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CELLULOSE PLATELETS-RICH PLASMA (CPRP): MODIFIED NANOTHERAPY FOR CUTANEOUS WOUND HEALING IN IRAQI ARABIAN MARES

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Abstract

The aim of this work was to investigate the effect of cellulose platelets-rich plasma CPRP as a modified nanotherapy on cutaneous wound healing in Iraqi Arabian horses. Cellulose was extracted from the frond mid rib of date palms and mixed with autologous PRP at ratio 2.5:1 Six Iraqi Arabian mares were divided randomly into three groups, treatment group-1 was treated with CPRP, treatment group-2 was treated with PRP, and control group-3 was treated with normal saline. Square incisions 5cm^2 were done surgically in the back region of each mare. Treatments daily for one week were used topically. The wound contraction of treatment group-1 incision was superior significantly. The macroscopic examination showed less scar tissue after 75 days and the histopathological evaluation showed marked healing characterized by very narrow scar tissue, proliferation of epidermal layers in both edges of incisions, profuse collagen and fibrosis with mild infiltration of inflammatory cells and formation of new blood vessels. The CPRP is very useful as a new nanotherapy medicine for veterinarians.

Key words*:* cellulose, platelets plasma, cutaneous wound healing, Arabian mares

Introduction

Wound healing is a complex dynamic process involving many phenomena's cellular, molecular, biochemical and physiological events which are ended in repair of the connective tissue and formation of the fibrous scar tissue (Bowler, 2002). In biomedical devices, the biomaterials play an essential role and help in tissue engineering. This fact helps in regenerative potential for developing tissues and organs which lead to normal body functions (CM *et al.,* 2017). The use of PRP has been documented in many fields in a lot of papers of Orthopedics, Neurosurgery, Ophthalmology, and Wound healing etc. The bio-effect of PRP is mainly due to the releasing of growth factors and cytokines by the concentrated platelets in plasma (Mishra and Pavelko, 2006).

PRP is autologous plasma and known as one of the constituents of peripheral blood. This modern therapy is rapidly extending to many multiple fields because of the easy of its use and its high safety as a regenerative medicine (Narhi and Nordstrom, 2014). It is regarded as a tissue sealant and drug delivery system according to its pharmaceutics effects (Marx, 2004) by its growth factors release and by granules degranulation (Pietramaggiori *et al*., 2006; Gonshor, 2002). Cellulose prepared as scaffolds have been considered suitable for tissue engineering. It is well known that cellulose is a hydrophilic material, so the mammalian cells do not adsorb to its surface (Wuc *et al*., 2003; Pelton, 2009). Cellulose requires the addition of matrix legands such as collagen which has an ionic charge to facilitate cell attachment to their surfaces (Martinez *et al.,* 2015; Torres *et al.,* 2015; Modulevsky *et al.,* 2014; Singh *et al.,* 2013).

Materials and Methods

The study was performed in the Department of surgery and obstetric, College of Veterinary Medicine, University of Al-Qadisiyah from November, 2018 to February, 2019.

A- Extraction of cellulose from [frond midrib] of

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date palm:

1. Grinding of the dry [frond midrib] was done to obtain its powder.

2. The cellulose was extracted from the [frond midrib] powder by using conventional Soxhlet extractor with absolute ethanol at 45ºC for 6hrs.

3. The extracted cellulose was dried by oven at 40ºC to evaporate the absolute ethanol and preserved at 4ºC.

B- Preparation of cellulose Platelets-rich plasma [CPRP]:

Whole blood at 150ml was aspirated from the peripheral autologous blood circulation [jugular vein] of each horse, centrifuged twice at 4000rpm/10min. The yellow layer [containing plasma, platelets, and growth factors] was aspirated gently, added 10% calcium chloride, and then the solution was kept in an incubator at 37°C for 24hrs to activate platelets and release high concentration of growth factors. Then, cellulose was mixed with PRP solution at ratio 2.5:1 and preserved at -80°C.

