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Research Article

Low Level Laser and Bovine Amniotic Fluid-Derived Cream Accelerating Skin Neck Wound Healing and Reducing Inflammation and Wound Scar in Rat Animal Model

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ABSTRACT

Background: Nowadays, wound healing is one of the main problems of patients. Therefore, extensive research is underway to discover mechanisms associated with non-scarring of wounds. Using amniotic fluid and laser may potentially play a key role in wound healing and scar reduction due to its presence in tissue growth and repair agents.

Aim: The present study evaluated the effect of Bovine Amniotic Fluid-Derived cream (BFA) and Low-Power Laser (LPL) on accelerating skin wound healing and reducing scarring in an animal model.

Methods: Therefore, 72 male Wistar rats were randomly divided into three groups (Each group: 24). A wound 6 mm in diameter was then inflicted on the rats' backs. In the first group that was the control group, the wound was only used. Moreover, a BAF was implemented for the second group, and in the third group, LPL radiation was utilized. On the 1st and 3rd, 5th, 14th, and 21st days, the healing condition of the wound and scar created was examined.

Results: Hence, evaluation of wound healing status on days 5 and 14 showed that the wound healing scale in the BAF group and LPL group was significantly better than the control group. On the 21st day, the average Scar Scoring Scale in the BFA and LPL groups was significantly lower than the control group.

Conclusion: To conclude, considering the positive effect of LPL and BAF on wound healing and less scarring, it seems that LPL and BAF can heal wounds faster. Moreover, they can be used to prevent scarring.

Keywords: Amniotic fluid; Scar; Wound healing; Low power laser

INTRODUCTION

The skin is an effective barrier against adverse external conditions and has properties that can prevent microbial, mechanical, chemical, osmotic, and thermal damage. Wound healing is a complex and dynamic process that affects the quality of life during recovery. It leads to high costs for the health system worldwide. Adverse wound healing, especially in exposed areas of the body, is not attractive in appearance; however, it predisposes a person to tissue infection, necrosis, and other severe consequences. Scar formation and delay in wound healing are the main challenges in skin tissue trauma treatment. Scar formation is caused by increased fibroblast activity during the wound healing process. When a wound form, the epidermis thickens, collagen and glycoprotein deposition increases, and collagen fibers become thicker than normal and parallel to the epithelium. Higher levels of β1-TGF, I-Col, III-Col, fibronectin, and SMA- α have been observed in wounds compared to normal skin. Despite advances in wound healing, the complexity of this process remains a significant clinical barrier. Some common

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treatments used to reduce or eliminate scars include surgery, corticosteroid injections, radiation therapy, topical silicone gel, and interferon injections. Due to the unknown mechanism of scar formation, treatment has been unsuccessful in most cases, so they have encountered various side effects. Some substances with specific conditions such as tissue oxygenation, small blood vessels, age, underlying diseases, decrease or increase in nutritional status, or drug interactions can reduce or increase the time required for tissue repair by affecting each step in the wound healing process be damaged. One of the substances that possibly contain a variety of molecules that may be involved in wound healing and scarring is amniotic fluid. This substance has been used in the healing of bone lesions. It has also been effective in healing damage to peripheral nerves and tendons. The effect of amniotic fluid on wound healing in the fetus has also been studied, and the presence of hyaluronic acid has been suggested as a possible reason. In addition, the presence of stem cells in this fluid indicates the richness of the substances in it for cell growth. The advantages of this material are easy to access and low cost of its preparation, which can be used to repair skin wounds if it proves its effectiveness in accelerating the wound healing process and reducing the resulting scar. LPL has recently been used to treat skin wounds and positive results have been obtained. Due to the importance of this physiological process and to weave new factors affecting this process, by conducting this experimental study, the effect of LPL and BFA in each stage of tissue repair in the animal model will be studied [1].

MATERIALS AND METHODS

Amniotic fluid preparation

Amniotic fluid was prepared from a pregnant cow in the 20th week after pregnancy using a veterinarian. In addition, the cow underwent veterinary examinations for no specific disease (TB or brucellosis).

