# RESEARCH ON CHANGES IN SOME CHARACTERISTICS OF ACTIVATED UMBILICAL CORD BLOOD PLATELET-RICH PLASMA AFTER STORAGE AT NORMAL COLD TEMPERATURE

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**Abstract**

***Objectives:*** To evaluate sensory changes, infection, epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) concentrations in activated umbilical cord blood platelet-rich plasma (PRP) after cold storage at 2 - 6 degrees Celsius (°C). ***Methods:*** The study was conducted on 8 umbilical cord blood samples and 8 venous blood samples of adults. ***Results:*** EGF concentrations in PRP from umbilical cord blood and adult blood after activation were 115.72 (51.59 - 217.61) pg/mL and 113.04 (69.71 - 155.73) pg/mL ($p > 0.05$), with VEGF concentration being 193.99 (75.58 - 320.25) pg/mL and 16.12 (11.70 - 49.03) pg/mL, respectively, $p < 0.01$. After cold storage at 2 - 6°C for 7 days, 10 days, and 14 days, the concentrations of EGF and VEGF in PRP from activated umbilical cord blood showed no difference compared to immediately after activation ($p > 0.05$). PRP remained clear and yellow, with no signs of infection. ***Conclusion:*** PRP from umbilical cord blood has high VEGF concentration, no significant change in appearance, EGF and VEGF concentrations, and sterility after cold storage for 7 - 14 days.

**Keywords:** Platelet-rich plasma; Umbilical cord blood; Change; Cold storage.

## INTRODUCTION

Platelet-rich plasma contains many growth factors that stimulate wound healing stages. These factors are much higher than normal when concentrated from plasma [1]. Autologous PRP has many advantages in treating chronic wounds but encounters some difficulties when implementing. The source is umbilical cord blood with a lot of potential [2, 3].

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Stored umbilical cord blood is used to treat many diseases in the form of allogeneic [2, 3]. In Vietnam, umbilical cord blood is mainly used for stem cell products to treat diseases or as a precaution for potential future autologous disease treatment. Blood and blood products are also studied for application in cold storage conditions, usually at 2 - 6°C, which is convenient for the actual treatment process [4]. Umbilical cord blood contains hematopoietic stem cells and mesenchymal stem cells; however, the number of stem cells is quite low, leading to slow graft growth and a high risk of infection [5]. Therefore, the application of activated umbilical cord blood PRP in wound treatment mainly depends on the role of growth factors. EGF and VEGF are two of the growth factors from platelet alpha granules having an important role in initiating and stimulating the four stages of wound healing [6, 7]. This study aims to: *Evaluate the changes in some characteristics of PRP from activated umbilical cord blood after conventional cold storage to support further understanding in the application of umbilical cord blood PRP in clinical wound treatment.*

## MATERIALS AND METHODS

### 1. Subjects

8 samples of cord blood immediately after birth from healthy pregnant women and 8 samples from healthy adult donors.

* *Inclusion criteria:*

Pregnant women aged 20 - 45; no history of obstetric diseases, no concomitant diseases such as congenital diseases, tuberculosis, cancer, autoimmune diseases, mental illness, screened for HIV, HBV, HCV with negative results before giving birth, no obstetric complications; gestational age > 36 weeks; born within 24 hours of rupture of membranes; newborn weight ≥ 2.800g.

Volunteer donors: Agree to give blood samples; no acute or chronic diseases; HIV, HBV, HCV screening tests with negative results; no fever (< 38°C); aged 20 - 45 years old.

* *Location and time:* Umbilical cord blood was collected at the Department of Obstetrics and Gynecology, Military Hospital 103. Blood from healthy adults was collected, and PRP extraction, hematology, biochemistry, and bacterial culture were performed at the Department of Paraclinical Medicine, Le Huu Trac National Burn Hospital. EGF and VEGF were tested at the Military Medical Research Institute, Vietnam Military Medical University. The study was conducted from May 2023 to September 2024.

* *Raw materials and equipment:* PRP separation kit New-PRP Pro kit; Thermo Fisher Scientific's human EGF, VEGF test kit; syringes, blood collection tubes for hematology and biochemistry tests, bacterial culture kits; newborn weight ≥ 2.800g; K4500 machine with 18 hematological parameters by Sysmex of Japan; AU480 blood biochemistry analyzer; ELISA machine; DLAB centrifuge; biological and immunological laboratory equipment; freezer -80°C; refrigerator for storage at 2 - 6°C.

