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A Role of Bovine Cardiac Myosin-Hydrogel Polymere to Accelerate Wound Healing of Autograft Skin in Rabbits

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Abstract: ***The present study were showed more developed in 2nd and 3rd treated groups compare with control and 1st trated groups which were treated by bovine cardiac myosin-hydrogel polymer in three concentration 25%, 50% and 75%, while control group treated with gentamicine and hydrogel only, gross pictures were showed similar in all groups at 1-3 days after surgery, 2nd and 3rd groups were loss scar tissue above wound and wound line were showed until 9 days in 3rd treated group, while wound line in 2nd group were disappeared until to 10-13 days after surgery. Histopathological changes is very important to evidence the bovine cardiac myosin-hydrogel was effect to accelarete wound healing and speed recovery, collagen and fibers matrix in histopathology slides reveal to speed healing and the role of bovine cardiac myosin in wound healing, in conclusion the bovine cardiac myosin is highly effect in wound healing, in recommended ; to more study in this protein specially biochemical study and cytologyical study to knowledge the main elements whose play roles in wound healing.***

Keywords: bovine myosine, polymere, hydrogel, wound healing, cardiac myosine

1. Introduction

Bovine cardiac myosin (BCM) is a complex protein that is regarded to the superfamily prototype of motor protein (1), converts chemical energy in adinotriphosphate (ATP) to motor mechanical energy, thus generate forces and movements (2). BCM is a protein which extract from cattle heart and the calves have lage large quantity of myosin (3). Superfamily of myosin include myosin I, myosin II, myosin III, myosinIV, myosinV, myosin VI, and other type of myosin that related with main types of myosin such as myosin binding protein (MyBP) which subdivided into two types include MyBP-C and MyBP-H(1 1nd3). One of the best charesterstic properties of MyBP is its relatively strong affinity for actin protein (4), and react with C^{2+} to form force generation after C^{2+}-activiation which stimulate ATPase that convert chemical energy to movement the molecular motor (5,6). Myosin is a hexapolypeptide constituted by light and heavy chain the isoform of which segregate in bovine cardiac is polypeptide with molecular mass of about 20-200 kilodalton (KD) (7). Association the complex reaction of wound healing is calmodulin (CaM) that is highly react with C^{2+}. Calmodulin (CaM) found in all eukaryotic cells are multifunctional C^{2+}-binding regulatory protein that mediates many C^{2+}-related cellular events (9). Re-vascularization and vascular permeability are occasionally depend on myocin phosphorylation and calcium C^{2+}- calmodulin CaM activation to stimulate myosin chain bioactivity in permeablized endothelium, ATP activity, and C^{2+} motion, these processes are called myosin chain phosoryltion (MCP) are absolute requirements for bio-reaction and intercellular gap formation in the wound (10). The activity of myosin motor is modulated by phosphorylation of regulatory myosin chain kinase and phosphotase (11). Myosin ligand-based C^{2+} signaling may important in fibroblast-mediated wound repair, but the mechanism supporting transiet changes in C^{2+} are unkown (11). Magnesium (Mg^{+2}) play an important role in myosin bioactivity, ATPase activity of myosin is activated by C^{2+} at millimolar rang (12) , the high rate of ATP hydrolysis by myosin in the presence of millimolar C^{2+} , therefore stimulate Mg^{+2}-ATPase to chelate structure involving the two sulfohydral sits (H1 sit and H2 sit) of myosin protein (8). Myosin activity regulates cells migration, these dynamic of cells migration signals through protease-activited receptors (Par1 and Par2) such as epidermal growth factor (EGF), platelet-drived growth factor (PDGF), vascular endothelial growth facor (VEGF) (13). Actin-activited ATPase is stimulated when myosin regulatory light and heavy chains are phosphorylated by Myosin kinase(14).

Hydrogel is polymeric materials, that is hydrophilic structure of which capable to holding large amount water in their three-dimensional networks (15), it ability to absorb water arises from hydrophilic function attached to the polymeric backbone (15). Most researches were documented the hydrogel didn't effect on the process of wound healing, except burn wound healing due to the hydrogel have large amount of water to moist the burn area and prevent the infection (16).

Cutaneous wound injery, a series of coordinated events occurs include bleeding, coagulation, acute inflammation, cells migration, proliferation and protein synthesis as well as remodeling of extracellular matrix (17), cutaneous skin mesenchymal stromal cells(MSCs) regulate immune and inflammatory reseponces and enhance cutaneous wound healing (17). Cutaneous wound healing is a complex and well orchesteated biological process requiring the coordinated migration and profiliration of both keratinocytes and fibroblasts (18). Ca^{+2} play main role in wound healing process via intracellular- Ca^{+2} concentration channels (19), that promote wound healing as well as that is related with myosin phosphorylation (19). Several factors

affects on the process wound healing such as oxygenation, infection, sex hormones and stress factors as well as corticoid steroids drugs (20).