Experimental groups

In this study, 72 male Wistar rats weighing 250-200 g were randomly divided into three groups (Each group: 24) and kept in temperature control and 12-hour light-dark cycle and standard feeding in the nest, after anesthesia with intravenous injection intra Peritoneal ketamine/xylocaine, and hair removal in the lumbar region was created with a skin puncture of a wound with a diameter of 6-mm in the lumbar region. After examining and observing the wound area for infection and bleeding the closed containers were used on different days to maintain sterile conditions. On the 1st, 3rd, 5th, 14th, and 25th days, a group LPL (Diode laser, 810 nm, 60 s, 4 J/cm^2) and a group of BFA were sacrificed according to the standard method, and skin sampling was performed on the wound area [2].

Sampling and analysis

Samples from the 1st, 3rd, and 5th days after wounding were counted in 5 microscopic fields: in terms of inflammatory cells and fibroblasts, neutrophils on the first day; macrophages, and lymphocytes on the third day; and lymphocytes and fibroblasts on the 5,15 days, respectively. The standard deviation of each percentage was calculated. Also, to examine the scar tissue in the samples related to the 5th day a microscopic field count was performed. Besides, its mean and standard deviation were calculated. To evaluate the collagen accumulation in the samples related to the 14th day, pathology analysis was used using Image Analysis Software (the pathology analysis software registered in the Supreme Informatics Council of Iran affiliated to President No. 102543). Wound healing scores were evaluated in samples related to days 5, 14, and 21 by 3 physicians without knowing the control and LPL, BFA groups, according to Krasner scoring. Finally, in the samples for the 21st day, the Oscar scoring criteria (20 and 16) were evaluated according to the Vancouver burn scar scoring [3].

Histological examination

Wound sampling was performed after sacrificing mice. First, the samples were fixed in 10% formalin for fixation. The tissues were then fixed, and 5-micron sections were prepared from the skin. Hematoxylin and eosin staining were finally performed.

Real time PCR

In this assay, gene RT-qPCR was performed on samples PE, (Applied Biosystems, CA). For a whole volume of 20 µl, the sample reactions contain SYBR green PCR master mixture (TaKaRa), cDNA template, primer forward and reverse, and distilled water. The level of relative gene (Bax, Bcl2, Caspase 3, GAPDH) expression was measured using the ΔΔ Cq method. GAPDH was used to normalize gene expression. The primers sequence used are listed as follows in Table 1 [4].

Table 1: The primary sequence of gene expression.

Gene name	Product size	Sequence	Accession No
GAPDH	175	GTCTCCTCT GACTTCAAC AGCG	NM_001256799.3
		ACCACCCTG TTGCTGTAG CCAA	
BAX	210	TCAGGATGC GTCCACCAA GAAG	NM_001291428.2
		TGTGTCCAC GGCGGCAAT CATC	
BCL2	194	ATCGCCCTG TGGATGACT GAGT	NM_000633.3
		GCCAGGAGA AATCAAACA GAGGC	
CASPASE3	220	GGAAGCGAA TCAATGGAC TCTGG	NM_004346.4
		GCATCGACA TCTGTACCA GACC	

Statically analysis

After collecting and editing, the data were entered into the computer and analyzed by SPSS software version 24. The following tests were implemented to analyze the data. Student T-test to compare between quantitative data of two samples, Chi-square test to compare between qualitative data, and Mann-Whitney test for comparison between ranking data were utilized.

RESULTS AND DISCUSSION

In the present study, 72 male rats were selected and randomly assigned to 3 groups. Then, a 6 mm wound was made on the back of the mice, no treatment was used for the control group, and 1 ml of BFA was applied topically for the experimental group. Then, on the first and third days, the type and severity of inflammation were evaluated. On the fifth day, the type and severity of inflammation were re-evaluated. In addition, fibroblast, angiogenesis, and wound healing scale were evaluated. On the 14th day, collagen accumulation was examined, and the wound healing scale was re-evaluated. Finally,
evaluated (Table 2).