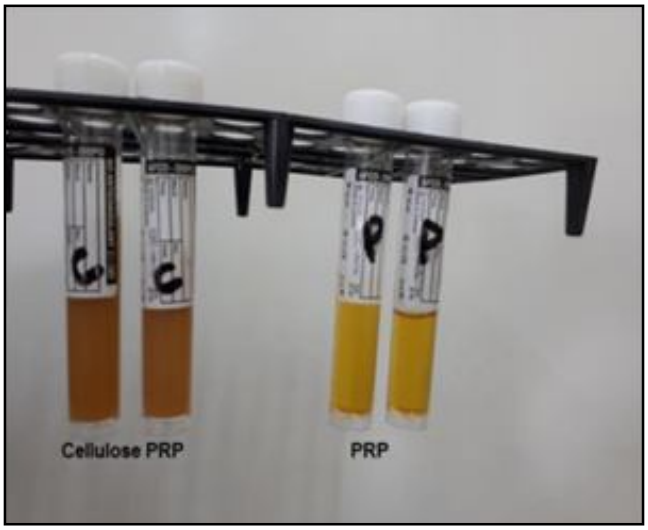

Fig. 1: The differences of colors between CPRP and PRP.

C- Experimental design:

The study was conducted on six Iraqi Arabian mares lived in a private stall. Their ages were ranged from: ±12 years old, and their weights were 385 ± 10kg. These mares were divided randomly into three groups equally, treatment group-1 [TG1] treated with CPRP, treatment group-2 [TG2] treated with PRP, and control group-3 [CG3]. Complete square cutaneous incisions at 5Cm2 were done under routine surgical procedures in the back regions of all groups. CPRP was thawed at 37ºC and provided daily for one week topically in a dose of few drops on the incisions of TG1. This approach was followed at the same manner for the PRP of the TG2 and the normal saline at 0.9% N for CG3.

D- Measurement of wound contraction:

All incisions were measured daily for one week by drawing them on transparent paper and measuring the length of the side with caliper to calculate the rate of the wound contraction.

E- Histological evaluations:

Biopsies were taken from the edges of the healed incisions at the time points of day-7, day-14 and day-21 for histopathological examination to evaluate the healing processes of the wounds. Smears were stained with E&H stains and examined under a light microscope at 10X and 40X.

Results

Wound contraction:

The wound contraction of CG3 was the lower value, 3.8214 ± 0.3234, than TG2, 4.2500 ± 0.2034 and TG1, 4.5000 ± 0.1577, as shown in Fig. 2.

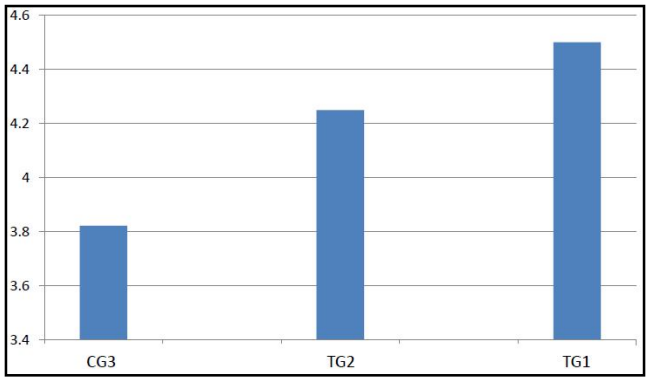

Fig. 2: Shows the wound contractions of the treatment groups for one week.

Macroscopic evaluation:

The macroscopic evaluation revealed clear differences between PRP and CPRP treatments in contraction and scar formation of the wounds.

