RESULTS OF WOUND HEALING OF PLATELET RICH PLASMA GEL FROM UMBILICAL CORD BLOOD IN AN EXPERIMENT

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ABSTRACT

Objective: *To evaluate the wound healing effect of PRP gel from human umbilical cord blood in experimental studies.*

Subjects and methods: *The extra-long dorsal skin flaps on 18 white rats were treated for wound healing by injecting PRP gel from human umbilical cord blood and the control was injected with 0.9% NaCl at a dose of 0.5 mL/injection point.*

Research results: *The living area of the skin flaps injected with PRP gel from human umbilical cord blood on days 3; 7 and 14 were 4.1 ± 0.2, 3.9 ± 0.1 and 3.8 ± 0.1 cm^2, respectively, significantly different from the control group ($p < 0.05$). The average number of blood vessels and the average total blood vessel area increased over time when compared with the control group ($p < 0.05$).*

Conclusion: *PRP gel from human umbilical cord blood stimulates angiogenesis and promotes wound healing in experimental studies.*

Keywords: *PRP from human umbilical cord blood, dorsal-long skin flap in white rats*

1. INTRODUCTION

Wound healing is a very complex process, overlapping between inflammation, proliferation and regeneration. The multifactorial nature of the wound environment, systemic and local responses, in addition to the migration, proliferation, interaction and differentiation of many types of cells, many biological molecules, synthesis of matrix components and other signaling networks contribute to the complexity of this process [1].

Autologous platelet-rich plasma (PRP) has been known to contain various biological factors involved in tissue and vascular repair such as specialized granules, alpha granules, platelet-derived growth factor, transforming growth factor - beta, epidermal growth factor and others. Author Jason E et al. (2019) [2]: PRP is effective in tissue regeneration, helping to increase the ability to heal wounds. In clinical practice, there are many elderly patients, chronic diseases, exhaustion... blood collection to produce PRP is difficult and of low quality, human umbilical cord blood (HUCB) has been proven to have many outstanding biological characteristics, is easy to collect and produce PRP gel. In

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order to evaluate the stimulating effect in wound healing treatment, we conducted this study to: Comment on the treatment effectiveness of PRP gel from human umbilical cord blood on the experimental model of extremely long skin flap wound healing.

2. SUBJECTS, MATERIALS AND METHODS OF RESEARCH

2.1. Subjects and materials of research

- Research material: PRP gel from HUCB incubated for 10 minutes in the refrigerator with $CaCl_2$ (10%) and thrombin in a ratio of 4/2/1 (5ml).

Figure 2.1. PRP gel product from human umbilical cord blood

- The research animals were white Wistar rats, 8 - 10 weeks old, weighing 150 -180g, healthy, modeled with wound healing by creating an extremely long dorsal flap of the mouse and treated with PRP gel injections at the Department of Practical and Experimental Surgery - Military Medical University during the period of 9/2023 - 6/2024

2.2. Research method

- Research design: Cross-sectional, longitudinal, controlled study.

- Sample size: Apply sample size estimation according to one-way ANOVA:

n = DF/k + 1 (DF (Degree of Freedom): degrees of freedom; k: number of groups; n: number of samples per group).

From there, based on the distributed file system (DFs - Depth First Search): $10/k + 1 \leq n \leq 20/k + 1$. Choose n = 6/group.

- Research steps and research criteria

+ Proceed to create PRP gel from HUCB: Umbilical cord blood collected from healthy pregnant women is pumped into 10ml tubes. Place these tubes in a centrifuge chamber symmetrically, spin at 3000rpm for 20 minutes and aspirate the dark yellow and light yellow layers. Mix 10% $CaCl_2$ with thrombin (4/2/1) and leave at 4 - 6^0C for 10 minutes.

