Feasibility Study on Using Syringes to Separate Human Peripheral Blood for Preparing Platelet-Rich Plasma (PRP)

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Abstract: This paper focuses on exploring new methods for preparing platelet-rich plasma (PRP) to improve its clinical application and popularization. As a natural growth factor, PRP has significant advantages in promoting tissue repair and alleviating inflammation, but limitations in preparation methods hinder its widespread use. The study uses clinical medical syringes as the centrifuge container for PRP preparation. Without disrupting the stratification of the blood, the method employs a pushing technique to move the density gradient centrifuged blood through a medical three-way valve, separating the different blood components to obtain the target PRP layer. This method is simple to operate, has a higher recovery efficiency, and ensures the blood is handled in a closed environment throughout the process, reducing environmental contamination. It can be performed in ordinary settings. The authors seek a feasible, easy-to-operate, environmentally low-requirement, and low-cost separation method to facilitate the clinical popularization of PRP.

***Keywords*:** PRP (platelet-rich plasma), PRP preparation, syringe as PRP centrifuge container, pushing technique, separation of blood components

1. Introduction

Platelet-rich plasma (PRP) is an important biological resource with significant effects in promoting tissue repair and alleviating inflammation. In recent years, with the continuous development and application in clinical medicine and sports medicine, the research on PRP preparation methods has also received widespread attention. Finding a simple, efficient, and stable PRP preparation method to improve its clinical application and popularization has become a current research hotspot.

The centrifugation method and component separation scheme for PRP preparation are crucial. Centrifugation has a long history, dating back to the 18th century. After the Industrial Revolution and the rapid development of the textile industry, a cotton fabric dehydrator appeared in 1836. With technological advancements and clinical needs, medical centrifuges have become increasingly important in today's medical field. They can quickly sediment red blood cells and rapidly extract serum and plasma, playing a vital role in clinical treatment and auxiliary medical processes. Medical centrifuges can efficiently solve many medical problems, such as hematopoietic stem cell collection, renal function decline, blood component replacement, and clinical serological examinations.

This paper introduces a new PRP preparation method and explores its properties and advantages through experiments. Using the pushing technique after blood density gradient centrifugation is an excellent method for separating blood components. Its characteristics include: 1) not disrupting the relationship between the layers after blood centrifugation; 2) high platelet recovery rate; 3) simple operation process; 4) low cost, conducive to clinical promotion.

In clinical PRP preparation, it has been found that current mainstream PRP preparation kits have limited concentration and volume ranges. This limitation is related to the volume, structure, and PRP layer harvesting method used in the PRP centrifuge container. Is it possible to separate the blood layers without altering the relationship between the layers after density gradient centrifugation? After continuous exploration and thinking, we utilized common clinical medical syringes, medical three-way valves, modified centrifuge adapters, and created a small pushing device. In an ordinary environment, sterile blood component separation operations can be completed, achieving layer separation for PRP harvesting. This method can also be used for extracting other important cell components from blood, such as nucleated cells.

2. Materials and Methods

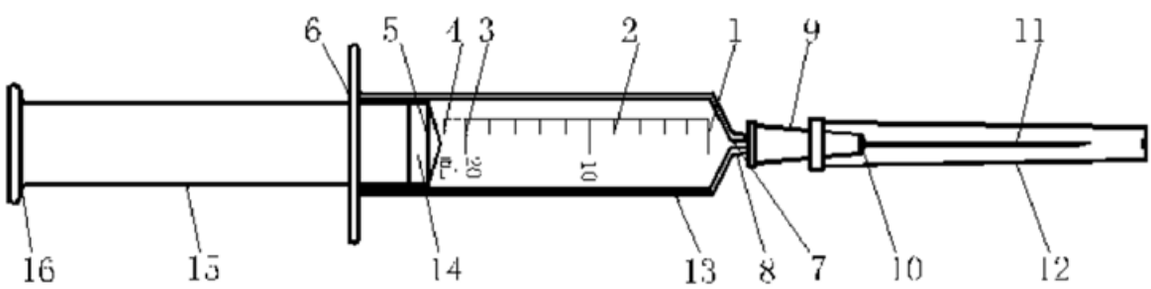
2.1 Feasibility Study of the Experimental Plan