**3. Methods**

* *Study design:* Umbilical cord blood and venous blood were collected according to aseptic procedures, blood was taken into test tubes in the PRP extraction kit, transferred to the laboratory of the testing department immediately after collection, and PRP was extracted using the double centrifugation method. PRP was activated with $CaCl_2$ (ratio 1:10). Activated PRP was stored at 2 - 6°C, and characteristics and test indexes were collected and evaluated the day after activation, 7 days, 10 days, and 14 days after activation.

* *Method of implementation:*

Umbilical cord blood is aspirated with a sterile 10mL syringe immediately after the aseptic procedure, the blood is collected into the test tube of the kit. A negative pressure needle is used to insert directly into the test tube of the kit to collect peripheral blood from the volunteer donor according to the procedure. PRP was separated according to the procedure of the kit. Whole blood is partially collected in the same process, and a quantity of PRP after activation is tested, evaluated, and compared.

PRP preparation process as described in the instructions of GeneWorld:

- Phase 1 (collection of unactivated PRP): Take venous/umbilical cord blood into test tubes, each tube with a total of 10mL (including 1.5mL of available anticoagulant). Centrifuge the first blood tube to separate plasma, red blood cells, and platelets at 2,000rpm x 10 minutes. Gently aspirate the yellow plasma layer on top, aspirate the white layer next to the red blood cell layer (buffy coat layer), and put it into a centrifuge tube labeled PLASMA (total volume recovered from 3 tubes is about 12 - 16mL). Centrifuge the second time at 3,500rpm x 5 minutes with a PLASMA tube with a counterweight to obtain a solution that separates into 2 layers. Gently aspirate the yellow liquid on top and discard it, leaving 6mL of liquid at the bottom of the tube. This is the unactivated PRP.

- Phase 2 (activate PRP with $CaCl_2$): Aspirate the remaining 6mL of PRP into another test tube that contains a small amount of $CaCl_2$ activator solution. Add and mix well for 2 - 5 minutes until a solid mass appears. Use a sterile pipette to gently rotate the solid mass until it separates from the tube wall. Wait 5 - 15 minutes until the solid mass shrinks completely, then aspirate the solid mass. The final product is a clear yellow activated PRP solution with a volume of about 4 - 5mL.

* *Measurement of EGF and VEGF:* Using the ELISA reaction method. Quantify the presence of antibodies/ antigens by the "sandwich" method. A layer of antibody specific for EGF/VEGF is coated on the well plate. The base sample, test sample, and antibody are added to the well if the biotin of the EGF/VEGF receptor is detected. At the bottom of each well, EGF/VEGF will attach itself to the available antibody at the bottom and to the biotin-linked detection antibody after incubation. Remove all non-specific binders, and add streptavidin - HRP. Wash and add substrate solution to the well. A color reaction occurs in which the intensity of the color matches the concentration of EGF/VEGF. Stop the reaction with another solution. Measure the color intensity, then calculate the concentration of EGF/VEGF in the sample.

* *Bacterial culture:* Take 0.1mL of activated PRP and culture bacteria according to the routine procedure of the microbiology lab, and determine the species and number of bacteria (if any grow).

* *Research indicators*

Color characteristics, clarity-turbidity, quantitative test indexes of EGF and VEGF of activated PRP (T1), after activation, stored cold at 2 - 6°C for 7 days (T7), 10 days (T10) and 14 days (T14); PRP infection testing culture were performed at the above times.

* *Data processing:* The obtained data are calculated as the average in the form of $\overline{X} \pm$ SD compared by T-test or in the form of Q2 (Q1 - Q3) compared by Mann Whitney U-test, Wilcoxon test. Analyze data using SPSS 22.0 software. There are statistically significant differences when $p < 0.05$.

**4. Ethics**

The research content was approved by the Expert Subcommittee for evaluation according to Decision No. 4692/QD-HDTSSDH dated November 18, 2022, and approved by the Ethics Council in Biomedical Research of

Military Hospital 103 (certification No. 14/CNChT-HDDD dated January 6, 2023). Military Hospital 103 granted permission for the use and publication of the research data. The authors declare to have no conflicts of interest when implementing or publishing the results of this study.

## RESULTS

**Table 1.** Bacterial culture results of PRP after storage period.

| Time after storage | PRP from umbilical cord blood (n = 8) | PRP from adult human blood (n = 8) |
|---|---|---|
| Immediately after activation (T1) | Negative | Negative |
| After 7 days of storage (T7) | Negative | Negative |
| After 10 days of storage (T10) | Negative | Negative |
| After 14 days of storage (T14) | Negative | Negative |

PRP samples stored at 2 - 6°C after 7, 10, and 14 days did not exhibit colonial growth.

