

A comparison effects of autologous platelet-rich plasma gel and low-level laser therapies on induced cartilage defects: An experimental study

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Abstract

Most surgeons have tried to use modern techniques to treat lots of surgical cases in veterinary medicine. This study aims to compare the effects of autologous platelet-rich plasma (PRP) and laser therapies on induce elastic cartilage defects in sheep. Twelve healthy sheep were divided into 3 groups, G1 was treated with autologous PRP gel with different platelet counts, G2 was treated with laser radiation with different doses and G3 was treated with normal saline and was used as control group. the left ear cartilages of all animals were incised at 5 cm, while at 6 mm diameter, a full thickness hole was inserted in the right ear. G-1 was treated with platelet-rich plasma gel, 3 ml daily for one week. Two of G2 was treated with 820 nm laser and the other two were treated with 860 nm laser daily for one week. In addition, G-3 were treated with normal saline daily for one week. Biopsies were taken at 1, 6 weeks for histo-pathological examination to compare the healing performance of each type of treatment. Histopathological evaluation showed the beneficial therapeutic effect of autologous PRP compared to laser therapy and the PRP therapy has more beneficial effect than laser therapy, its preparation is very simple and cheaper than laser which requires expensive equipment and dangerous side effect when used.

Keywords: autologous, platelets, rich, plasma, gel, laser, cartilage.

Introduction

A lot of researchers have tried many biological therapeutic techniques, among them, platelet-rich plasma and laser therapies are the modern therapies mostly used by veterinarians. Often, animals are exposed to many ear cartilage defects which are regarded as a serious problem in the field; besides, it is well known that cartilage healing has limited regenerative ability (1, 2).

The cartilage found in the external ear is of fibro elastic nature which consists of typical chondrocytes and yellow elastic fibers provide the matrix except around the lacunae. Cartilage are known as avascular tissues, because most of their cells are far from exchange vessels which are located mostly in the perichondrium while the nutrients diffused to the chondrocytes depending on the difference between the concentrations(3,4). For these reasons, cartilage has little ability for regeneration and it is well known that they heal by forming fibrous tissue scar (5).

Platelet-rich plasma (PRP) is defined as a volume of the plasma fraction of autologous blood which has a platelet concentration above baseline (6).

Over the past decade, the growing concern in the use of PRP to optimize the healing process of tissues has sparked the market development of a plethora of commercial procedures that are designed to concentrate platelets and suspend them in plasma or a fibrin construct of varying densities (7, 8, 9).

Consequently, it contains a high concentration of platelet-derived GFs with a potential promoting effect on tissue healing and regeneration (10).

The use of autologous PRP is a possible strategy to enhance the wound healing cascade in both soft and hard tissues(11). Activation of the platelets of PRP leads to release of conspicuous amounts of various growth factors

(PDGF), transforming growth factor (TGF), platelet-derived angiogenesis factor (VFGF), insulin-like growth factor (IGF)-I, platelet-factor (PF-4) and epidermal growth factor (EGF)(12,13,14,15).

The use of low-level laser in medical science has gained popularity over the past 30 years [16]. Studies on the use of low-level laser on rabbit musculoskeletal tissue and cartilage appear to have conflicting findings (17,18) , with reports of favorable (19, 20), and unfavorable (21) results. The therapeutic effect of laser therapy in wound healing has been identified such that a better knowledge about the mechanism of tissue repair using light energy has been obtained in the areas of skin, muscle, ligaments, nerves, bone and cartilage which respond to doses of light with wavelengths range of 600-1000 nm. However, the amount of energy absorb varied from one tissue to another even when the wavelength remain constant (22,23).

In addition, low-level laser therapy is a procedure that uses large portions of the visible and infra-red light spectra to improve the healing process by stimulating vascularization, fibroblasts proliferation and the deposition of collagen (24,25).

The aim of this study is to compare between two modern therapies, PRP gel and low-level laser, and their effects on cartilage healing.