(1) Blood is a tissue that circulates within the heart and blood vessels. In adults, blood constitutes about one-thirteenth

of the body weight and consists of plasma and blood cells. Its pH ranges from 7.3 to 7.4, and its osmotic pressure is approximately 313 milliosmoles per liter. The hematocrit in adults is about 37-50%. Blood cells are categorized into three types: red blood cells, white blood cells, and platelets. White blood cells and platelets account for only 0.15%-1% of the total blood volume. The relative density of whole blood in adults is about 1.050-1.060, with plasma having a density of approximately 1.025-1.030, red blood cells and polymorphonuclear leukocytes about 1.092, mononuclear cells 1.075-1.090, and platelets 1.030-1.035 [1]. Due to the different densities of blood cell components, the density gradient centrifugation method can be used to separate various target cells.

(2) Medical centrifuges can be broadly classified into two types: angle rotor centrifuges and horizontal centrifuges [2]. Angle rotor centrifuges are typically used for separating plasma or serum, with the separated layers forming an angle with the container. This experiment does not suit this type. Horizontal centrifuges operate with low noise, have a maximum speed of 5000 r/min, and a maximum capacity of 1000 ml. They offer low chamber temperature rise and high separation efficiency. The entire machine is computer-controlled, easy to operate, and equipped with an electronic door lock, ensuring safety and reliability. After separation, the blood layers are parallel to the container's opening, making it suitable for separating blood cell components [3].

(3) Can medical syringes (Figure 1) replace centrifuge tubes in this experiment? We need to harvest the blood layers after centrifugation using a horizontal method. Current cell centrifuge tubes cannot achieve this through layer pushing. Medical syringes, with their plunger, tapered tip, and tip hole, are the best choice for horizontal pushing experiments. To determine the pressure resistance of medical syringes, we conducted an experiment. A 20 ml syringe was filled with water, the plunger removed, the tip hole sealed with a scalp vein needle, and the distal end of the needle heat-sealed, creating a closed system. The syringe was fixed in the centrifuge basket, with balanced weights on both sides. At a speed of 4000 rpm (commonly 2000 rpm for cell separation) for 20 minutes, no leakage was observed.



1-zero graduation line; 2-graduation capacity line; 3-nominal capacity graduation line; 4-total graduation capacity line; 5-baseline of the syringe; 6-flanging of the syringe jacket; 7-cone-shaped head hole of the syringe; 8-cone-shaped head of the syringe; 9-needle hub of the syringe; 10-connector of the syringe; 11-needle tube of the syringe; 12-injection needle sheath; 13-syringe barrel; 14-syringe piston; 15-syringe plunger; 16-syringe press handle

Annotation: This schematic diagram only illustrates the structure of the syringe, and it is not the only form stipulated by the standard."

Figure 1. Medical Syringe

(4) To better secure the syringe, we created an adapter that fits the syringe, ensuring stable placement in the centrifuge basket.

(5) To achieve a more uniform pushing force, we developed an auxiliary pushing device for the medical syringe plunger.

(6) Other Equipment: The medical three-way valve can effectively direct the flow of blood components according to the pushing process.

2.2 Experimental Instruments and Reagents

(1) According to the experimental requirements, the selection of reagents and instruments must be standard, especially when handling blood. Although this experiment is a physical one, it considers future practical applications, so the selection should align with actual cell separation scenarios. The chosen medical syringes, medical infusion three-way valves, and medical scalp vein needles are all commonly used clinical medical devices (Figure 2). The horizontal centrifuge used in the experiment is the TDL-40C model produced by Shandong Baiou Medical Technology Co., Ltd. (Figure 3). The biological safety cabinet is the Airstream A2 type Class II biological safety cabinet (AC2-4S8-NS series) by ESCO (Figure 4). The

automated blood cell counter used is the XS-1000i model by Sysmex (Figure 5).

