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Therapeutic Effects of Autologous Platelets-Rich Plasma with Hydrofiber Dressing on Cutaneous Wound Healing in Experimental Rabbit Model

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ABSTRACT

Autologous platelet-rich plasma (PRP) in combination with hydrofiber dressing (HFD) has been suggested for cutaneous wound repair. However, the scientific proof validating its application in animals experiencing cutaneous wound restoration is limited. Thus, this study was aimed at exploring whether PRP in combination with HFD benefited rabbits's ability to recover cutaneous wounds. A total of eighteen local adult male rabbits weighing 2.5–3.0 kg and aged 4-6 months were selected and allocated randomly into three groups: group A (control), group B (PRP), and group C (PRP+HFD), with six rabbits in each group. In each animal, two centimeters from the midline, full-thickness (2 x 2 cm2) skin incisions were made on the right dorsal surface. Autologous PRP (0.4 mL) was injected subcutaneously in wounds of group-B rabbits, while wounds in group-C rabbits underwent a subcutaneous injection of autologous PRP (0.4 mL), followed by hydrofiber dressing application on days 1, 7, and 14. The wounds of rabbits in group A did not go through any treatment and were only irrigated with sterile saline. Re-epithelization and neovascularization were assessed on day 21 by H&E staining, while collagen amount and collagen organization were assessed by Mason's trichrome staining. Oxidative stress markers (MDA, catalase) and inflammatory factor C-reactive protein were also assessed using blood samples. All the data were statistically analyzed. Results showed that PRP+HFD and PRP-treated wounds showed highly significantly increased levels of re-epithelialization, number of fibroblasts, neovascularization, and collagen amount with organization on day 21 than control wounds, while PRP wounds had a non-significant difference compared with PRP+HFD wounds. The levels of MDA significantly decreased, while catalase activity non-significantly increased in PRP and PRP+HFD-treated wounds compared to the control wounds on days 7, 14, and 21. However, the level of C-reactive protein decreased non-significantly between the different groups at different time intervals. In conclusion, PRP combined with HFD-treated wound healing accelerated wound healing by boosting re-epithelialization, neovascularization, collagen organization, and inhibiting oxidative stress in treated groups than control group. Therefore, this study recommends the application of autologous PRP with HFD to treat cutaneous wounds in rabbits, which is a reliable and practical approach.

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HA, AS and AZD conceptualized the hypothesis of this manuscript. FKP conducted the research. MAH, AS and HA statistically analysed the data. FKP performed the experiments and wrote the manuscript. HA and MAH critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Key words
Histopathology, Rabbits, PRP, Hydrofiber dressing, Wound healing

INTRODUCTION

The skin, being the most significant organ in the body, plays a critical role in shielding it from wounds and hazardous microorganism (Joanna, 2015). Skin wounds of numerous etiologies are regularly encountered in a veterinary clinical center. The healing of catastrophic wounds influenced by a variety of variables, like the course of therapeutic and treatment techniques, might end up in distinct therapeutic outcomes that determine the velocity as well as effectiveness of tissue restoration

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(Wang *et al*., 2011). Skin injuries are prevalent in horses, donkeys, mules (DeRossie *et al*., 2009), dogs (Qi *et al*., 2021), cats (Angelou *et al*., 2022), rats (Carvalho *et al*., 2022), and rabbits (Meira *et al*., 2020). Wound recovery encompasses an assortment of proliferative variables, chemical facilitators, cytokines, and various varieties of cells. Whichever change takes away this system could end up in resilient injuries that are incapable of healing. A wound that remains chronic fails to recover in the specified pattern of phases or has not received any recommended therapy in the suggested timeframe (Yolanda *et al*., 2014). Considering the latest innovations in skin-closing technologies and processes, surgeons continue to believe the healing process for wounds is problematic (Sadek *et al*., 2020). Yet, the sole method to effectively treat serious cutaneous wounds is to resolve them via a secondary intentional method due to tissue damage and not being able to close the wound originally. Thereby, with the goal of achieving optimal outcomes, it is vital to use cutting-edge advancements to boost the repair phase.