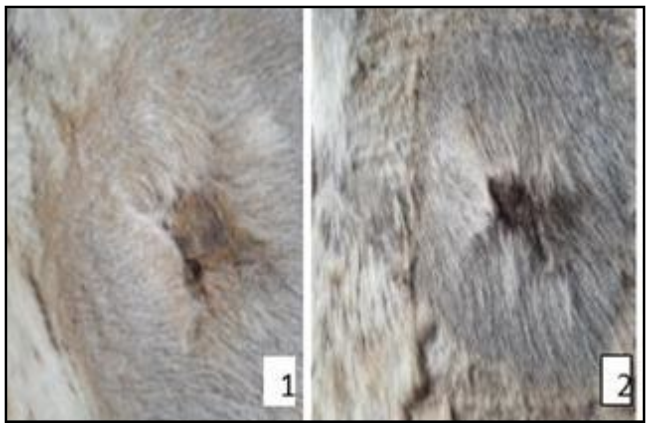

Fig. 3: The incision of TG2 after 14 days (1) and the incision of TG1 after 14 days (2).

Histopathological evaluation:

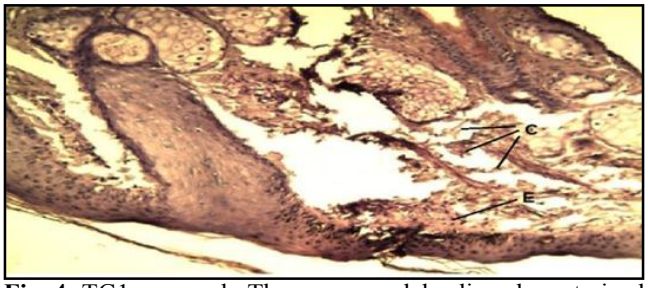

Fig. 4: TG1 one week: There was weak healing characterized by thin threads of collagen (C), proliferation of epidermal layers (E). X10, H & E.

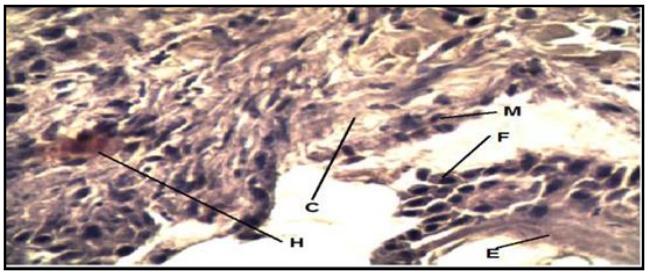

Fig. 5: TG2 one week: High infiltration of inflammatory cells (F), macrophages (M), threads of collagen (C) with mild proliferation of epidermal layer and hemorrhage (H) X40, H & E.

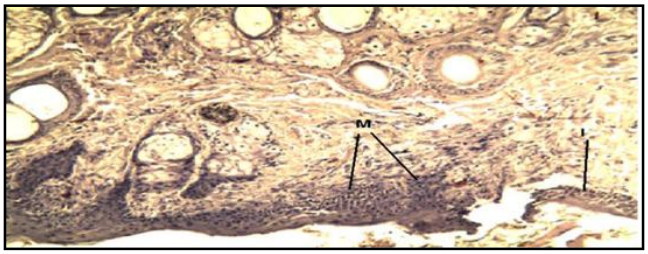

Fig. 6: CG3 one week: There are no signs of healing, the site is filled with macrophages (M) and severe infiltration of inflammatory cells (I) X10, H & E.

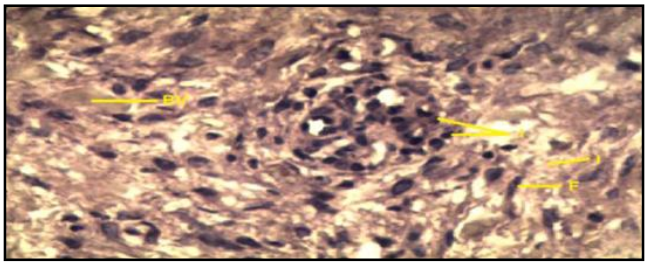

Fig. 7: TG1 two weeks: Higher magnification, high infiltration of macrophages (M), proliferation of fibroblasts (F) and hemorrhage (I) with formation of new blood vessels (BV) X40, H & E.

Discussion

The world now is heading toward the biological remedies which considered as revolutionary and effective cures for a number of surgical cases that the antibiotic and drugs can't provide a treatment for them.