(2) Blood requires an anticoagulant. We used the JieRui 200 ml blood bag (Figure 6), which contains 28 ml of 4.0% sodium citrate anticoagulant. The citrate ions form a soluble complex, calcium citrate, with the calcium ions in the blood. This complex is water-soluble but not easily dissociated, inhibiting the coagulation process and thus preventing blood clotting. The required sodium citrate/peripheral blood ratio for anticoagulation is 1:10-15.

(3) Developing a new PRP separation scheme may require custom experimental instruments. The centrifuge adapter for the medical syringe used in the experiment (Figure 7) and the pushing device for the syringe plunger (Figure 8) were designed and fabricated according to the experimental needs.

(4) The Zhenye Cell Tissue Sample Bank and the Henan Tissue Cell Bank provided the facilities and sample sources for the experiment.

(5) The blood samples required for the experiment were obtained through voluntary donations. All collection processes were conducted with full informed consent, with consent forms signed, and the donors were healthy individuals screened for blood-borne infectious diseases and related blood disorders.

Figure 2. Medical three-way valve

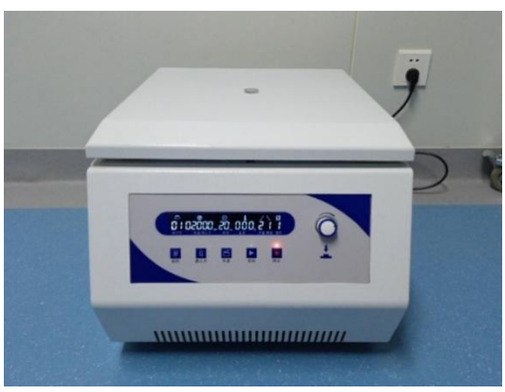


Figure 3. Baiou Horizontal Centrifuge TDL-40C

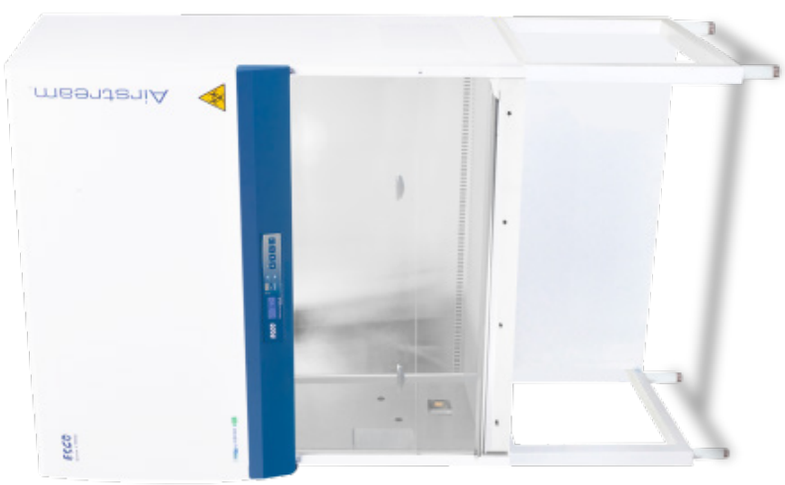


Figure 4. ESCO Airstream A2 Type II Biological Safety Cabinet (S Series AC2-4S8-NS)

