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

Ibrahim MH Alrashid¹, Shireen J Khaleel²

²Department of Surgery and Obestetric, University of Basrah/ Basra / Iraq

²Department of Anatomy and Histology / College of Veterinary Medicine / University of Basrah/ Basra / Iraq

Abstract: The present study were showed more developed in 2^{nd} and 3^{rd} treated groups compare with control and 1^{st} 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, 2^{nd} and 3^{rd} groups were loss scar tissue above wound and wound line were showed until 9 days in 3^{rd} treated group, while wound line in 2^{nd} 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 C²⁺-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 C²⁺. Calmodulin (CaM) found in all eukaryotic cells are multifunctional C²⁺-binding regulatory protein that mediates many C²⁺-related cellular events (9). Re-vascularization and vascular permeability are occasionally depend on myocin phosphorylation and calcium C2+- calmodulin CaM activation to stimulate myosin chain bioactivity in permeablized endothelium, ATP activity, and C2+ 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). Actinactivited 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

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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 $20C^{\circ}$, 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

drying followed acrylonitrile, separation and 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⁺ 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) , liquidsold =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



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.

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Paper ID: ART20163557

DOI: 10.21275/ART20163557

Experimental Design

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

			•	-			
	Time	Groups C	Control	Treated 1	Treated 2	Treated 3	
	3 days	8 rabbits Conventional treatment, only gentamicine		8 rabbits	8 rabbits	8 rabbits	
				25% myosin	50% myosin	75% myosin	
				75% hydrogel	50%	25%	
					hydrogel	hydrogel	
		ointment a	and %				
		hydrog	gel				MALL MOPPLY A
				Para meter	rs		
	7 day						
WBCs cou	u110 day	iy Immune Respon		se Test	Gross Pictures		Microscopy Freatheant method of bovine cardiac myosine-
	15 day	5 day					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 1 cm length X 1 cm 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. (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 and 15 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.

Period	WBCs *10 ⁹	Neutro*10 ⁹	Lympho*10 ⁹	Mono *10 ⁹	Eosino*10 ⁹	Baso*10 ⁹	I HT						
3 days	$7\pm2.1^*$ 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	-	-						

Table 1: WBCs and indirect hemoaglutination test

IHT , Indirect hemoaglutination test, *Mean and standard deviation, 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.

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DOI: 10.21275/ART20163557



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DOI: 10.21275/ART20163557



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.

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DOI: 10.21275/ART20163557

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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

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pictures at 1-3 day post surgery and treatment don't appear clearly at different groups except in 2^{nd} and 3^{rd} 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 redifferentiation, proliferation vascularisation; the 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 Ca2+ 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. Ca²⁺ ions plays two roles in acceleration of wound healing, 1st role when Ca²⁺ react with calmodulin with ATP, this phenomena is very important in cutaneous skin wound healing (37), while 2^{nd} rule of Ca^{2+} that react with sarcoplasmic reticulum occurs when Ca²⁺ influx from pump of Ca²⁺ 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

I would like to thank Ali A Alrushud/ college of sciencesdepartment of chemistry /Basrah university.

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