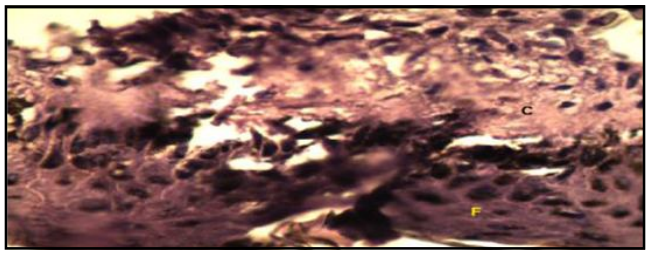

Fig. 8: TG2 two weeks: Higher magnification, proliferation of stratum basal of epidermis (F), proliferation with few collagen (C) in the dermis and few infiltration of macrophages X40, H & E.

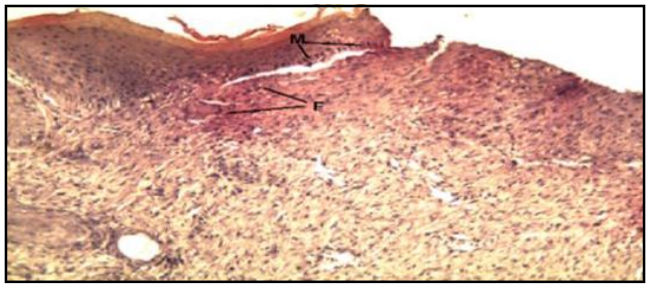

Fig. 9: CG3 two weeks: Higher magnification, proliferation of stratum basal of epidermis, proliferation of fibroblasts (F) and infiltration of macrophages (M) in the dermis X10, H & E.

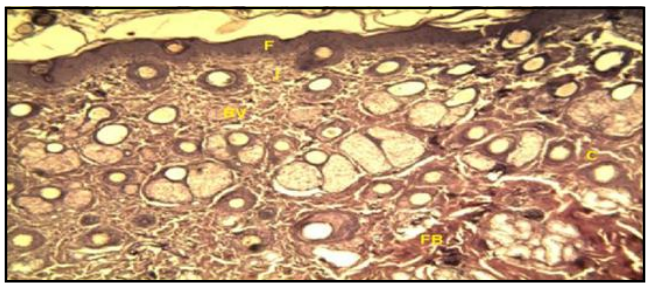

Fig. 10: TG1 three weeks: There was marked healing characterized by very narrow scar tissue (F), proliferation of epidermal layers in both edges of incisions, profuse collagen (C) and fibrosis (FB) with mild infiltration of inflammatory cells (I) and formation of new blood vessels (BV). X10, H & E.

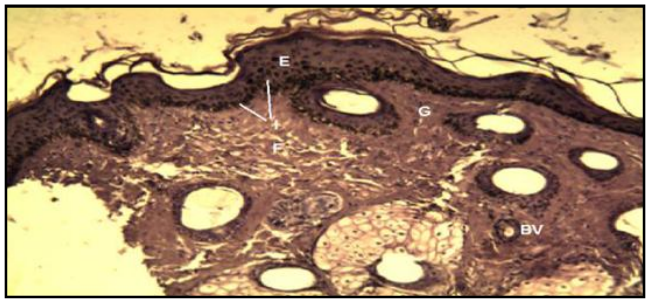

Fig. 11: TG2 three weeks: There is downward hyperplasia of epidermal layer (E), high granulation tissue (G) which characterized by formation of new blood vessels (BV) vertically on the site of incision, fibrosis which is shown horizontally and there is infiltration of inflammatory cells (F). X10, H & E.