Figure 5. Sysmex XS-1000i Fully Automated Hematology Analyzer

Figure 6. 200ml Blood Collection Bag for Medical Use

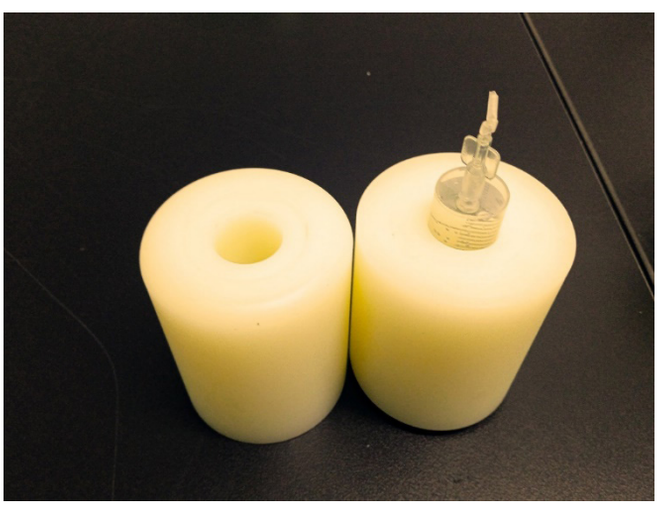


Figure 7. Adapter for Medical Luer Lock Syringe Centrifuge

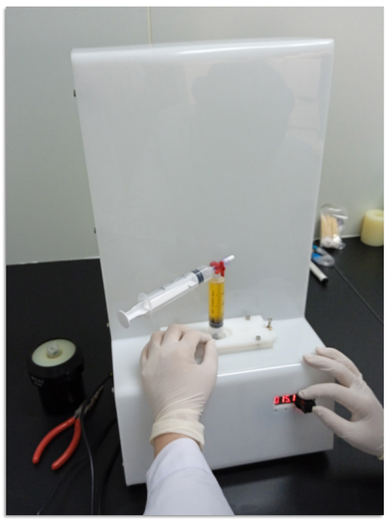


Figure 8. Syringe Plunger Pusher

2.3 Roles of Instruments and Reagents

(1) Role of the Biological Safety Cabinet: Provides a sterile, dust-free, and well-lit platform for the experiment.

(2) Role of the Horizontal Centrifuge TDL-40C: In the experiment, the 20 ml medical syringe containing blood needs to undergo density gradient centrifugation using this equipment to obtain stratified blood samples, making it an essential instrument.

(3) Role of the Sysmex XS-1000i Automated Blood Cell Counter: Used for cell counting at various stages of the experiment.

(4) Role of the JieRui 200 ml Blood Bag: Contains 28 ml of anticoagulant, used for anticoagulating the experimental blood samples.

(5) Role of 20 ml, 5 ml, and 1 ml Luer Lock Medical Syringes: Serve as containers in place of centrifuge tubes during blood centrifugation and for collecting target cells.

(6) Role of the Medical Three-Way Valve: After centrifugation, the 20 ml syringe containing blood is connected to waste collection syringes and target cell layer collection syringes using the medical three-way valve. During the pushing process, it directs blood components into the respective collection syringes.

(7) Role of the Centrifuge Adapter for Fixing the 20 ml Syringe: Secures the 20 ml medical syringe in the centrifuge basket to prevent tilting, shifting, or imbalance during centrifugation and to prevent the syringe from rupturing during centrifugation.

(8) Role of the Syringe Pusher: During blood separation, the 20 ml medical syringe's plunger needs stable, appropriately paced, and forceful pushing action. The syringe pusher provides this force, enabling the plunger to move forward and, through the medical three-way valve, collect the target PRP layer.

2.4 Experimental Protocol

(1) Selection of Experimental Blood:Choose healthy adults without hematological diseases or blood-related infectious diseases such as hepatitis B, hepatitis C, HIV, or syphilis.Obtain informed consent from donors who voluntarily sign the consent form.

Collect 20 blood samples, each divided into an experimental group (20 ml) and a control group (20 ml).