Platelet-rich plasma (PRP) is being thoroughly investigated as an innovative natural material that is capable of speeding up the rehabilitation of injuries on tissues such as muscles, skin, ligaments, tendons, bones, and cartilages (Molina-Miñano *et al*., 2009; Vendramin *et al*., 2010; Barrionuevo *et al*., 2015; Marques *et al*., 2017; Ferraciolli *et al*., 2018). Plasma having elevated numbers of platelets is referred to as PRP, and such plasma can be generated via seriated centrifuge technologies. Platelets use growth factors (PDGF, TGF-alfa, TGF-β, VEGF, IGF-I, PDECGF, and EGF) to reproduce and repair tissues through various processes such as mitogenesis, angiogenesis, chemotaxis, proliferation, as well as cell and fibroblast differentiation (Molina-Miñano *et al*., 2009; Vendramin *et al*., 2010).

During the last couple of years, a wide range of wound dressings have been developed, dramatically improving the area of wound healing. Given that hydrofiber dressings, a comparatively recent category of dressings, are believed to be more unlikely to trigger skin blistering, postoperative surgical area illness, or recurrent replacement of dressings, their adoption has expanded. Hydrofiber technology incorporated in these dressings gives them the ability to yield such outcomes (Mundi *et al*., 2023). Additionally, there are some dressings that have silver in them. Since the silver contained in these dressings is released in a regulated and stretched way, they are believed to have beneficial antimicrobial and therapeutic characteristics. These are additionally believed to be beneficial since they do not require daily replacement in accordance with the condition of the wounds, which could reduce unease and accelerate the recovery process (Dhivya *et al*., 2015; Edwards, 2013; Hurlow, 2012; Jahromi *et al*., 2018). By absorbing wound exudate and transforming it into a gel, this hydrofiber material offers the optimal circumstances for wound repair. Furthermore, these dressings are impermeable and saturated on the outside of the hydrocolloid circumference, which might boost their antimicrobial abilities (Mundi *et al*., 2023).

This study is aimed at exploring the therapeutic value of PRP in combination with hydrofiber dressing in the recovery of rabbit cutaneous wounds. We hypothesised that PRP with a hydrofiber dressing promoted neovascularization and angiogenesis to promote wound healing, making it a safe and effective clinical treatment.

MATERIALS AND METHODS

Animals

In this study, a total of 18 male rabbits, 4–6 months old, 2.5–3 kg were purchased from the Lahore city market and housed in the animal shelter at UVAS, Lahore. They had access to industrial feed, vegetables, and water at all times. All rabbits were housed on a 12-h light/12-h dark cycle (artificial lighting). The animals were acclimatized to being approached and handled for 15-20 days prior to the start of the investigation.

Preparation of platelet-rich plasma (PRP)

The rabbits were anaesthetized with Xylazine @ 5 mg/kg (Xylaz 20 mg/mL, Mylab Pakistan Pvt. Ltd.) and Ketamine @ 35mg/kg (Ketamine 50 mg/mL, Panpharms, France) intramuscularly. Antisepsis of the jugular vein was preceded by a 5% povidone-iodine solution and 5 mL of blood was collected using a 10 mL syringe in two sterile 3.6-mL capacity tubes containing sodium citrate to prepare PRP, and 0.8 mL of blood was collected into another tube. Once the platelet count was done, the PRP preparation was carried out by double centrifugation in accordance with Morato (2013). Platelets isolated by centrifugation at 1600 rpm for 10 min, were further centrifuged at 2000 rpm for 10 min. The pellet was platelet-rich. The platelet concentration was measured using an automatic device with 80 µL of PRP sample to ensure a platelet count over 1,000,000/µL. At the end, 0.3 mL of calcium chloride (TDF-HECBDH9224; VWR PROLABO Chemicals, USA) was added for activation purposes.