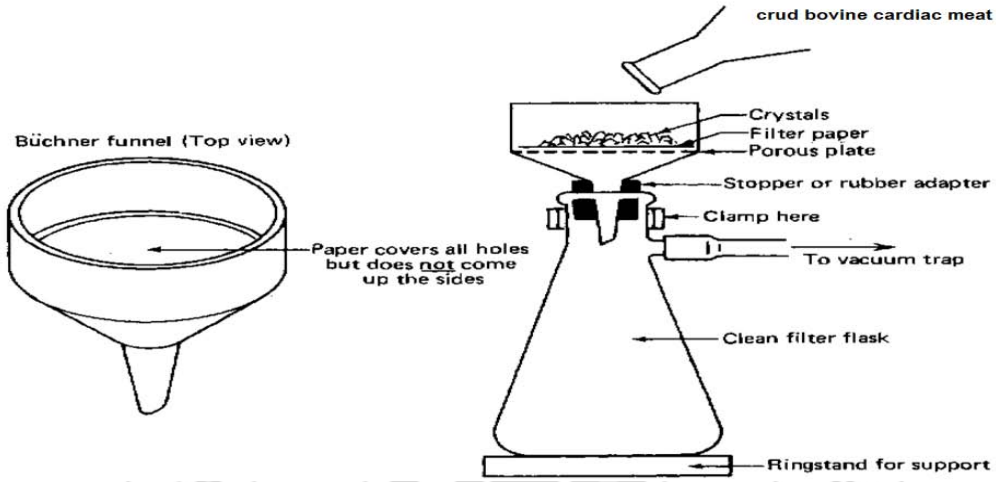
2. Materials and Methods

Bovine Cardiac Myosin Preperation

Extraction and Isolation

Bovine cardiac myosin extract was describe by Feuer *et al.* and Spudich *et al.* (21), these method was called *Acetone Powder Myosin Extraction,* calf hearts were brought at abattoir from fresh sloughted calves, right and left ventricals were sperated from other parts of heart, packed in ice box in 30 minutes of death, these tissues were then cut into small pieces and stored in a thin layer papers at -15 C° within 2hr in freez. A 400g meat batch was removed from the freezer, quickly chopped, and minced meat while still frozen and wash in 1.5 liters of 0.1M KCl for 10 minute with continuous strring at 4 C° to digest the minced meat (23). The residue was collected in fine nylon gauze over a *Büchner filter flask* and rewashed in 1.5 liters of 0.1 M KCl for 10 minutes, these procedure was repeated in 1.5 liters of 50mM Sodium Bicabonate for 7 minutes within 1.5 liters 1 mM EDTA for 7 minutes, after than rewashed 2 minutes in 1.5 liters of fresh water at 4 C° were performed and care was taken to remove as much of the water prior to extraction in acetone at 20C°, a 50% of aceton concentration in the picese, 2.5 liters volume of acetone was made ready in a beaker and the residue was crumbed by manual hand in a second beaker, the acetone was add rapidly within 10-20 second only, the residue was extract through clean nylon gauze over *Büchner filter flask,* finally the residue was quickly air dried with fan (22). Pic (1)

Picture (1) diagrammatic procedure of preparation of myosin



Purification

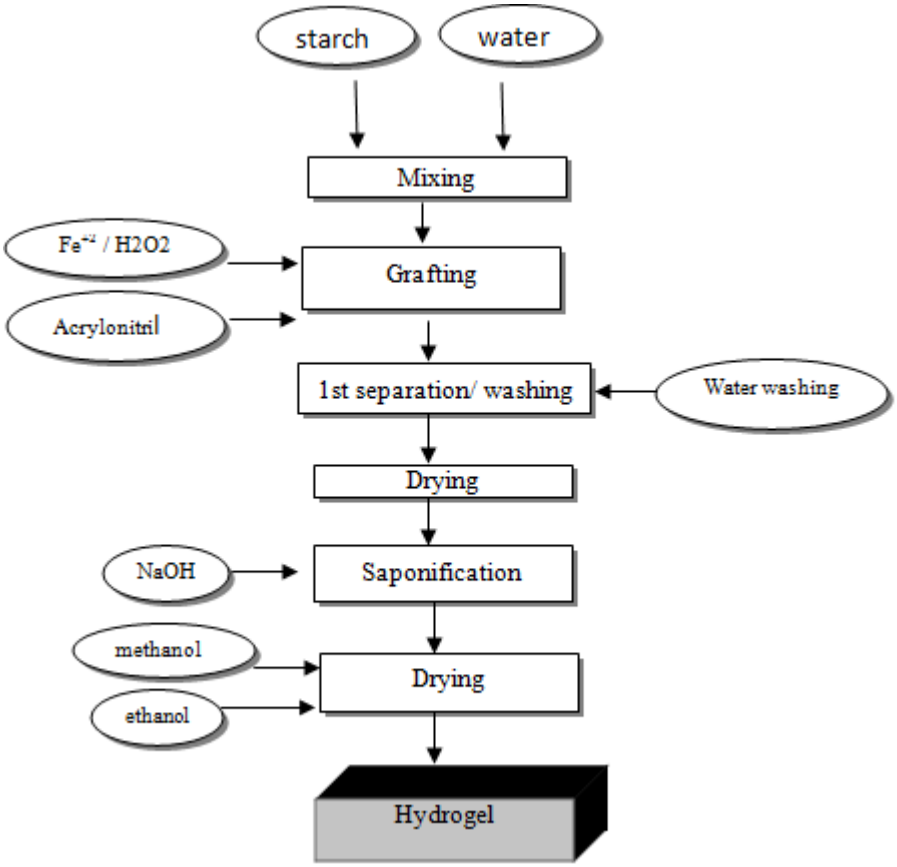
Bovine cardiac myosin was purified at 6.5-7.0 PH in 1 mM Di-Thio-Threitol (DTT)* and 50% glycerol at 20C° , further purification by protolytic degradation products of cadiac myocin (50 klotz)

Identification

To ensunce and more identification was tested by sepharose gel filteration electrophorsis (24).