(2) Single Collection Preparation.Prepare two 20 ml medical syringes, each drawing 1.5 ml of sodium citrate from the blood bag, and replace the syringe needle with a butterfly needle for blood collection.Use a 5 ml tube for routine blood tests. After disinfecting the donor's skin, use the prepared 20 ml syringe with sodium citrate anticoagulant to collect venous blood up to the 20 ml mark. Tie and seal the butterfly needle tubing, then gently shake for 1 minute to mix the anticoagulant with the blood thoroughly.

(3) Break off the plunger rod of the 20 ml syringe filled with blood, ensuring the plunger end is flush with the syringe barrel edge.Verify the syringe tip is sealed with a butterfly needle, then place the syringe in the centrifuge adapter, ensuring the adapter is placed in the centrifuge basket.Balance the centrifuge load and proceed with centrifugation.

(4) Based on literature, various blood cell densities are as follows: whole blood (1.050–1.060), plasma (1.025–1.030), red

blood cells and polymorphonuclear leukocytes (1.092), mononuclear cells (1.075–1.090), and platelets (1.030–1.035) [1].

(5) Establish centrifugal force, time for ramp-up/down, and duration based on literature.Utilize the density gradient centrifugation method to stratify blood components, applying either sedimentation velocity or isopycnic sedimentation equilibrium.Use a horizontal centrifuge (TDL-40C) set to 20 minutes at 714 G (2000 rpm) with a ramp-up of 5 and ramp-down of 1. This initial spin aims to stratify plasma from red blood cells to harvest plasma and platelets.Ensure the centrifuge basket is balanced.

(6) After running the biosafety cabinet for 10 minutes, spray the exterior of the centrifuged 20 ml syringe with 75% alcohol for disinfection.Place the syringe in the biosafety cabinet and connect it via a medical three-way valve to another 20 ml syringe.

Maintain the blood level horizontal to the ground while connecting, then proceed to push (manually or with a pushing device), aiming to collect the upper plasma layer into the 20 ml syringe.

(7) Slowly push the plunger of the centrifuged 20 ml syringe while controlling the three-way valve, collecting all the plasma into the 20 ml syringe.Carefully slow down as the buffy coat nears the three-way valve, stopping collection at the buffy coat.

(8) Seal the syringe tip with a butterfly needle and trim the protruding plunger rod flush with the syringe barrel edge. Place it in the centrifuge for a second spin, set to 20 minutes at 2187 G (3500 rpm) with a ramp-up of 6 and ramp-down of 1. This aims to sediment platelets to the plunger end.Ensure the centrifuge basket is balanced.

(9) Disinfect the exterior of the centrifuged syringe with 75% alcohol, then place it in the biosafety cabinet.Connect via a three-way valve to another 20 ml syringe (for waste) and proceed with pushing (manually or with a device) to collect the desired plasma volume based on clinical PRP requirements.For this study, retain 3 ml of plasma in the syringe, then seal the tip with a butterfly needle.

(10) Use a vortex mixer to mix platelets adhered to the syringe plunger with the remaining 3 ml of plasma for at least 30 seconds, creating platelet-rich plasma (PRP).

(11) Using the three-way valve, transfer the PRP to a 5 ml syringe for injection and future use.

2.5 Experimental Results Data Statistics and Analysis

(1) Experimental Results:

Table 1. Comparison of Platelet Harvest Rates for 20 Samples in the Experimental Group

Sample Number	Platelet Routine Count (109)	Total Platelet Count in 20 ml Blood(109)	Total Platelet Harvest in 20 ml Blood(109)	Harvest Rate %
1	204	4.08	3.72	91.18%
2	235	4.70	4.20	89.36%
3	169	3.38	3.00	88.76%
4	222	4.44	4.01	90.32%
5	279	5.58	4.79	85.84%
6	190	3.80	3.36	88.42%
7	238	4.79	4.22	88.10%
8	160	3.20	2.91	90.94%
9	198	3.96	3.56	89.90%
10	237	4.74	4.29	90.51%
11	147	2.94	2.46	83.67%
12	288	5.76	4.94	87.13%
13	183	3.66	3.13	85.52%
14	265	5.30	4.79	90.38%
15	221	4.42	3.86	87.33%
16	178	3.56	3.05	85.67%
17	253	5.06	4.44	87.75%
18	210	4.20	3.74	89.05%
19	167	3.34	2.94	88.02%
20	140	2.80	2.34	83.57%
Average	209.2	4.19	3.69	88.07%