Table 2: Wound healing scoring scale.

Condition of sore bed	Epithelialization	Ready for autograft	Infection	Depth	Area	Score
Healed	Complete	-	-	-	-	2
Excellent	Well in minimum time	Yes	Decreased	Decreased	No	2
Good	Good	No	No	Unchanged	Unchanged	1
Bad	Hasn't done well	No	Yes	Increased	Increased	0

Inflammation

On the first day, the severity of inflammation was evaluated in the LPL, BFA, and control group, in which for 4 samples the severity of inflammation was +4 and for 2 samples was +3. If in the control group all 6 samples had inflammation of +3 and according to Whitney test, the severity of inflammation on the first day was significantly different in the experimental and control groups (p=0.019). On the first day, the type of inflammation was acute in all samples and there was no case of i subacute or chronic inflammation. The results are shown in Figure 1 [6].

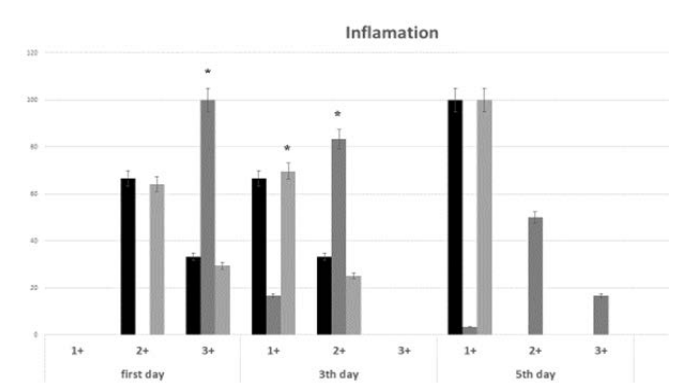

Figure 1: Frequency distribution of inflammation intensity on the first day in control and experimental groups (*P<0.05).

On the third day, in LPL, BFA groups, and control group, inflammation was observed +1 and also inflammation +2. In addition, there was no case of severity of inflammation +3 in any of the samples. Mann-Whitney statistics on these data showed that the severity of inflammation on the third day was not significantly different between the two groups (P=0.093), On the third day, some samples BFA and LPA groups had subacute inflammation (66.7%) and some had chronic inflammation (33.3%). There was also no case of acute inflammation in any of the samples. On the 5th day, all samples in LPL, BFA groups had +1 inflammations, while in the control group, 2 samples had +1 inflammation, 3 samples had +2 inflammation and 1 case had inflammation of +3. Mann-Whitney test also showed that the severity of inflammation on the 5th day in the LPL group was significantly lower (p=0.021). On the 5th day, there were no samples with acute inflammation in the experimental groups, but in the control group, 1 sample had acute inflammation. Also, on the fifth day, there was no case of subacute inflammation, while in the control group, 3 samples had subacute inflammation, and all samples in the LPL, BFA group had chronic inflammation, but in the control group, 2 samples had chronic inflammation (Figures 2 and 3).

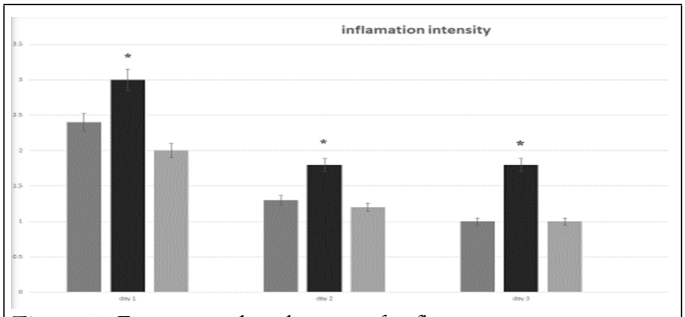

Figure 2: Frequency distribution of inflammation intensity on the 1,2 and three day in control and experimental groups (*p<0.05).