Hydrogel Preparation

The hydrogel was synthesis from starch, the main process of this procedure is mixing of starch and water, inserting with acrylonitrile, separation and drying followed by saponification with alkali at 95C° for 1 hr, precipitation with methanol, washing with water free ethanol, and drying under vacuum at 60 C° for 3hr. A redox system (Fe^{+2} / H2O2) has been employed as a source [OH^-] free radical. Acrylonitrile (AN) / starch = 1.4 , oxygen peroxide (H2O2) 1.2 , 1.5 g starch. H2O2/FeSO4. 7H2O = 6(w/w) , liquid-sold =10-1, grafting temperature 30 C° , grafting time 90 min., saponification time 90 min. , 9 ml NaOH(0.7N)/g grafting starch, saponification temperature 95C°, methanol precipitation and washing (20 ml/g grafting starch), water and drying temperature 60 C°, and drying tim 3 hr. (25). The procedure is summarized by diagram pic. (2)



Myosin-Hydrogel Polymerization

Myosin protein after preparation with asptical technique was mixed with hydrogel polymer, packaging with sterial container, the ratio according experimental design (W/V) and kept under 20 C° , Pic (3)

Picture (3) myosine-hydrogel tubes with different concentration

Control group Hydrogel	Treated I group 25% myosin	Treated II group 50% myosin	Treated III group 75% myosin

Animals

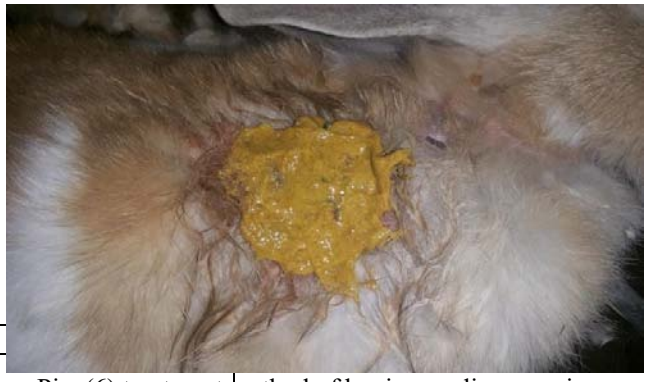
In the present study, thirty two rabbis were used (*Lepus cuniculus)*, same genera (male), age 7±2 months, one in similar condition and fed with bread and hay, which were divided into four groups, according to the experimental design.

Experimental Design

The animals were subdivided into 4 groups, each group includes 8 rabbits, regardless their genus

Time	*Groups*	*Control*	*Treated 1*	*Treated 2*	*Treated 3*
3 days	8 rabbits Conventional treatment, only gentamicine ointment and % hydrogel		8 rabbits 25% myosin 75% hydrogel	8 rabbits 50% myosin 50% hydrogel	8 rabbits 75% myosin 25% hydrogel

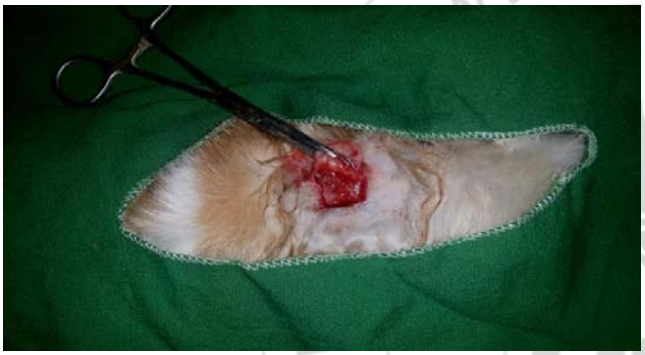
	Para meters		
WBCs count	7 day 10 day 15 day	Immune Response Test	Gross Pictures	Microscopic Pictures



Pic. (6) treatment method of bovine cardiac myosine-hydrogel

Procedure and Treatment

Carefully, under general anesthesia(12 mg , kg/BwKetamine+ 3 mg, Kg/Bw)(26), and aseptic technique, the skin were inscised at a dorsal aspect of rabbits with a (deep 3-4 mm X 1cm length X 1cm wide) and removed the patch and re-suture the patch at same place (27). After surgery the polymere was injected intr-wound daily. Pic (5) and pic (6).



Pic. (5a) site of skin wound surgery (skin fold autografting)

Pic. (5b) site of skin, wound sutur (skin fold autografting)

Immune Response Test

Indirect hemoaglutination test and WBCs count were used to know the immune response of the body against to the polymer (28).

Gross Pictures

Wound were imagined after 7 and15 days by canone digital camera 16 migabixal to vesion the development the wound.

Microscopic Pictures

Samples from surgical site were taken after 7 and 15 days post induce wound and made histopathology slide were prepared with routine manner and stained by haematoxylin and eosin.(29)

3. Results

WBCs and indirect hemoaglutination test

The WBCs and immunoassay were indicated to the safety of new polymers that were used for wound treatment and accelerated healing, the table (1) illustrated the chages in some blood parameters after polymere treatment in all groups.