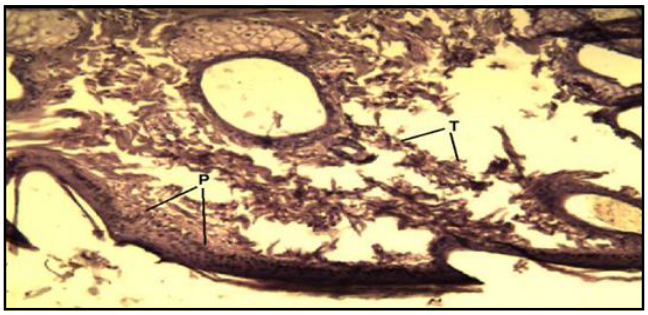

Fig. 12: CG3 three weeks: There was weak healing characterized by thin threads of collagen (T), mild proliferation of epidermal layers (P). X10, H & E.

The aim of our research was to study the use of moderate Nanotherapy [Cellulose Platelets rich-plasma] for the wound healing in Iraqi Arabian mares. Many researchers try to mix nano-materials with PRP like purified fat graft in the aesthetic plastic surgery (Cervelli *et al.,* 2009) but according to our knowledge there are no previous studies regarding this approach. Cellulose is a nanomaterial which extracted from two sources, plant 9 and bacteria. Plant cellulose has very wide distribution on earth, cheap, and is considered as a cold material that doesn't react with the immune system as in cases of materials that are from animal sources.

Fig. 2 shows the gradual reducing of length of the side of the experimental square incisions of all groups but the superiority of this reduction is for the TG1 [3.8214 ± 0.3234] that is treated with cellulose PRP. The first stage of healing in TG1 is the removing of the unhealthy tissue, and the granulation tissue grow healthy red filling the floor of the wound. We believe that cellulose acts as scaffold for fibroblast migration. This result is clear also with TG2, 4.2500 ± 0.2034, which is treated with PRP only which maybe the platelets content of PRP accelerates the regeneration of these cells and these platelets may be secreted growth factors which also play an essential role in accelerating the proliferation of tissue cells. In addition to this fact, the origin of the cellulose is the date palm plant that means it's a cold material and there is no reaction against it applied from the immune system.

The contraction and the scar tissue formation with no fibrous tissue formation are better in TG1 than TG2 as shown in Fig. 2 that reveals the macroscopic appearance. This result also may show the effect of the cellulose which is extracted from plant source, cellulose nano-particles don´t show any immune reactions like itching, redness, swollen….etc.

The histopathological evaluation of TG1 as shown in Fig. 10 after three weeks. There is marked healing which is characterized by very narrow scar tissue, proliferation of epidermal layers in both edges of incisions, profuse collagen with mild infiltration of inflammatory cells, and formation of new blood vessels. This result may prove our 10 explanations about the role of growth factors which are released by platelets and the cellulose scaffold mechanism for cells growth.

Also this research shows the easy administration of this new therapy without the use of any unfeasible instruments and could be applied topically using few drops only.

The results clearly indicate that the cellulose PRP is a new, cheap, and effective biological therapy useful for wound healing. More studies are needed to be conducted in the future to unveil the real effects of these new nano-materials which, in fact, act as a new scaffold for wound regeneration and the mechanism of the bioactive factors which is represented in the PRP. Our research is very useful medically for veterinarians and medical companies.

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Ethical approval

The care and use ethical approaches were followed according to all important and ethical guidelines known globally and locally.

Disclosure of potential conflicts of interest and current submission

No current submission, peer-reviewing, or publishing processes are present. No conflicts of interest are present.

References

Bowler, P.G. (2002). Wound pathophysiology, infection and therapeutic options. *Ann. Med. (Internet). [cited 2019 Jan 25];,* **34(6):** 419-27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12523497.

C.M., S.S., A.G, S.N., M.K., K.C. (2017). Bio-Engineering of Wounds by PRP Led Regeneration. *J. Tissue Sci. Eng. (Internet). Oct 15 [cited 2019 Jan 25];* **08(03):** 1-4. Available from: https://www.omicsonline.org/open-access/bioengineering-of-wounds-by-prp-led-regeneration-2157-7552-1000208-94991.html.

Mishra, A. and T. Pavelko (2006). Treatment of Chronic Elbow Tendinosis with Buffered Platelet-Rich Plasma. *Am. J. Sports Med. (Internet). 2006 Nov 30 [cited 2019 Jan 25];* **34(11):** 1774-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16735582.