Experimental design and treatment

Experimental rabbits were assigned randomly into three groups: group A (control), group B (PRP), and group C (PRP+HFD), with six rabbits in each group. Using a sharp surgical knife, full-thick rectangular wounds of 2 × 2 cm2 were made around 2 cm from the midline, to the right of the dorsum, under aseptic condition. The wounds

of rabbits in group A did not go through any treatment and were only irrigated with sterile saline. 0.4 mL of autologous PRP was injected into rabbits in group B; rabbits in group C underwent a subcutaneous injection of 0.4 mL of autologous PRP followed by hydrofiber dressing application on days 1, 7, and 14.

Each full-thickness wound (2 x 2 cm2) was dehaired on the dorsum, shaved its margins, and detached it from the subcutaneous tissue using Noorani Surgical 25 cm/10 scissors. Each site was cleaned with sterile saline (NaCl 0.9 percent, Geofman), injected with 0.4 mL of PRP, and then covered with a hydrofiber dressing. The bandage was removed after seven days, the incision was cleaned with sterile saline, PRP was injected, and it was covered with a hydrofiber dressing. The treatment was given every seven days until the fourteenth day. On day 21, the final parameters were obtained. Similarly, the cutaneous wounds of the animals in control group A were treated with sterile saline before being covered with a cotton bandage (Surgitex, Rehman Rainbow Pvt. Ltd.). For animal welfare 0.2 mg/kg, meloxicam (Vetcon Pharma Pvt. Ltd.) was supplied intramuscularly twice daily for the first three days, and then the rabbits were housed in hygienic cages with limited exercise.

Histopathology of skin

Scrubbing of the biopsy site was done with saline solution by applying it to gauze. A biopsy punch (Kai Medical@Japan) with a 6-mm diameter was used to collect 3–4 mm of full-thickness tissue for histopathology. All the samples were initially preserved in 10% neutral-buffered formalin for a period of 24 h, transferred to a 70% alcohol fixative, embedded in paraffin and sectioned into 1.5-mm-wide sections. Additionally, samples were stained with hematoxylin and eosin to observe tissue anatomical evaluation with the help of a standard light microscope. Biopsied tissues histology showed semi-quantitative dynamics, such as the degree of vascularization, re-epithelialization, fibroblast count, and polymorphonuclear leukocyte (PMNL) presence. The preceding factors were scored using a semi-quantitative scoring system as follows: 0 represents absent, 1 represents minimal, 2 represents mild 3 represents moderate, and 4 represents marked (Sabol *et al*., 2012).

For Masson's trichrome stain, collagen fiber staining was carried out by using protocols devised and implemented by the Centre for Musculoskeletal Research (CMSR) at the University of Rochester Medical Centre. The biopsied tissues were stained for 5 min with Biebrich scarlet acid fuchsin (Fisher Scientific). Thereafter, for 2 min, samples were stained again using a 1% phosphomolybdenum-phosphotungstic acid solution (Fisher Scientific). The washing of the samples was done with distilled water, followed by staining with an aniline blue solution as a counterstaining agent for 5 min. Later on, rinsing of samples using a 1% aqueous solution of acetic acid was performed. At the end, drying out, clearing debris, and mounting slides were done. In this study, a simple descriptive scale of 0 to 3 was used to assess collagen content and organization in trichome-stained slides. Samples with a score of 0 demonstrated a lack of collagen bundles or organised collagen fiber production. A score of 3 indicated adequate collagen fibers and organized collagen fiber production.

Oxidative stress marker and C-reactive protein analysis

On days 0, 1, 7, 14, and 21, 2 mL of blood were drawn from each animal's jugular vein and then transferred into a vacutainer. A micro-lab 300 chemistry analyzer (Vital Scientific BV, Netherlands) was used to measure the C-reactive protein in the serum samples.

The quantification of serum MDA (µmol/mL) was performed in accordance with the procedures outlined by Ohkawa *et al*. (1979). The MDA concentration was measured as µmol/mL (ε = 1.56 × 105 mmol/L/cm). Serum catalase concentration was analysed in accordance with Aebi (1984). The catalase activity of a substance is defined as the quantity of catalase enzyme needed to reduce 1 mole of hydrogen peroxide per second at 25°C.