Table 1: WBCs and indirect hemoaglutination test

Period	WBCs $*10^9$	Neutro$*10^9$	Lympho$*10^9$	Mono $*10^9$	Eosino$*10^9$	Baso$*10^9$	I HT
3 days	$7 \pm 2.1^*$	3.6 ±1.5	3.5 ±1.1	2.4±0.1	1.03 ± 2.7	0.001± 2	-
7 days	6.2±1.7	2.8 ±3.9	3.0 ±1.7	2.7±0.1	1.02 ± 2.7	0.001± 3	-
10 days	7.5±0.1	2.9 ±0.5	3.2 ±0.13	2.7±0.11	0.06 ± 2.7	-	-
15 days	7.6±1.7	3.9 ±7.1	2.5 ±0.1	2.5±0.15	0.13 ± 2.7	-	-

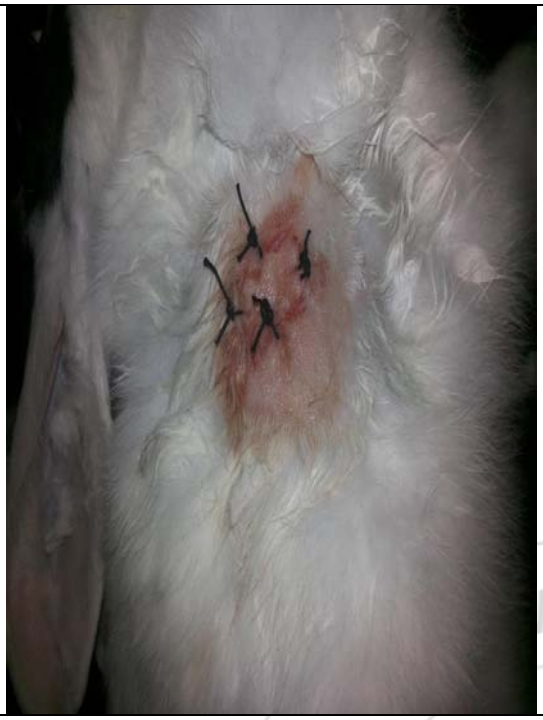
IHT , Indirect hemoaglutination test, *Mean and standarddeviation, P value ≤ 0.05

Gross Pictures Exammination

Proliferation of cells during cutaneous wound healing or cutaneous scar formation are discriminative signs of wound healing and the size of scar tissues, raise of scar above skin and regularity of scar tissue reveals to the grade of wound healing, table (2; a,b,c,d), shows cutaneous wound healing with defferant modle.

Table (2): Gross Pictures

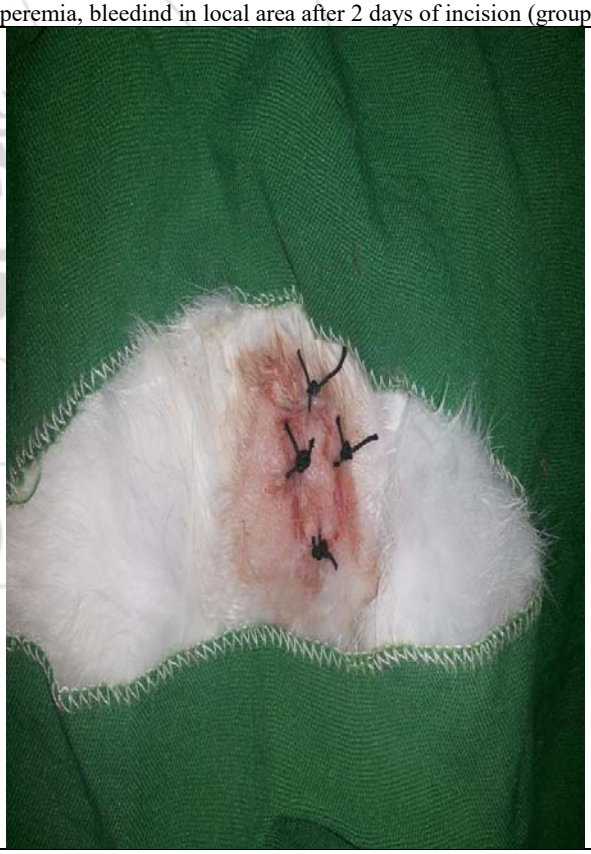
After 3-7 day post operation



Picture (1) rabbit skin cross picture , note wound line and hyperemia in local area after 3 days of incision (contrl group)

Picture (2) rabbit skin cross picture , note wound line and hyperemia, bleedind in local area after 2 days of incision (group I)