Materials and Methods

Twelve healthy sheep as an experimental model were selected from the College of Veterinary Medicine farm, University of Al - Qadisiyah. These sheep were fed by concentrate diet and alfalfa with drinking water *ad libitum*. Their ages range between 18- 24 months, both sexes and weighing 150 ± 30 kg. This experiment was carried out according to the guidelines of the ethics committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

The sheep were divided randomly and equally into three groups:

1 – Group-1 (G-1) was treated by platelet-rich plasma. Whole blood of 18 ml was collected from the jugular vein of each animal in anticoagulant tubes, and centrifuged in a cool centrifuge at 3000 rpm for ten minutes. Thereafter, the supernatant plasma was transferred to another sterile tube and recentrifuged at 1000 rpm for 10 min to

concentrate the platelets. Platelet count was measured by Blood auto analyzer (Mindray, mod. BC-3000 Plus, USA). The platelet-rich plasma was mixed with autologous calcified thrombin at 1: 1 ratio to manufacture platelet-rich plasma gel and to activate the platelets to release growth factors(27).

2 – Group- 2 (G-2) was treated by low level laser (LLL) therapy by Laser diode (Omega laser systems Ltd., UK) as shown in Table- 1.

Table-1: The ages, sex, laser dose, wave length, time of dose, energy density, pulsing rate, and pregnancy.

| No . | Age (months) | Sex | Laser dose (nm) | Wave length (mw) | Time (dose) (sec) | Energy density (J/Cm ²) | Pulsing rate/sec | Pregnancy |
|------|---------------|--------|-----------------|------------------|-------------------|-------------------------------------|------------------|-----------|
| 1 | 19 | Female | 820 | 820 | 1000 | 16 | 146Hz | Nil |
| 2 | 22 | Male | 820 | 820 | 1000 | 16 | 146Hz | - |
| 3 | 24 | Male | 860 | 660 | 50 | 4 | 146Hz | - |
| 4 | 18 | Female | 860 | 660 | 50 | 4 | 146Hz | Nil |

3 – Group – 3 (G-3) are regarded as control group and were treated with normal saline.

Experimental design:

Under routine surgical approach, the left ear cartilages of all animals were incised at 5 cm, while at 6 mm diameter, a full thickness hole was inserted in the right ear. G-1 were treated with platelet-rich plasma gel, 3 ml daily for one week. Two of G2 were treated with 820 nm laser and the other two were treated with 860

nm laser daily for one week. In addition, G-3 were treated with normal saline daily for one week.

Biopsies were taken at 1, 6 weeks for histopathological examination to compare the healing performance of each type of treatment.

Results

The platelet counts of G-1 were $2 \times 10^9/L$, $16 \times 10^9/L$, $6 \times 10^9/L$ and $8 \times 10^9/L$, respectively as shown in Table-2.

Table- 2: The ages, sex, platelet counts, and the pregnancy states of G-1 sheep

| No. | Age (months) | Sex | Platelet count/L | Pregnancy |
|-----|--------------|--------|------------------|-----------|
| 1 | 20 | Male | 2×10^9 | Nil |
| 2 | 18 | Female | 16×10^9 | Nil |
| 3 | 21 | Female | 6×10^9 | Nil |
| 4 | 23 | Female | 8×10^9 | Nil |

Histopathological evaluation:

At one week, G1(Fig.-1) showed mild healing in the lower layers of epidermis and thin keratinized layer with mild collagen in the dermis. Moreover, G2(Fig.-2) showed mild healing of epidermal layers, absence of keratinized layer with hemorrhage and mild fibrosis, and G3(Fig.-3) showed infiltration of inflammatory cells- macrophages with hyperplasia of stratum basal.