Statistical analysis

All data were statistically assessed by one-way ANOVA between the groups using the GraphPad Prism version 8 software (Version 8 ,GraphPad Software Inc., San Diego, CA, USA). All data were showed as mean±standard error (Mean ± SD). The level of significance "*" showed ($P<0.05$) and "**" showed ($P<0.01$).

RESULTS

Histopathological evaluation

The results of histopathology for re-epithelialization showed that the wounds that were treated with PRP and HFD healed on day 21. This gives a clue for faster epithelium growth and improved arrangement of connective tissue. On the other hand, in the control group, the wound showed a slight improvement in epithelium growth, minor growth in the basal lamina, and insignificant growth in the connective tissue on day 21 (Fig. 1). Statistically, the PRP-treated and PRP-HFD-treated wounds showed a significant growth of basal lamina as compared to the control group on day 21, while non-significant growth of basal epithelium was

recorded between the both treated groups (Fig. 2).

Histopathological examination of the angiogenesis indicated minimum growth of blood vessels in the control group, maximum development in PRP, and marked performance in PRP+HFD-treated groups at day 21 (Fig. 1). Statistically, compared with control wounds, PRP-treated and PRP+HFD groups showed a significant increased level of neo-vascularization, while both treated groups had a non-significant increase in neo-vascularization on day 21. In this study, keratinized epithelium tissues were visible on the edges of the PRP and PRP+HFD-treated wounds. However, scabs and degenerative PMNL covered the edges of the wounds. On the other hand, less PMNL was noted in the PRP and PRP+HFD wounds than in the control wounds. Histopathological examination of fibroblast scoring indicates a significant increase in PRP and PRP+HFD wounds compared to the control wounds, but a non-significant increase in fibroblast numbers was examined between the two treatment groups (Fig. 2).

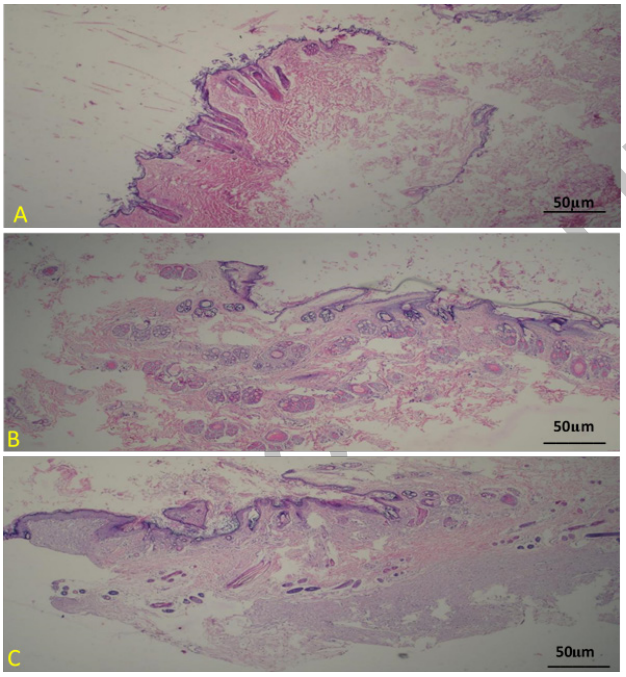

Fig. 1. Re-epithelization in the cutaneous wound of control and treated groups. **A**, control wound on day 21 showed minimal number of fibroblasts, moderate PMNL cells, some dead tissue mass along with less re-epithelialization. **B**, PRP treated wound on day 21 showed increased thickness of epithelium, presence of few fibroblasts, new blood vessels. **C**, PRP+HFD treated wound on day 21 indicated marked mature epithelial cells, excessive number of fibroblasts and new blood vessels formed. Stain: H & E. Bar=50µm.

Collagen fibers

The findings of Misson's trichome staining showed that collagen fibres were significantly increased in PRP and HFD wounds compared to the control wounds on day 21. The qualitative examination of collagen fiber represented the increased and well-organized collagen fiber in both treated groups as compared to the control group (Fig. 3). Statistically, the abundance and arrangement of collagen fibers significantly enhanced (P<0.01) in both treatment groups compared to the control, while both treatmentgroups had a non-significant increase in collagen fiber number and arrangement on day 21 (Fig. 4).