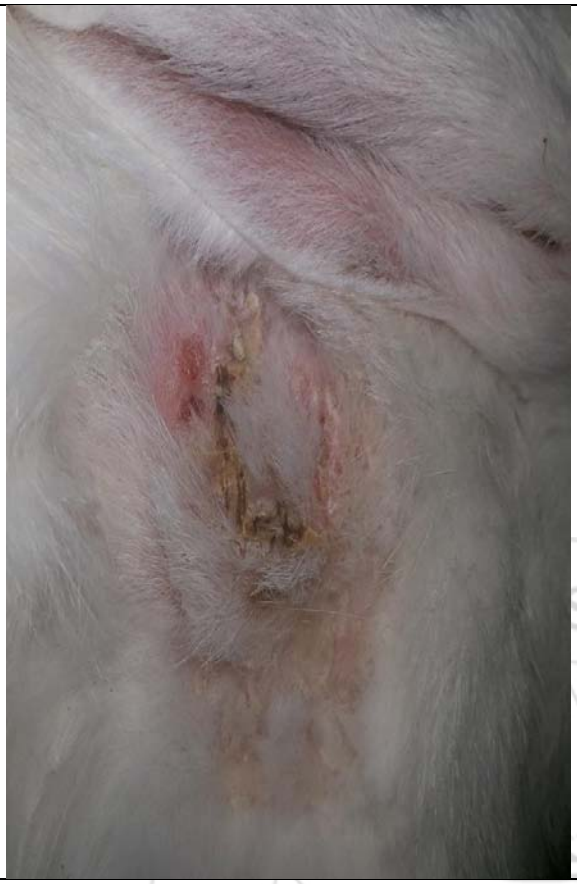
Picture (4) rabbit skin cross picture , note wound line and soft scar tissue after 5 days of incision (group III)



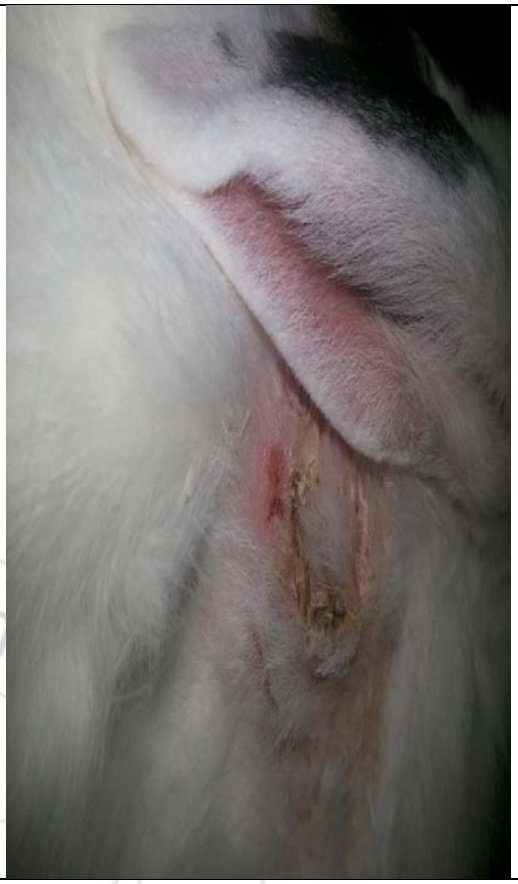
Picture (3) rabbit skin cross picture , note wound line and hyperemia in local area after 3 days of incision (group II)

Table(2) Gross Pictures

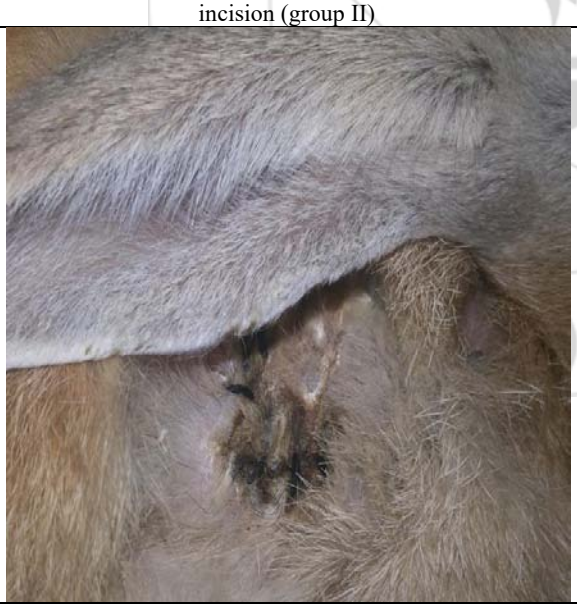
After 8-15 day post operation



Picture (5) rabbit skin cross picture , note wound line and and hard scar tissue with normal tissue in one line after 11 days of incision (group II)



Picture (6) rabbit skin cross picture , note wound line and and hard scar tissue with normal tissue in one line after 8 days of incision (group III)



Picture (7) rabbit skin cross picture , note wound line and scar tissue in local area after 8 days of incision (group I)

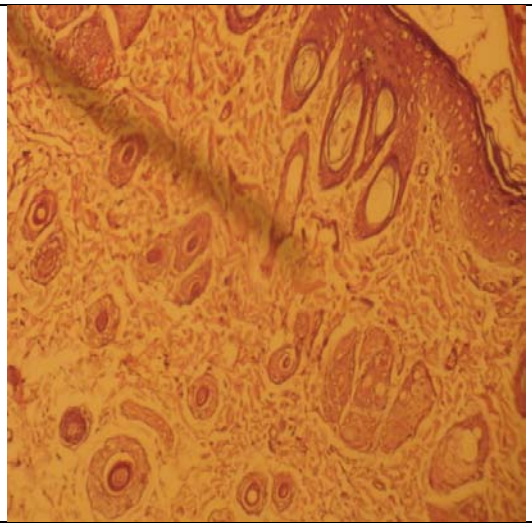
Picture (8) rabbit skin cross picture , note wound line and hyperemia, scar tissue in local area after 9 days of incision (contrl group)