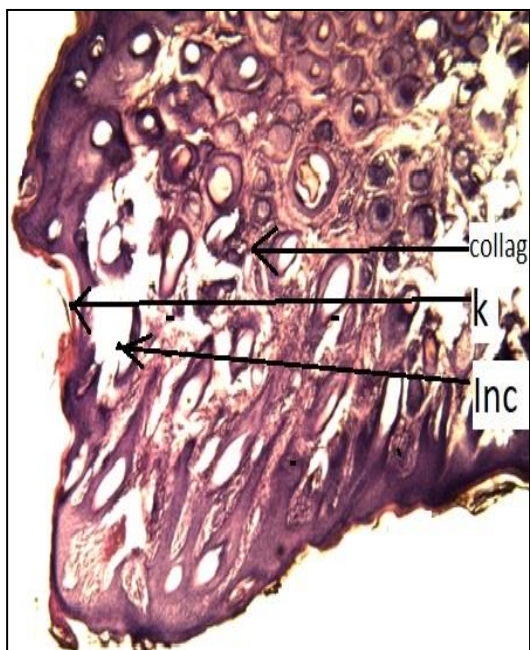


Fig.-1: G1 at one week: mild healing in the lower layers of epidermis and thin keratinized layer (k), also there is mild collagen in the dermis 10x(H&E)



Fig-2: G2 at one week: mild healing of epidermal layers(s) with absence of keratinized layer(k), hemorrhage (H) and mild fibrosis (F)10X(H&E)

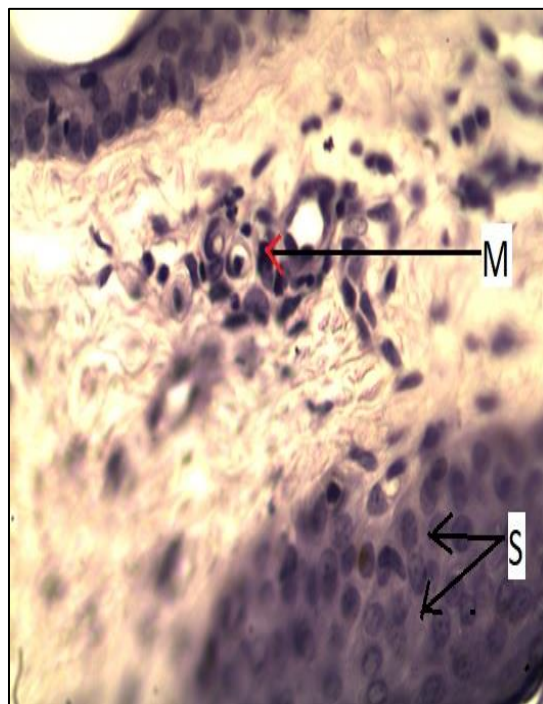


FIG-3: G3 at one week: infiltration of inflammatory cells (macrophages M) with hyperplasia of stratum

At 6 weeks, G1(Fig.-4) showed mild healing in the lower layers of epidermis and thin keratinized layer with mild collagen in the dermis. Furthermore, G2(Fig.-5) showed mild healing of epidermal layers, absence of keratinized layer with hemorrhage and mild fibrosis, and G3(Fig.-6) showed infiltration of inflammatory cells- macrophages with hyperplasia of stratum basal.

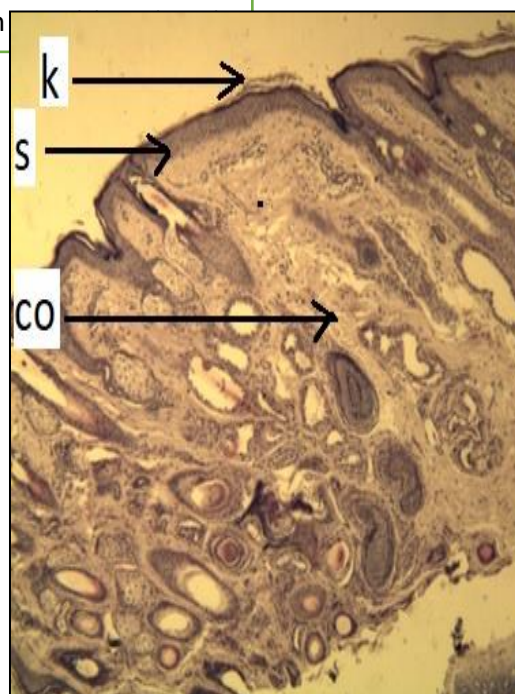
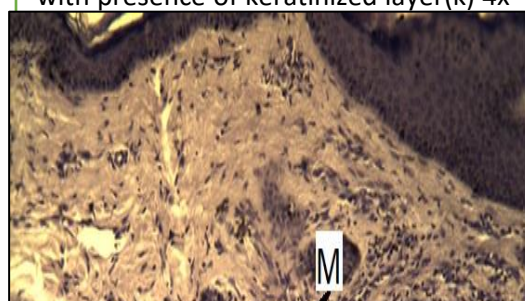