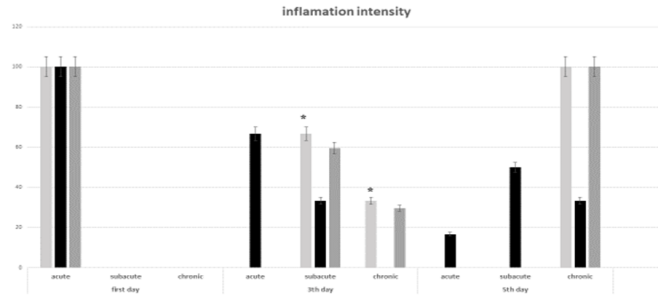

Figure 3: Frequency distribution of inflammation intensity on the five day in control and experimental groups (*P<0.05).

Fibroblast formation

On the fifth day of the intervention, the samples were examined for fibroblast formation, angiogenesis, and wound healing score. The status of fibroplasia was examined in all groups, of which 2 samples from the experimental groups had +3 fibroblasts and 4 samples had +3 fibroblasts. In the control group, 5 samples had +1 fibroblasts and 1 sample had no fibroblasts. Fisher's exact test on these data also showed that fibroblast formation was significantly higher in the LPL, BFA group comparison to the control group (P=0.002) [7].

Angiogenesis

The mean angiogenesis in the experimental and control groups was 11.7. 3.7, 6.5, 1.9, 9.1, and 3.9 respectively, and according to the student t-test, the mean angiogenesis in the case group was significantly higher (P=0.013). The results are illustrated in Table 3.

Table 3: Comparison of mean and standard deviation of angiogenesis in the two groups.

Standard deviation	Angiogenesis percent	Group
3/7	11/7	LPL
1/9	6/5	Control
3/9	9/1	BFA

Wound healing status

On the fifth day, out of 6 samples from the experimental group, 2 samples had partial wound healing and 4 samples had complete wound healing, but in the control group, 4 samples, no wound healing, and 2 samples had partial wound healing. Fisher's exact test also showed that the wound healing status in the case group was significantly better (P=0.039). Results for the On the fourteenth day, 6 samples from the experimental groups and 6 samples from the control group were examined for wound healing status and collagen tissue formation at the wound site. The 5 samples from the experimental group had complete wound healing. And the sample had a relative wound healing. In the control group, 4 samples had no wound healing and 3 samples had relative wound healing. Fisher's exact test also showed that the wound healing status on the fourteenth day in the case group was significantly more favorable (P=0.006).

Collagen tissue formation

The frequency distribution of collagen accumulation intensity in the two groups and according to the Mann-Whitney test, the collagen accumulation intensity in experimental groups was significantly higher than the control group (p=0.037) [8].

Wound scar score

On the 21st day, the rest of the samples, which included 12 samples from the experimental groups and 13 samples from the control group, were examined and 2 Scar scoring scales were examined in them; the mean Scar scoring scale in the control and experimental groups was 4.1. 1.2 and 5.6 6 1.6, respectively, and according to the scar scoring test. Student t in the case group was significantly lower. (P=0.025) The results are presented in Table 4.

Table 4: Comparison of mean and standard deviation of wound scar in two groups.

LPL		Control		BFA		Groups Collagen
Percent	Number	Percent	Number	Percent	Number	
16/7	2	33/3	2	0	0	+1
25	3	50	3	0	0	+2
16/7	2	0	0	33/3	2	+3
41/7	5	16/7	1	66/7	4	+4
100	12	100	6	100	6	total

Histological result

The macroscopically and histologically changes in LPL, BFA, and control group. The results showed that after skin lesion and healing in different groups, the rate of improvement in the laser and fluid group was faster than in the control group. Histological images showed that fibroblasts and collagen increased in the wound healing area, indicating a positive effect of LPL and BFA (Figure 4).