Histopathological Picture Exammination

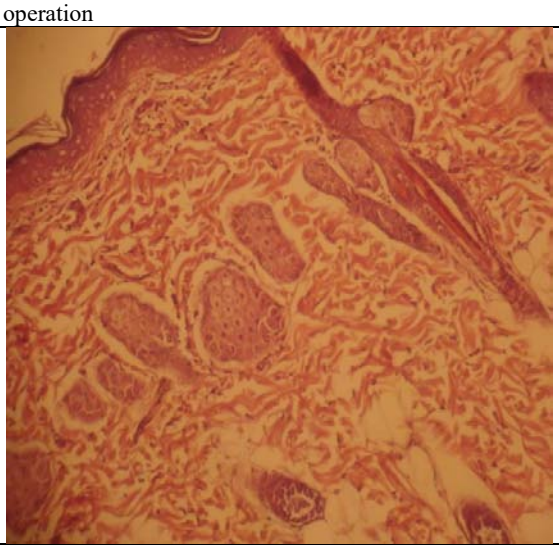
Parameters skin wound tissues changes accordingly the polymers consists with bovine cardiac myocine. The changes occurs in wound edges and wound hole. The skin with scar formation filled with necrotic debris, fibrin and vascularized granulation tissue consisting myofibroblast and immature capillaries. Table (3;a,b,c,d) shows histopathological changes amonge groups of treated animals.

Table (3) Histopathological Pictures

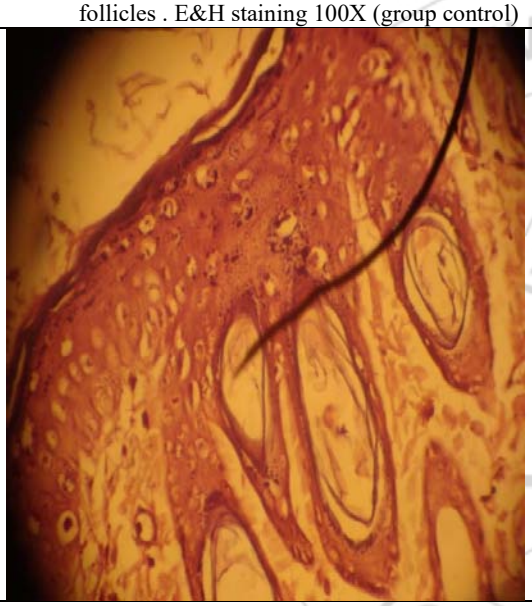
After 7 day post operation



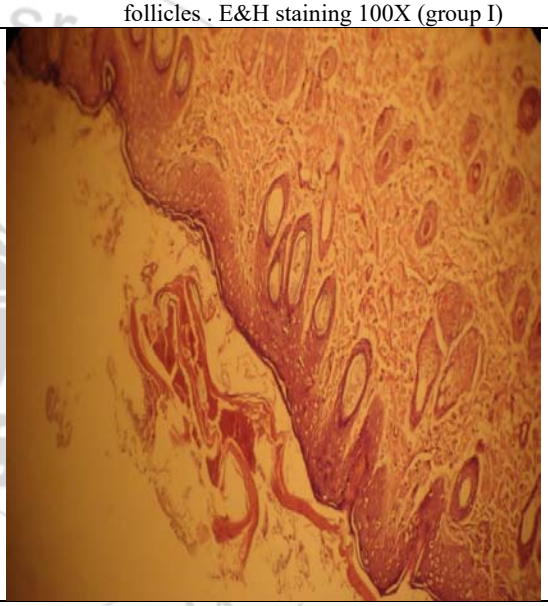
Picture (1) rabbit skin histopathology area of thickened epidermis with hyperkeratosis, note dilated cystic hair follicles . E&H staining 100X (group control)



Picture (2) rabbit skin histopathology area of thickened epidermis with hyperkeratosis, vessiles dilated, cystic hair follicles . E&H staining 100X (group I)



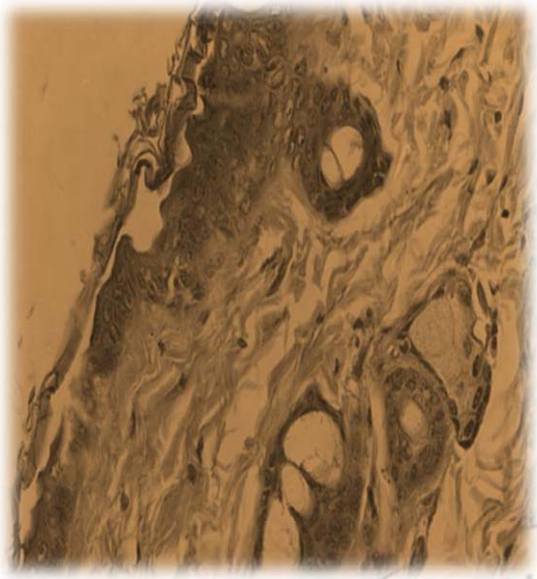
Picture (3) rabbit skin histopathology area of thickening epidermis with hyperkeratosis, collagen deposition hair follicles . E&H staining 100X (group II)



