial and vitreous fluid formulation. This formulation requires additional analyses to identify related molecular mechanisms.

Declaration 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.

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

References