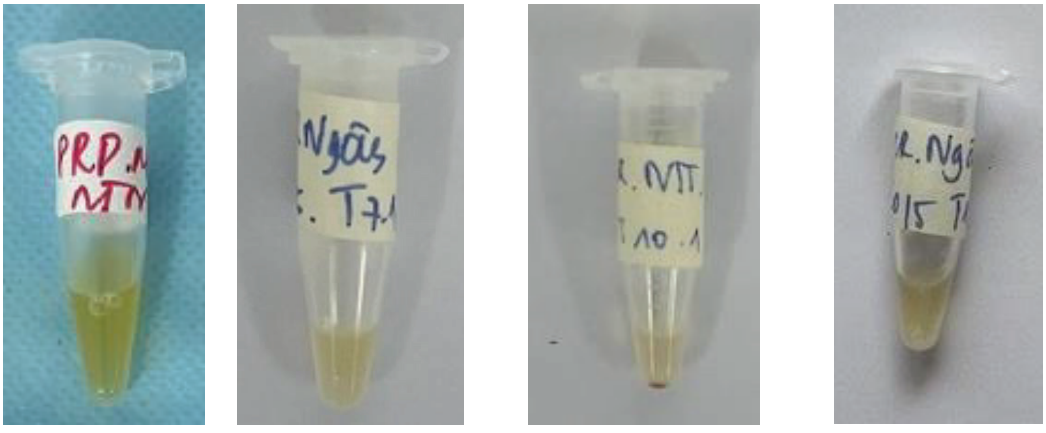


**Figure 1.** Activated PRP after 14 days of cold storage.

PRP samples stored at 2 - 6°C after 7 days, 10 days, and 14 days showed no change in color (yellow), no turbidity, and no air bubbles (clear).

**Table 2.** EGF concentrations in activated PRP stored after different storage periods[+].

| EGF concentration in PRP Q2 (Q1 - Q3) | Cord blood (n = 8) | Adult human blood (n = 8) | p |
|---|---|---|---|
| Immediately after activation (T1) (1) | 115.72 (51.59 - 217.61) | 113.04 (69.71 - 155.73) | > 0.05* |
| After 7 days of storage (T7) (2) | 91.82 (47.71 - 258.20) | 164.91 (96.11 - 438.32) | > 0.05* |
| After 10 days of storage (T10) (3) | 109.02 (53.79 - 329.35) | 149.67 (86.35 - 198.39) | > 0.05* |
| After 14 days of storage (T14) (4) | 109.80 (45.42 - 284.79) | 192.77 (99.43 - 459.71) | > 0.05* |
| $p_{21}$** | > 0.05 | < 0.05 | |
| $p_{31}$** | > 0.05 | > 0.05 | |
| $p_{41}$** | > 0.05 | < 0.05 | |

*([+]: Storage conditions: Cold storage 2 - 6°C; *: Mann Whitney U test; **: Wilcoxon test)*

During storage time points, the EGF concentration in activated PRP from umbilical cord blood was not statistically different compared to that from adult blood ($p > 0.05$). After 7, 10, and 14 days of cold storage at 2 - 6°C, the EGF concentration in activated PRP from umbilical cord blood and from adult blood was not statistically different compared to the initial time point ($p > 0.05$).

**Table 3.** VEGF concentrations in activated PRP preserved after different storage periods$^+$.

| VEGF concentration in PRP Q2 (Q1 - Q3) | Cord blood (n = 8) | Adult human blood (n = 8) | p |
|---|---|---|---|
| Immediately after activation (T1) (1) | 193.99 (75.58 - 320.25) | 16.12 (11.70 - 49.03) | < 0.01* |
| After 7 days of storage (T7) (2) | 240.61 (49.68 - 462.65) | 11.70 (11.70 - 59.08) | < 0.05* |
| After 10 days of storage (T10) (3) | 211.78 (40.22 - 266.13) | 11.70 (11.70 - 49.86) | < 0.05* |
| After 14 days of storage (T14) (4) | 147.44 (50.53 - 254.44) | 17.90 (11.70 - 54.16) | < 0.05* |
| $p_{21}$** | > 0.05 | > 0.05 | |
| $p_{31}$** | > 0.05 | > 0.05 | |
| $p_{41}$** | > 0.05 | > 0.05 | |

*($^+$: Storage conditions: Cold storage 2 - 6°C; *: Mann Whitney U test; **: Wilcoxon test)*

During storage time, the concentration of VEGF in activated PRP from umbilical cord blood was statistically higher than that from adult blood ($p < 0.05$). After 7, 10, and 14 days of cold storage at 2 - 6°C, the concentration of VEGF in activated PRP from umbilical cord blood and from adult blood was not statistically different compared to the initial time ($p > 0.05$).