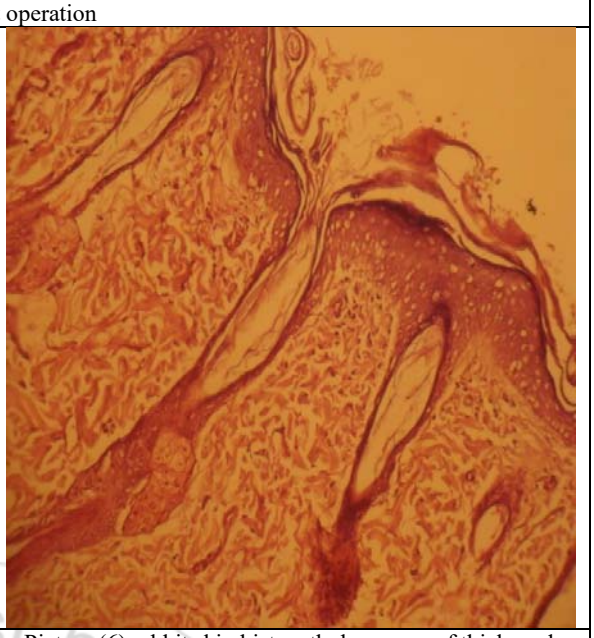
Picture (4) rabbit skin histopathology area of thickened epidermis with hyperkeratosis, collagen deposition and fibrosis hair follicles . E&H staining 100X (group III)

Table (3) Histopathological Pictures

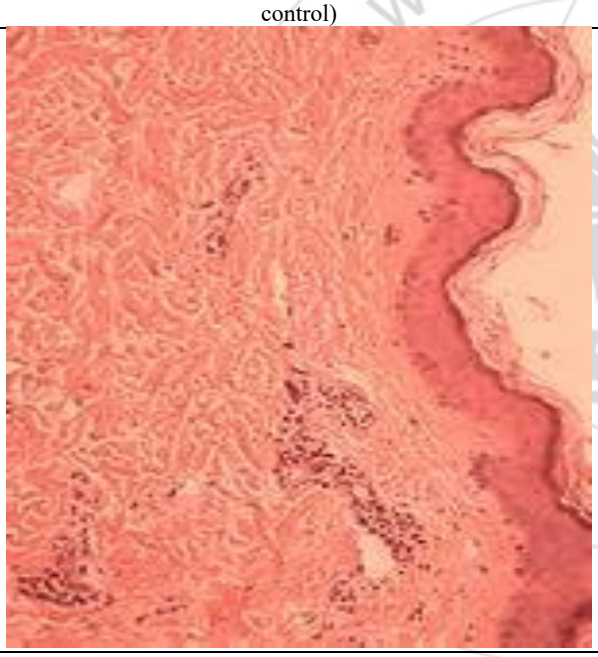
After 15 day post operation



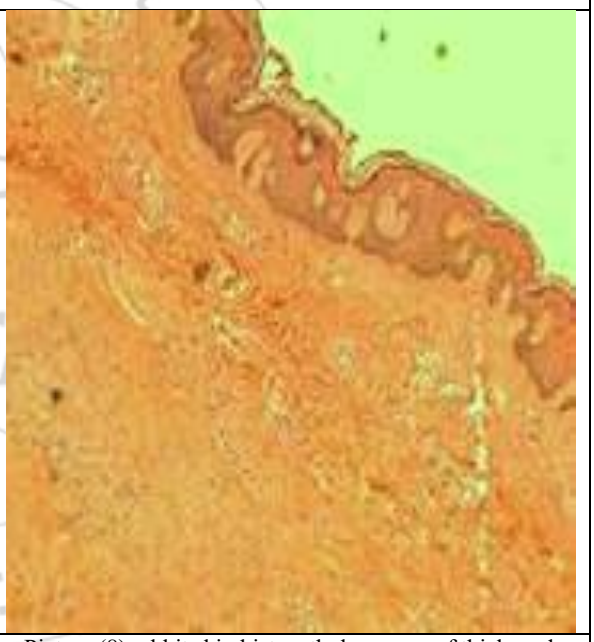
Picture (5) rabbit skin histopathology area of t epidermis with collagen concentration and fibrosis . E&H staining 100X (group control)



Picture (6) rabbit skin histopathology area of thickened epidermis hair follical . E&H staining 100X (group I)



Picture (7) rabbit skin histopathology area of epidermis with spotted of blood , note dilated cystic hair follicles . E&H staining 10 X (group II)