Närhi, M.O. and K. Nordström (2014). Regulation of cell-based therapeutic products intended for human applications in

the EU. *Regen Med. (Internet). May 17 [cited 2019 Jan 25];* **9(3):** 327-51. Available from: https://www.futuremedicine.com/doi/10.2217/rme/14.10.

Marx, R.E. (2004). Platelet-rich plasma: evidence to support its use. *J. Oral Maxillofac. Surg. (Internet). 2004 Apr [cited 2019 Jan 25];* **62(4):** 489-96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15085519.

Pietramaggiori, G., A. Kaipainen, J.M. Czeczuga, C.T. Wagner and D.P. Orgill (2006). Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds. *Wound Repair Regen (Internet). 2006 Sep [cited 2019 Jan 25];* **14(5):** 573-80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17014669.

Gonshor, A. (2002). Technique for producing platelet-rich plasma and platelet concentrate: background and process. *Int. J. Periodontics Restorative Dent (Internet). 2002 Dec (cited 2019 Jan25);* **22(6):** 547-57.Availablefrom: http://www.ncbi.nlm.nih.gov/pubmed/12516826.

Wu, C.Y., S.Y. Suen, S.C. Chen and J.H. Tzeng (2003). Analysis of protein adsorption on regenerated cellulose-based immobilized copper ion affinity membranes. *J. Chromatogr A. (Internet). 2003 May 9 [cited 2019 Jan 25];* **996(1-2):** 53-70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12830908.

Pelton, R. (2009). Bioactive paper provides a low-cost platform for diagnostics. *TrAC Trends Anal Chem (Internet). 2009 Sep 1 [cited 2019 Jan 25];* **28(8):** 925-42. Availabl from: https://www.sciencedirect.com/science/article/pii/S0165993609001307.

Martínez Ávila H., E.M. Feldmann, M.M. Pleumeekers, L. Nimeskern, W. Kuo, W.C. de Jong, *et al.,* (2015). Novel bilayer bacterial nanocellulose scaffold supports neocartilage formation in vitro and in vivo. *Biomaterials (Internet). 2015 Mar [cited 2019 Jan 25];* **44:** 122-33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25617132.

Torres Rendon, J.G., T. Femmer, L. De Laporte, T. Tigges, K. Rahimi, F. Gremse, *et al.,* (2015). Bioactive Gyroid Scaffolds Formed by Sacrificial Templating of Nanocellulose and Nanochitin Hydrogels as Instructive Platforms for Biomimetic Tissue Engineering. *Adv. Mater (Internet). 2015 May 1 [cited 2019 Jan 25];* **27(19):** 2989-95. Available from: http://doi.wiley.com/10.1002/adma.201405873 13.

Modulevsky, D.J., C. Lefebvre, K. Haase, Z. Al-Rekabi and A.E. Pelling (2014). Apple Derived Cellulose Scaffolds for 3D Mammalian Cell Culture. Kerkis I, editor. *PLoS One (Internet). 2014 May 19 [cited 2019 Jan 25];* **9(5):** e97835. Available from: https://dx.plos.org/10.1371/journal. pone. 0097835.

Singh, N., S.S. Rahatekar, K.K.K. Koziol, T.S. Ng, A.J. Patil, S. Mann, *et al.,* (2013). Directing Chondrogenesis of Stem Cells with Specific Blends of Cellulose and Silk. *Biomacromolecules (Internet). 2013 May 13 [cited 2019 Jan 25];* **14(5):** 1287-98. Available from: http://pubs.acs.org/doi/10.1021/bm301762p .

Cervelli, V., L. Palla, M. Pascali, B. De Angelis, B.C. Curcio and P. Gentile (2009). Autologous platelet-rich plasma mixed with purified fat graft in aesthetic plastic surgery. *Aesthetic Plast Surg (Internet). 2009 Sep 9 [cited 2019 Jan 25];* **33(5):** 716-21. Available from: http://link.springer.com/10.1007/s00266-009-9386-0.