* Evaluation criteria: Platelet count in PRP gel: Platelet count/ml PRP gel with Model Z3 hematology analyzer and PRP gel quality based on DEPA classification table of Magalon J., et al. (2016) [3]:

Table 2.1. Evaluation of PRP gel quality

Variable	Score			
	1+	2+	3+	4+
Platelet count (µL)	< 1	1 - < 3	3 - < 5	≥ 5
Purity (%)	< 30	30 - < 70	70 - < 90	≥ 90
PRP gel activation process creates an opaque white color	4/1	3/1	2/1	1/1

+ Modeling and PRP treatment: Animals were anesthetized with Ketamine injected into the peritoneum at a dose of 0.001mg/g body weight. Created an extremely long skin flap model. Treated with PRP gel injection points in a 1cm square on the left and 0.9% NaCl on the right. Ended the experiment with CO2 asphyxiation, took pathological specimens on days 3 (M1); 7 (M2); 14 (M3).

* Evaluation criteria: Wound area over time (calculated in cm^2 using Image J software) and wound pathology results [4]: Including average vascularity (total number of blood vessels/total number of microscopic fields), percentage of vascular area (total blood vessel area/total flap area)

2.3. Data processing

The collected data will be collected and analyzed using SPSS 20.0 software

3. RESEARCH RESULTS

- Results of PRP gel from HUCB:

Table 3.1. Average platelet count

	Platelets: Mean ± SD (T/L)
HUCB	168 ± 10.5
PRP gel	858.4 ± 13.4

The average platelet count in PRP gel was approximately 5.1 times higher than that in whole blood.

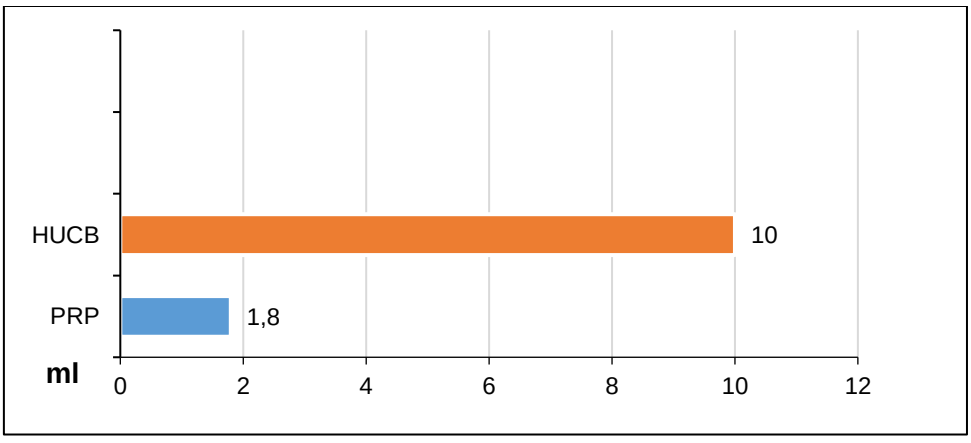

Figure 3.1. PRP volume obtained from 10ml of HUCB

The volume of PRP obtained was 18% of whole blood and averaged 1.9 ± 1.5ml.

Table 3.2. PRP gel quality

Criteria	Score
Platelet count (µL)	3.8+
Purity percentage (%)	3.9+
PRP gel activation rate (4/2/1)	3.8+

PRP obtained with good quality, high purity, best white opaque activation ratio reached 4/2/1.

- Treatment results of PRP gel on extremely long skin flap model:

+ Survival rate (%): Survival rate reached 100%.

+ Length of living skin flap over time.

Table 3.3. Skin flap length over time

Time	Length of skin flap Mean ± SD (cm)					
	M1		M2		M3	
	gel PRP	NaCl	gel PRP	NaCl	gel PRP	NaCl
After 3 days (n = 6)	4.1±0.2	3.9±0.1	-	-	-	-
After 7 days (n = 6)	4.0±0.2	3.5±0.2	3.9±0.1	3.2± 0.2	-	-
After 14 days (n = 6)	4.0±0.1	3.8±0.5	3.8±0.2	3.0±0.3	3.8±0.1	2.8±0.2

The length of the dorsal skin flap, when injected with PRP gel, was larger than that when injected with NaCl. The distal end of the skin flap had necrosis in the PRP gel injection group more slowly and tended to stabilize during the period from day 7 to 14. Unlike NaCl injection, the necrosis of the skin flap still increased and changed with large SD.