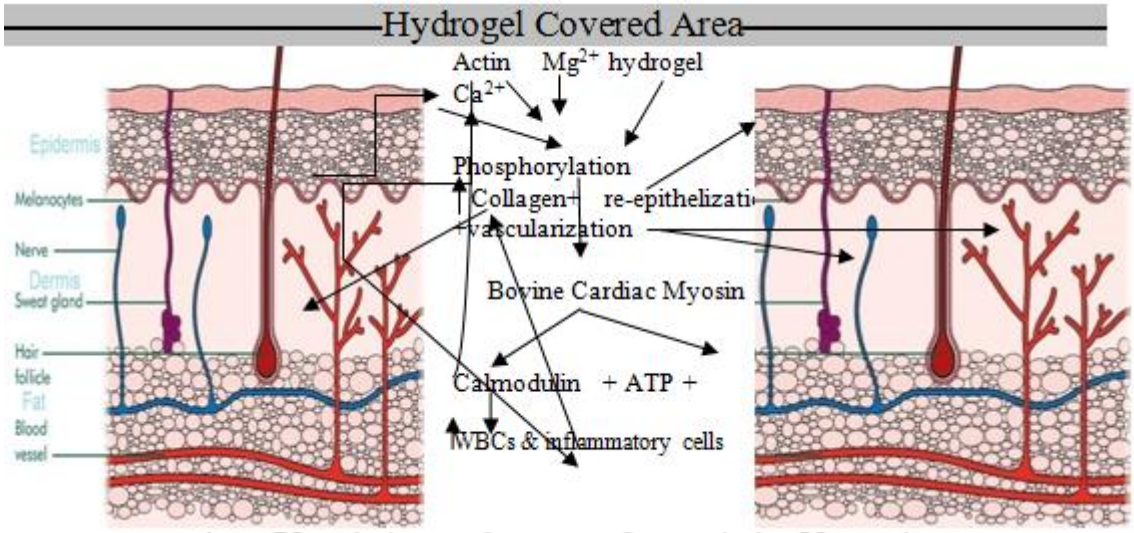


Picture (8) rabbit skin histopathology area of thickened epidermis with hyperkeratosis, collagen concentration and fibrosis. E&H staining 10 X (group III)

4. Discussion

Wound healing is a serious reaction include local rection and systemic reaction with multiple phases eg. hematoma, coagulation, inflammation, collagenation, fibroblast, myoblast migration as well as anti-inflammatory, intibiotic and other drugs may be accelerate or inhibit the processes wound healing (30). The present study indicate desirable systemic reaction, means the bovine cardiac myosin polymer has positive effect in skin wound healing, there weren't similar study to compare with present study, therefore should be succeeded the effect of myosin in wound healing dynamic. Surgical wound of skin graft was done under aseptic technique and remove the patch and re-suture at same anatomical situation are the edges of wound and two surfaces; cutaneous and cutaneous muscles, the first step of wound healing was hematoma and inflammatory phase. Hematoma includes RBCs and other inflammatory cells (TGF-β, PDGF, FGF, EGF, T-lymphocytes and other inflammatory cells), the reaction between bovine cardiac myosin polymer-hydrogel and inflammatory cells lead to increase inflammatory amount in the surgical area to the threshold pike, as well as initiate wound healing. Excess blood supply and local area temperature, gross pictures and microscopic pictures at 1-3 days post surgery (31). The metabolism of myosin by enzymatic ATPase, cAMG and myosin chain kinase, the phosphorylation of myosine product polypeptide, calmodulin and considerable ATPafter absorbtion and ooze the hematoma containt (32). The gross

pictures at 1-3 day post surgery and treatment don't appear clearly at different groups except in 2nd and 3rd treated groups were show more excessive soft scar tissue tend to redness. In debridement phase and invasion of mesenchymal cells, collagen, fibroblast cells, myoblast cells and progenic re-vascularisation; the differentiation, proliferation and migration of stromal cells depending on biological catalysts. Bovine cardiac myosine after phosphorylation release high energy ATP, therefore the re-epitheliazation occurs quickly and more collagen receipted with fibroblasts (33). The evidence to the role of myosin phosphorylation and product energy and increase biological processes were showed in microscopic picture 2nd and 3rd treated groups, there were more collagen and fibers, 1st treated group no evidence to change in collagen and fibers of control group. Gross pictures of 2nd and 3rd treated groups were shown after 5-9 days after surgery more development, after 10 days the line of wound in 3rd group were disappearing completely, due to the rection calmodulin and Ca^{2+} with ATP those enhanced local stem cells in cutaneous wound healing and tissue regeneration at 7 days after treatment of surgical wound (34). Calcium ions Ca^{2+} was resided from three sources, blood circulation Ca^{2+}, intracellular Ca^{2+} and extracellular Ca^{2+} in wounded area (35,36). In hematoma phase, the Ca^{2+} affinity with protein that transport is capacity and regulates ions that is important activities of cells accrose the plasma membrane. Ca^{2+} ions plays two roles in acceleration of wound healing, 1st role when Ca^{2+} react with calmodulin with ATP, this phenomena is very important in cutaneous skin wound healing (37), while 2nd rule of Ca^{2+} that react with sarcoplasmic reticulum occurs when Ca^{2+} influx from pump of Ca^{2+} channel and binding myosin and actin protein to initiate cytoplasmic bridges between two wound edges. Mg^{+2} ions don't had individual play role in collagen and fibers develop, therefore the histopathology in all groups no evidenent to Mg^{+2} had role without Ca^{2+} (12). Hydrogel was maintained the wound moist without contamination because of high sterile aqueous counting in hydrogel base (38).



5. Conclusion and Recommendation

- The bovine cardiac myocin-hydrogel polymer improve wound edges biological processing as well as maintance the wound sterility.
- The polymer can applied to improve healing in tendon injury, bone fracture, nerve damage, and cartilage erosion.

6. Acknowledgement

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