## DISCUSSION

The umbilical cord consists of 2 smaller arteries spiraling around a larger vein. The umbilical vein supplies oxygen-rich blood and nutrients to the fetus. The arteries return deoxygenated blood to the placenta. The umbilical vein is large and easily visible. When taking umbilical cord blood, blood is taken from the umbilical vein because it is large and easy to see. This ensures easy collection and the quality of blood because this blood contains a lot of nutrients and is transported to the fetus from the placenta through the osmotic pathway in the placenta, not directly

from the mother's blood [5]. The source of umbilical cord blood has a lot of potential as an abundant blood source, ensuring economic and medical ethics, especially being able to bring a good quality product and creating a ready-made PRP product for wound treatment. As this source is very accessible, donors do not face safety risks, infectious diseases have a very low risk of transmission, and umbilical cord blood also produces low immunity [2, 3]. Preserved umbilical cord blood can be used for autologous transfusion for patients with blood diseases. It is also used in cases of patients with neurological diseases, pulmonary diseases, systemic skin diseases, etc., in the form of allogeneic [2, 3]. Studies have shown that autologous PRP has a wide range of clinical applications. However, it may also contain many inflammatory cytokines, and its quality depends on several factors, such as the number and quality of platelets, the age of the patient, and the treatments the patient has received. Studies have shown that umbilical cord PRP contains more anti-inflammatory and growth factors than peripheral blood PRP [8]. Therefore, research on the application of umbilical cord blood PRP will open up a new direction in the treatment of chronic wounds today.

EGF is secreted from platelets and is also produced by macrophages and fibroblasts. It is present throughout the epidermis, especially in the basal layer. EGF plays a major role in re-epithelialization by stimulating the proliferation and migration of keratinocytes, increasing the tensile strength of the new skin layer [6]. VEGF is not only secreted from platelets but also from many other types of cells, such as fibroblasts, endothelial cells, smooth muscle cells, macrophages, and neutrophils. VEGF plays a major role in promoting the process of neovascularization, increasing permeability through capillaries during wound healing [7]. In acute wounds, VEGF begins to increase from day 1, increases significantly from day 3 - 5, and returns to normal from day 7 - 14 after injury. In chronic wounds, VEGF decreases abnormally [7]. Chronic wounds are weakened by excessive vascularization, leading to the lack of oxygen and micronutrients, thereby causing more tissue damage [6]. This phenomenon causes a decrease in many cytokines, chemokines, and growth factors necessary for wound healing, including EGF and VEGF [6]. Many studies have mentioned the use of exogenous EGF and VEGF in the treatment of chronic wounds. EGF and VEGF are found in high concentrations in PRP, so PRP is a good source of EGF and VEGF in the treatment of chronic wounds [6, 7].

Blood and blood products have long been commonly used in treatment. The preservation of blood and blood products is also regulated to be able to practice in accordance with actual conditions. According to Circular 26 of the Ministry of Health in 2013, the shelf life of plasma can be up to 14 days from the time of preparation in a closed system [4]. Thus, the preservation of blood products is easily implemented in clinical practice conditions, especially in grade 1 medical facilities. There are reports that at room temperature, PRP can be preserved for 5 - 8 days [9]. In this study, we preserved activated PRP at 2 - 6°C for 7 days, 10 days, and 14 days, the evaluation criteria were color, turbidity, bacterial and fungal culture, and measurement of EGF and VEGF concentration index. Through evaluation, we found that the color of PRP did not change, maintaining a clear yellow color during storage. PRP was also not infected with bacteria or fungi. The concentration indexes of EGF and VEGF in PRP from adult blood and umbilical cord blood did not fluctuate significantly compared to PRP immediately after activation ($p > 0.05$). Our results are similar to the findings of previous reports [9, 10]. Under the above storage conditions, the EGF concentration was not different between the origin of umbilical cord blood and adults, but VEGF in PRP from activated umbilical cord blood was significantly higher than that from adult blood, consistent with previous reports [8]. Thus, when storing PRP in cold conditions of 2 - 6°C, if PRP is extracted tightly and ensures the process, it can be stored for a long time, possibly 7 - 14 days, as in our evaluation. Application in clinical practice can be deployed to suit the conditions of each medical facility.

## CONCLUSION

Platelet-rich plasma from activated umbilical cord blood has higher VEGF concentrations than that from adult blood sources. Cold storage at 2 - 6°C for 7 - 14 days keeps PRP clear, yellow, and free of bacteria, and EGF and VEGF concentrations in PRP do not change significantly.

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