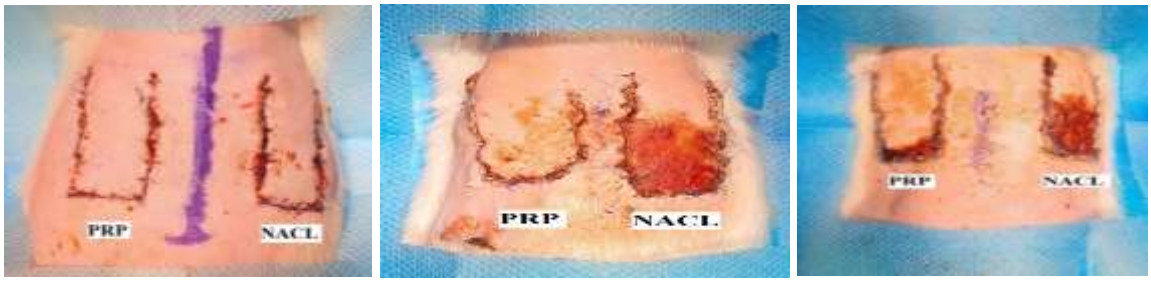

Figure 3.1. Percentage of skin flap survival at days 3, 7 and 14

+ Pathological results and average number of blood vessels:

Table 3.4. Number of blood vessels and average blood vessel area

Variable	M1	M2	M3	p
Average number of blood vessels	6.4 ± 0.6	14.2 ± 1.0	19.8 ± 3.2	p(3,7) < 0.05; p(7,10) < 0.05; p(10,14) < 0.05
Average vascular area (%)	3.3 ± 0.4	6.6 ± 1.2	13.8 ± 2.2	p(3,7) < 0.05; p(7.10) < 0,05; p(10.14) < 0.05

The number of blood vessels and the percentage of blood vessel area in the PRP-injected skin flap increased gradually from day 3 to day 14 and the difference was statistically significant ($p < 0.05$).

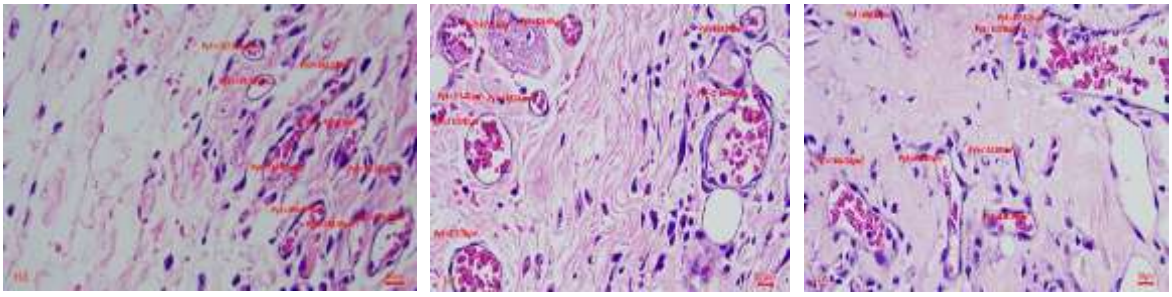

Figure 3.2. Microscopic images of blood vessels on days 3, 7, and 14 after PRP gel treatment

The images show that blood vessels were strongly stimulated to proliferate during the 14 days of the wound.

4. DISCUSSION

According to the research results, the average platelet concentration in PRP is 5.1 times higher than the average platelet concentration in whole blood, lower than the research results of Weiwei et al. (2012) of 5.7 and Chai J. et al. (2019) of 6.2 times [5], [6], however, the average number of platelets obtained is equivalent to other authors and with such quantity and purity, many studies have proven to have high biological activity.