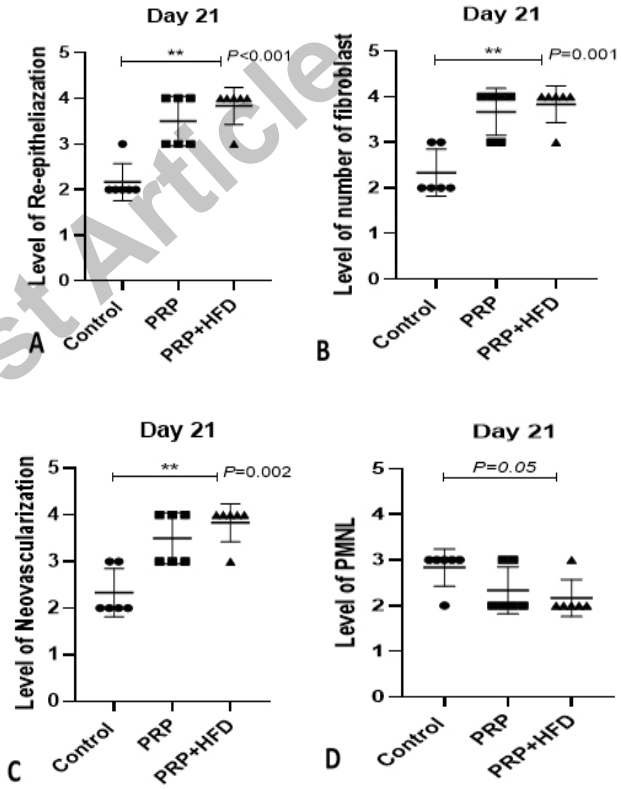

Fig. 2. Lesion score of wound healing in PRP and PRP+HFD treated wounds. **A**, Lesion score of wound re-epithelization on day 21. **B**, Lesion score of wound fiberoblast on day 21. C, Lesion score of wound Neo-vascularization on day 21; Lesion score of wound PMNL on day 21; "**" indicates that the PRP and PRP+HFD treated wound has a significant difference compared with the control wound (P < 0.01).

Oxidative stress markers and C-rreactive protein

Serum analysis of MDA concentration was significantly (P<0.05) lower in the PRP treated group on days 7 to 21, while it was significantly (P<0.05) lower on day 7 and highly significantly lower (P<0.01) on days 14 to 21 in the PRP+HFD treated groups than the

control group. The concentration of the catalase was non-significantly (P>0.05) increased from day 7 to day 21 between the groups. On the other hand, C-reactive protein decreased non-significantly (P>0.05) between the groups at different time interval (Fig. 5).

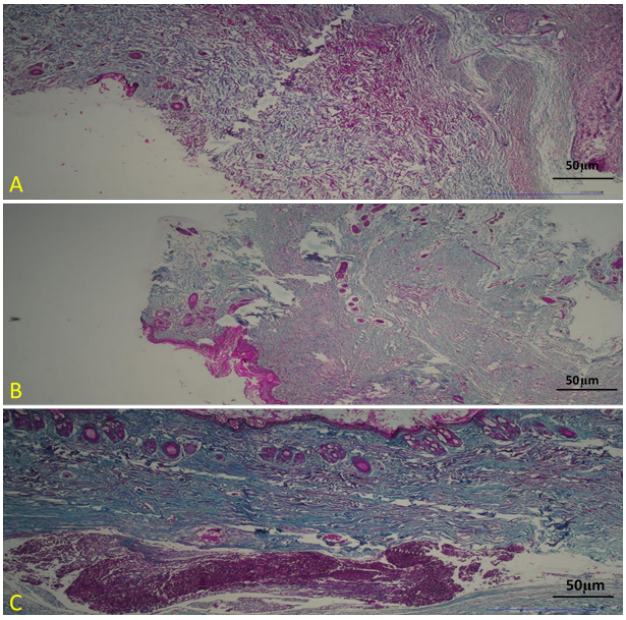

Fig. 3. Formation of collagen fiber in the cutaneous wound of different groups. **A**, Control wound showed disorganized collagen fibers on day 21; **B**, PRP wound showed minimal and organized collagen fiber on day 21 post treatment. **C**, PRP+HDF wound showed dense and tightly packed collagen bundles oriented parallel to the overlying epithelium. Massons trichrome stain, Bar=50µm.