Calculation Formulas:

Total Platelet Count in 20 ml Blood (10^9) = (Platelet Routine Count (10^9/L) * 20 ml) / 1000 ml

Platelet Harvest Rate (%) = (Total Platelet Harvest in 20 ml Blood (10^9) / Total Platelet Count in 20 ml Blood (10^9)) * 100%

(2) We refer to the data from two published articles as the objective control group for our experiment:Control Article 1: "Preparation and Clinical Application of PRP" used the manual test tube method to prepare PRP. The PRP had a concentrated platelet count of (880±143)×109/L, which is 4.2±1.1 times the physiological baseline value, and the platelet recovery rate was (80.7±13.5)%[4] .Control Article 2: "Chinese Journal of Joint Surgery," December 2016, Volume 10, Issue 6. In the article by Wang Shujun, Wen Congji, and Li Shiyan, "Comparison of Cellular and Cytokine Components in PRP Prepared by Different Kits," the platelet recovery rates were as follows:Arthrex PRP: 52.9±7.7%;Regen PRP: 66.2±11.9%;Weigao PRP: 67.5±9.3%;Harvest PRP: 68.4±10.0% [5].

(3) Comparison of Platelet Recovery Rates Using Syringe Pushing Method and Other PRP Preparation Methods

Table 2. Comparison of Platelet Recovery Rates Using Syringe Pushing Method and Other PRP Preparation Methods

group	number of samples	Platelet recovery rate %
Arthrex PRP	20	(52.9±7.7) %
Regen PRP	20	(66.2±11.9)%
Wego PRP	20	(67.5±9.3)%
Harvest PRP	20	(68.4±10.0)%
Manual test tube method	43	(80.7±13.5)%
Syringe pushing method (experimental group)	20	(88.07±2.59)%

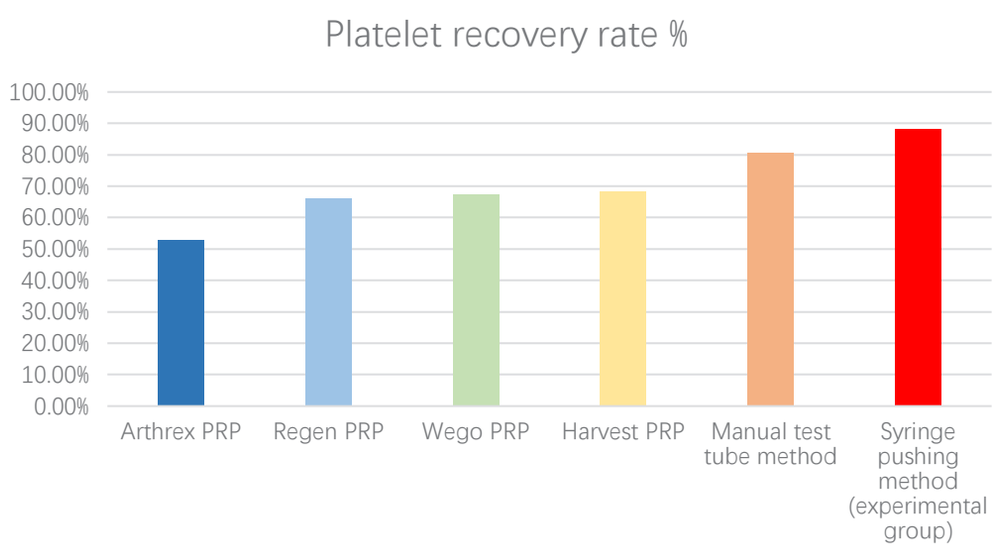


Figure 9. Comparison of Platelet Recovery Rates Using Syringe Pushing Method and Other PRP Preparation Methods