Fig-4: G1 at 6 weeks: There is normal epidermal layers and profuse collagen with presence of keratinized layer(k) 4x



All experimental procedures on mice were agreed by the Ethics Committee in the College of the Veterinary Medicine, University of Al-Qadisiyah.

Discussion

A lot of auricular cartilage defects in ruminants are presented to the Educational Veterinary Medical Hospital in Al- Dewania Province, Iraq. These defects may lower their economic values and they have to undergo classic surgery which may lead to prolong healing period, so in this study, we tried to compare two modern therapies, autologous PRP and LLL.

There are three types of cartilages – hyaline, fibrous, and elastic which have a distinct structure and biochemical composition depending on their specific functions and sites. They differ in their extracellular matrix, therefore to improve tissue-engineered cartilage, we must study the growth factors, scaffolds and other biological products.

Auricular elastic cartilage comprises mainly elastin fibers type II collagen fiber and matrix which consist of proteoglycans, carbohydrates, and proteins that fill the spaces in and around the fibers.

We believe that laser therapy has limited therapeutic value in acceleration of the cartilage healing. In this study, as shown in Table-1, we tried to treat sheep as a model of different ages and for both sexes, different laser doses of 820 and 860 nm, different time dose of 50 and 1000 s, different energy density of 4 and 16 J/Cm² with pulsing rate of 146 Hz for all the sheep. All these different parameters may clearly show us the comparison of the therapeutic effect of laser therapy. On the other hand, these important results also clearly show histologically, the comparison of the therapeutic effect of PRP as shown in Fig.-7 and Fig.-8.

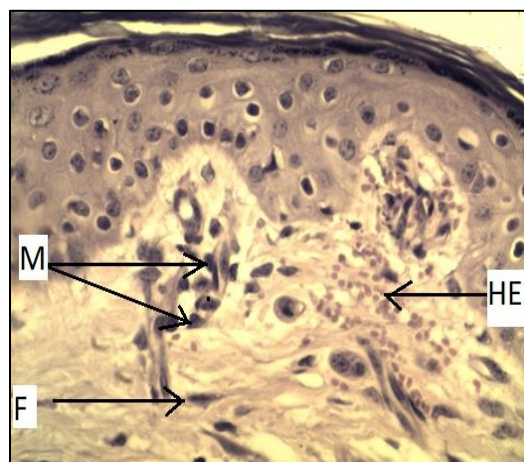
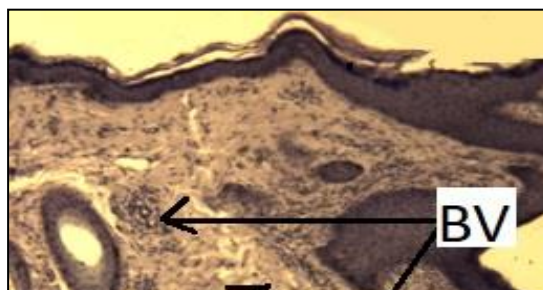


Fig-6: G3 at 6 weeks: profuse hemorrhage (HE) and infiltration of macrophages (M) in the dermis with proliferation of fibroblasts(F) 40X(H&E).