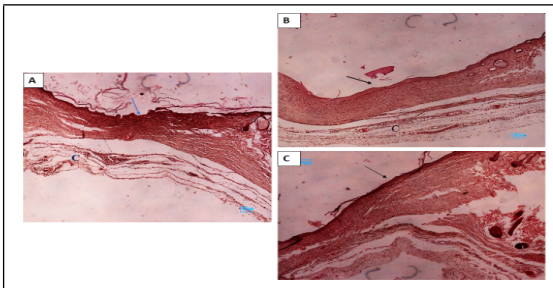

Figure 4 : Histological images show skin repair in the control (A), BFA (B) and LPL groups. Arrows showed that wound healing in different group and C: collagen fiber.

DISCUSSION

The overall aim of this study was to determine the effect of LPL and BFA in accelerating skin wound healing and reducing wound scarring in an animal model. Recent clinical and empirical evidence suggests a fundamental difference between fetuses and adults in terms of injury; fetal skin lesions heal quickly and without scarring. This phenomenon was first observed clinically during fetal surgery experience, and a new trade in several animal embryo models has supported the clinical observation of scar-free fetal wound healing. Progressive research has begun to elucidate the unique mechanism of wound healing in the fetus, and the process of fetal wound healing may provide a model for ideal wound healing in adults. Unlike chronic non-healing bone healing wounds, there is usually little concern about the time it takes for tissue to re-integrate and its tensile strength after injury, but the important point is to restore tissue structure and normal function without scarring. In one study, the effect of amniotic fluid on preventing intra-abdominal adhesions after laparotomy in rats was investigated and its effectiveness was emphasized. In another study, amniotic fluid improved peripheral nerve regeneration and had a preventive effect on epineuria scars. Another study demonstrated the effectiveness of the topical application of amniotic fluid in accelerating -re acceleration of corneal epithelialization. In another study, topical application of amniotic fluid after tenorrhaphy had a significant effect on preventing the formation of adhesions around the tendon without impairing tendon healing in the animal model. Numerous other studies have been performed on each of the growth factors that are abundant in amniotic fluid and are effective in wound healing. One possible mechanism for accelerating wound healing and scarring in the fetus is its placement in amniotic fluid rich in hyaluronic acid and other growth factors such as TGF-B is VEGF PDGF and so on. Therefore, it seems that one of the substances with different types of molecules that may be effective in the wound healing process and reducing its scar is amniotic fluid. Also, the presence of stem cells in this fluid indicates that it is rich in cell growth. Given the importance of the physiological process of wound healing and intending to find new factors affecting this process, we undertake an experimental study of the effect of using BFA on the wound healing process and its scarring in the animal model. In this study, in a group of rats that used BFA and LPL in their wounds, the type and severity of inflammation were significantly lower and the inflammation disappeared in a shorter time in the control group. Therefore, the effect of BFA and LPL can be considered effective in reducing inflammation [9]. The effects of LPL in the treatment of various diseases have been reported to be consistent with the results of our study. The status of fibroblast formation was examined in different groups, the results showed that LPL and BFA have positive effects on this process. The mean angiogenesis in LPL, BFA, and control groups was 11.7. 3.7, 6.5, 1.9, 9.1, and 3.9 respectively, and the mean angiogenesis was higher in the case group. However, angiogenesis should be lower in the group receiving the BFA, which may be related to other interfering factors during wound healing. Other variables such as collagen tissue formation and wound healing status and wound scar score in the LPL and BFA group were significantly more favorable than the control group. Finally, this study indicates thes effectiveness of BFA and LPL in reducing inflammation, increasing fibroplasia, increasing angiogenesis, increasing collagen formation, as well as improving wound healing and reducing scarring, but for a definitive conclusion, this study needs to be performed on a larger scale. Considering the positive effect of BFA and LPL in improving wound healing and less scarring, it seems that with thecontinuation of research, it will be possible in the future for a drug containing amniotic fluid with similar synthetic compounds to heal wounds faster and prevent scarring [10].

CONCLUSION

Considering the positive effect of bovine amniotic fluid cream on wound healing and less scarring compared to the laser group, it seems that with the continuation of research, in the future, a drug containing amniotic fluid or similar synthetic compounds can heal wounds faster and Use to prevent scarring after wound healing.

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CONFLICT OF INTEREST

The Author declares that there is no conflict of interest.

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