(4) Adjustment of Platelet Concentration and Volume in PRP Obtained by Syringe Pushing Method.The syringe pushing method used to obtain platelet-rich plasma (PRP) in our experiment involves platelet resuspension after sedimentation, allowing flexibility in adjusting platelet enrichment and final PRP volume according to clinical requirements. Unlike other PRP kits, which use fixed volume ratios, our method allows for broad adjustments in platelet concentration based on practical needs.

(5) Data Statistical Analysis.We compared the syringe pushing method (experimental group) with the manual test tube method (control group), which showed the highest platelet recovery rate among commercial kits.

Manual Test Tube Method:Average recovery rate: 80.7%;Standard deviation: 13.5%

Syringe Pushing Method (Experimental Group):Average recovery rate: 88.07%;Standard deviation: 2.59%

To assess the magnitude of the difference between the two groups:

Calculate the Difference in Means:

Average of syringe pushing method (experimental group): 88.07%

Average of manual test tube method (control group): 80.7%

Difference = 88.07% - 80.7% = 7.37%

Compare with Standard Deviation:

Standard deviation of syringe pushing method (experimental group): 2.59%

Standard deviation of manual test tube method (control group): 13.5%

Average standard deviation = (13.5% - 2.59%) = -10.91%

Since 7.37% > -10.91%, it indicates a significant difference in the means of the two groups.

(6) Conclusion:Preliminary data suggests that the syringe pushing method may yield higher platelet recovery rates compared to the manual test tube method. However, further research and validation are recommended to ensure the accuracy and reliability of these results.

2.6 Troubleshooting Experimental Interference

In scientific experiments, various methods are typically employed to eliminate factors that could interfere with the results. Commonly used approaches include the method of constancy and the method of exclusion. The method of constancy involves maintaining factors that may affect the experimental results in a constant state, such as using the same operator or operating environment. On the other hand, the method of exclusion aims to eliminate irrelevant variables that do not affect the experiment, thereby reducing their potential interference with the results. For example, 20 blood sample donors may have their samples collected at the same time.

3. Conclusion

The fundamental viewpoint of the paper is that through experimentation and statistical analysis, the "top-push separation method" for cell growth drugs is a more efficient method for whole blood cell separation than the traditional pipette aspiration method in cell laboratories. Furthermore, there were no differences in cell preparation time or the number of personnel involved. The experiment demonstrated an 11.09% improvement in cell recovery rate, indicating that the top-push method is more effective.

The main innovations of this experiment are threefold. Firstly, the top-push cell separation method innovates in terms of separation strategy, adhering to the principle of non-destruction of cell layer structure and achieving a continuous separation process. Secondly, all consumables used in the experiment are of three medical device categories, meeting clinical needs. Thirdly, two top-push devices have been developed according to demand and have been patented.

Regarding the predicted and evaluated academic and practical value of the paper, the top-push separation design represents an innovative approach and could potentially become one of the methods for blood component separation in the future. It is characterized by simplicity, feasibility, high cell recovery rate, low risk of contamination in the relatively closed environment of the syringe, minimal environmental requirements, and ease of widespread application. The two top-push devices developed can be further optimized to become tools for the top-push separation method. Of course, there will be numerous new products emerging in terms of other containers and corresponding equipment.

The limitations or unresolved issues in the study mainly lie in the need for validation with larger sample sizes and the test of market viability over time.

Suggestions for further research in this research direction include exploring various sample separation methods, such as sound waves or other vibrations that can cause the separation of substances with different densities and volumes (as in the case of separating stones from wheat by shaking), or microfluidic cell separation methods using different micro-pathways. All these methods are worthy of further in-depth research. All research endeavors contribute to the advancement of science, technology, and knowledge, making them worthwhile pursuits.

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