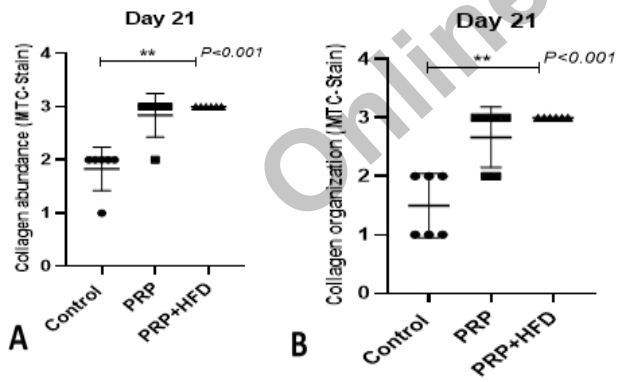

Fig. 4. Lesion score of collagen abundance and organization of cutaneous wound healing in treated groups. **A**, Lesion score of collagen abundance (MST stain) on day 21. **B**, Lesion score of collagen organization (MST stain) on day 21. "**" Indicates that the difference between the PRP and PRP+HFD treated groups has a significant difference than the control group (P<0.01).

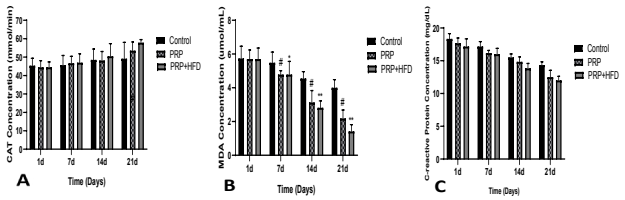

Fig. 5. Oxidative stress assessment and C-reactive protein. **A**, indicates CAT concentrations in control, PRP and PRP+HFD treated wounds. **B**, indicates MDA concentration in control and treated groups. **C**, indicates C-reactive protein concentration in control and treated groups. '*' and '#' Indicates that the difference between the treated wounds and the control wounds are significant (P<0.05); '**" Indicates that the difference between the PRP+HFD treated wounds and the control wounds is more significant (P<0.01).

DISCUSSION

Cutaneous wound healing is a complicated process that includes inflammation, tissue proliferation, granulation, epithelialization, and wound remodelling. Cutaneous wound healing has received a lot of attention in recent years. In this regard, the most recent breakthrough is the use of PRP, which has gained popularity as a silver-based hydrofiber dressing treatment in regenerative medicine. PRP is a high platelet concentration in a small volume of plasma that promotes the wound healing process in a number of surgical interventions (Lee, 2016). Several studies have previously been undertaken to assess the performance of a variety of injectable agents as healing promoters; however, PRP injection has been proven to be the most appropriate healing promoter for cutaneous wound repair in rabbits (Tahir *et al*., 2018). Thus, we investigated whether PRP with hydrofiber dressing promotes cutaneous wound healing in an experimental rabbit model.

PRP infiltration was implemented to promote re-epithelialization. Huang *et al*. (2016) used PRP to help create granulation tissue. This may be due to its absorption by surrounding tissue, which aids in the early stages of wound healing by promoting the formation of new blood vessels and re-epithelialization. Ostervall *et al*. (2015) found that PRP provided better histological results than control wounds at days 7, 14, and 21. Furthermore, on days 14 and 21, the average blood vessel density of PRP wounds was significantly higher than that of controls, with more microvessels and more epithelial junctions. The researchers found that PRP promoted angiogenesis, accelerated healing, and produced adequate granulation tissue compared with the control group. Ragab *et al*. (2021)