In this study, PRP was activated to form a gel with $CaCl_2$/thrombin at the tested ratios. These are platelet-activating factors that will release active factors, including a large amount of ADP and thromboxane A2. This activation helps increase the biological effects of platelets because platelets contain many growth factors such as VEGF, IGF, KGF,... which stimulate cell proliferation, encourage the formation of new blood vessels, increase collagen and fibroblast production, and help repair damage. We found that the ratio of 4/2/1 (ml) when incubated for 10 minutes at a temperature of 10 - 20^0C will form a clear white gel.

Unfortunately, in this study, we did not test the growth factors after activating the ratios for comparison. However, in vitro, research on fibroblasts and myofibroblasts by author Schere S.S. et al. 2012 found that platelet activation resulted in poorer wound healing than non-activation [7].

In fact, we found that platelet activation can be used well in wounds without skin loss or skin grafts because platelets will come into direct contact with collagen, which is also a natural activating factor.

Studying the wound healing results of PRP gel from umbilical cord blood on an extremely long skin flap model, it was found that the survival length of the skin flap was much larger than the control with the average length on days 3.7 and 14 being 4.1 ± 0.2, 3.9 ± 0.1 and 3.8 ± 0.1 cm, respectively, compared to the control of 3.9 ± 0.1, 3.2 ± 0.2 and 2.8 ± 0.2. This shows that PRP gel from umbilical cord blood has the ability to stimulate blood vessels to nourish the distal end of the skin flap. This result is similar to the study of Chai J. et al. (2019): The survival rate of the skin flap on the PRP-injected side was much higher than that of the control group from day 7 [5], the study of Weiwei et al in 2012: the skin flap survival rate was 61.2%, the control group was 28% from day 7 onwards [6]. This can be explained by the fact that activated PRP releases many growth factors such as

PDGF, TGFTGF-β, EGF, VEGF... which play an important role in the process of cell proliferation and tissue regeneration to promote wound healing. The pathological results also demonstrated this with an increase in the number and size of blood vessels in the skin flap injected with PRP gel compared to the control (Figure 4.1), in addition, the control side was also infiltrated with many inflammatory cells.

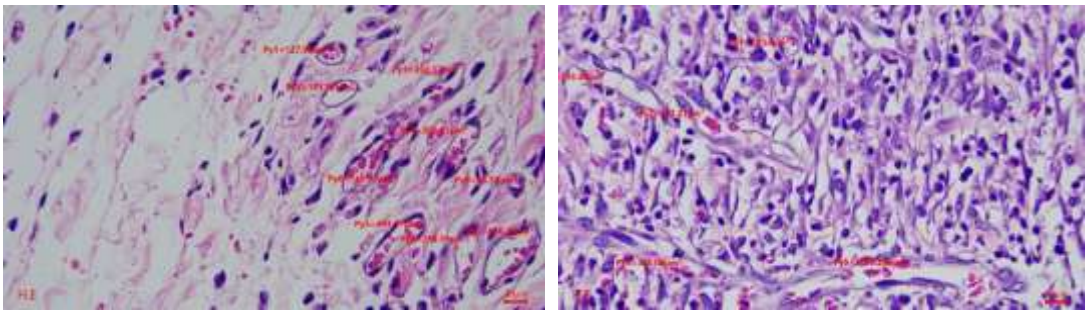

Figure 4.1. Microscopic image of skin flap on day 7

According to Chai J et al. (2019), the PRP activation process releases many growth factors such as PDGF which has been shown to increase the formation of capillaries to help wound healing and VEGF which is known to increase vascular permeability, improve the function of vascular endothelial cells, prolong the life of endothelial cells, and promote angiogenesis [5]. Interestingly, it can be suggested that this can be applied in the process of skin flap harvesting in skin grafting by injecting PRP gel beforehand to increase the survival of the skin flap.

5. CONCLUSION

Human umbilical cord blood PRP gel effectively promotes wound healing by stimulating angiogenesis and reducing inflammatory infiltration in an experimental ultra-long skin flap wound healing model.

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