Ethical approval



accelerate the cartilage healing. Their beneficial therapeutic effect may be very evident as shown at the first week of treatment. In Fig-1, there is mild healing in the lower layers of the epidermis and thin keratinized layer with mild collagen in the dermis. In this work, PRP is used topically which is in line with the study of (26) where PRP was used during surgical operation or at the end of it. Several studies have been published on humans which explain the use of PRP after surgery. Some chondrocytes may migrate from the surrounding tissue to fill the induce hole. This migration may be due to the effect of cytokines realized from the platelets [5] and act as promoters of the cartilage healing.

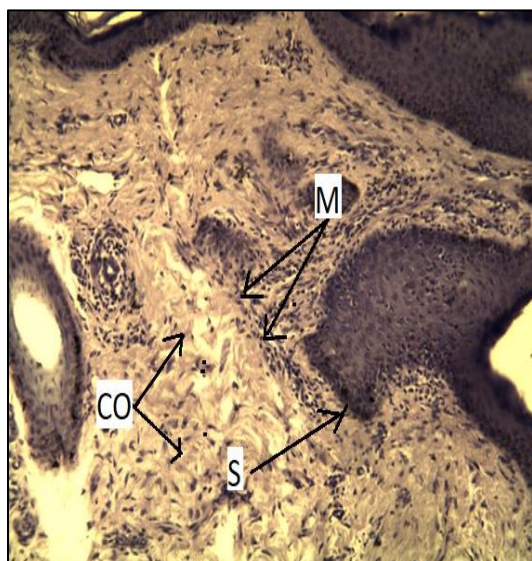


Fig.-8: G2 820 nm. At 6 weeks: Marked downward hyperplasia of stratum basal (S) with the infiltration of inflammatory cells (M) and profuse collagen fibers(CO) 40X (H&E).

In this study, we also used different doses of platelets ranging from 2×10^9 to 16×10^9 as shown in Table-2. This implies that there will be different levels of growth factors, so the healing in sheep No.2 is marked and characterized by normal epidermis and profuse collagen in the dermis, while the other sheep of G1 were less marked. We believe that these biological products- growth factors may

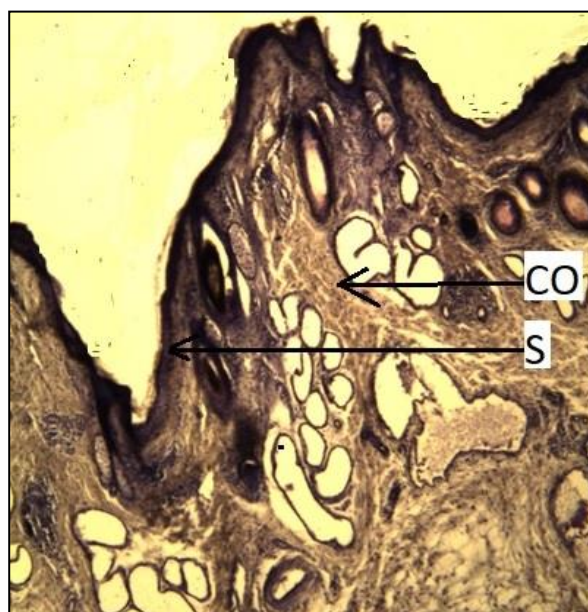
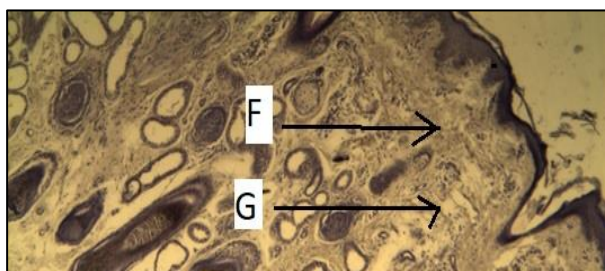


Fig.-9: G1 at 6 weeks: There is marked healing characterized by normal epidermal layers(s) and profuse collagen (co) in the dermis 10 x (H&E).



In conclusion, it is evident that PRP therapy has more beneficial effect than laser therapy, its preparation is very simple and cheaper than laser which requires expensive equipment and dangerous side effect when used.

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Conflict of interest

The authors affirm that they had no competing interests.

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