found that SNP-containing PRP significantly increased the healing of full-thickness surgical wounds in rabbits by increasing fibroblast counts, re-epithelialization, and angiogenesis. Strukova *et al*. (2001) reported that activated platelets reduce wound size by increasing the fibroblast-to-macrophage ratio and the number of proliferating fibroblasts. It can be induced by VEGF release, thereby promoting endothelial cell and fibroblast growth (Carter *et al*., 2003). In this study, treatment of autologous PRP with HFD increased wound epithelial cell thickness, resulting in the healing of all full-thickness skin wounds with more fibroblasts and neovascularization than control wounds.

Collagen fibres are part of the super cellular extracellular network that acts as the tissue's basic structure, directing cell proliferation and relocation during skin wound healing (Soundia *et al*., 2018). In this strategy, collagen filaments serve as a critical boundary for studying skin wound repair. According to Jee *et al*. (2016), the increase in collagen levels within the wound coincides with the rise in wound flexibility observed during the fibroblast phase. Xue and Jackson (2015) state that collagen fibres become dense, densely packed into bundles, and symmetrical in the later stages of wound healing. In this study, collagen fibres were arranged as thick, identical, wavy bundles in cutaneous wounds treated with PRP and PRP with HFD, but less in control wounds, indicating that PRP with HFD promoted granulation tissue development.

Researchers have recently discovered that reactive oxygen species (ROS) are directly linked to wound restoration and play an important role in wound healing (Yip, 2015). While a large concentration of ROS can disrupt cells, a low quantity of ROS that is less energetic promotes wound healing (Sen and Roy, 2008). ROS-induced lipid peroxidation produces a variety of end products, such as MDA. MDA levels reflect the degree of ROS breakdown (Soundia *et al*., 2018). Griffiths *et al*. (2002) found that the severity of injuries was associated with increased amounts of malondialdehyde in biopsied tissues and bodily fluids. Melnikova *et al*. (2021) found that wound therapy with both hydrophilic and lipophilic substances in rats resulted in increased antioxidant enzyme activity (CAT) in erythrocytes and decreases in MDA concentrations after 7, 10, and 21 days. It is attributed to lowering the intensity of free radical oxidation. On days 7, 14, and 21, MDA levels declined while CAT increased, which was in agreement with former studies (Melnikova *et al*., 2021; Mistry *et al*., 2020; Tort *et al*., 2020). Researchers first found the prototypical acute phase protein, CRP, in human blood during a pneumococcal infection. This is because CRP can react and precipitate with pneumococcal C-polysaccharide. Both people and rabbits show a 100–1,000-fold increase in CRP concentrations in serum during inflammation or tissue injury compared to the usual range. The different biological functions attributed to CRP have been related to the host defence against infectious agents and wound repair (Ahmadi-Abhari *et al*. 2013). Recently, Li *et al*. (2017) discovered that CRP could serve as a scavenger for chromatin that damaged cells secrete. CRP levels are frequently elevated in humans and dogs with neoplastic disorders, immune-mediated diseases, infection, or trauma (Ahmadi-Abhari *et al*. 2013; Li *et al*., 2017; Chan *et al*., 2009). In our study, the CRP level decreased at different time intervals, which indicates the supporting role of PRP and hydrofiber dressing on cutaneous wound healing in rabbits.

CONCLUSION

In conclusion, PRP combined with HFD-treated wounds accelerated wound healing by boosting re-epithelialization, neovascularization, collagen organization, and inhibiting oxidative stress in treated groups than control group. Therefore, this study recommends the application of autologous PRP with HFD to treat cutaneous wounds in rabbits, which is a reliable and practical approach.

DECLARATIONS

Funding

The study did not receive any funding.

IRB approval

The study was approved by Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan (DAS/1895, date: 26/10/2023).

Ethical statement

This study and all the procedures were approved and conducted in accordance with rules and regulations of the Ethical Review Committee (Ethical Approval No. DR/396; Dated: 04/09/2023) at the Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Availability of data and materials

All data supporting the study were obtained from the corresponding authors on reasonable request.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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