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Synthesis and use of red blood cells

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Abstract: Red blood cells and their functions are indispensable for the human body, many medical institutions are more or less facing the problem of blood supply shortage, red blood cells themselves are difficult to store, once removed from the human body, the shelf life is very short. This project is mainly to conduct research and exploration through scientific research experiments, which are also interspersed with some data inquiries. In this study, artificial red blood cells with normal physiological function were successfully obtained. The significance of this research is that it can help solve the problem of shortage of blood source and difficult storage of red blood cells. The successful synthesis of red blood cells helps to extend the storage time of red blood cells, reduce unnecessary blood waste, and further promote the in-depth understanding of human red blood cells.

1. Introduction

Red blood cells are one of the most common blood cells in the body, also known as red blood cells or red blood cells. They are non-nucleated cells, disc-shaped, produced through the bone marrow and circulated in the blood. The main function of red blood cells is to carry oxygen to the various tissues and organs of the body, and to carry carbon dioxide from the tissues back to the lungs for elimination. This is done by a protein molecule called hemoglobin that is present in red blood cells. Hemoglobin is able to combine with oxygen to form oxyhemoglobin, which then releases oxygen in the lungs while binding and transporting carbon dioxide to the lungs for elimination. The morphological characteristics of red blood cells give them a large surface area, which increases the efficiency of the exchange of oxygen and carbon dioxide. In addition, red blood cells have softness and deformability, allowing them to pass through tiny blood vessels and capillaries.

In both war and peace, blood shortages are inevitable because of the short life span of red blood cells and the limited technical means to store them. Many medical facilities face a shortage of blood to a greater or lesser extent, and red blood cells themselves are difficult to store and have a short shelf life once removed from the body. Red blood cells have a lifespan of about 120 days, after which they are broken down and cleared by the spleen and other tissues. Because of its importance, the research and development of artificial red blood cells is of great significance to solve problems such as blood shortage.

Artificial red blood cells can mimic the function of natural red blood cells, providing new options for medical applications and clinical treatments. Therefore, this study hopes to solve the above problems through artificial red blood cells.

Guo et al based on a silica cell bioreplication approach reported the design and construction of

synthetic rebuilt red blood cells (RRBCs) that fully mimic the broad properties of native RBCs[1], Ren et al focused on the progress of research and development of artificial blood at home and abroad in recent years, and makes a preliminary discussion on the clinical application value, development trend and future research and development direction of artificial blood, in order to provide new ideas for the development of this field[2]. In the pursuit of advancing the clinical application of artificial blood products, several notable studies have made significant contributions. Xie et al highlighted recent advances in the multifunctional manipulation of RBCs using optical tweezers, aiming to promote the clinical application of artificial blood products by enabling controllable deformation, dynamic stretching, RBC aggregation, blood separation, and Raman characterization[3]. In a comprehensive review, Allan Doctor discussed historical and new HBOC designs, including current state-of-the-art and novel bio-inspired artificial RBC designs in development, along with a critical analysis of successes and challenges in this field[4]. Furthermore, Hiroshi Azuma et al conducted an academia-initiated first-in-human phase 1 clinical trial to assess HbV safety and pharmacokinetics in healthy male adult volunteers from 2020[5]. Additionally, Ding et al provided a summary of the biology, synthesis, characteristics, and distribution of red blood cell-derived materials, offering insights into their potential applications and technical challenges in the treatment of malignant tumors[6]. Lastly, Qiao et al reported on a nanoplatform for postoperative on-demand pain management, which effectively enhances analgesic time while providing ultrasonic imaging. This involved incorporating levobupivacaine and perfluoropentane into dendritic mesoporous silica and covering them with red blood cell membranes to prolong pain relief in living organisms. These collective efforts represent significant strides in the advancement of artificial blood products and their potential clinical applications[7].

This project intends to artificially synthesize red blood cells through the following steps: Ficoll blood purification, polyformaldehyde fixation of red blood cells, extraction and measurement of hemoglobin, mineralization and enhanced mineralization of hemoglobin, gradient dehydration using anhydrous ethanol and HMDS, and construction of sodium alginate and chitosan structures on hemoglobin. Artificial red blood cells have a double-concave disk structure, oxygen carrying capacity and compliance similar to normal red blood cells, and can be stably preserved, which is expected to solve the medical situation of red blood cell shortage, and has great potential application value.

2. Materials and Methods

2.1 Materials(See Table 1 for details)

Table 1: Consumables, instruments and their specifications and brands

Material/ instrument name	Size	brand
Test tubes	5ml/15ml/50ml	SAINING
Pipettes	10μl/20μl/100μl/1ml	CORNING
Hettich ® MIKRO 220/220R Centrifuges	/	Sigma-Aldrich
PBS solution	500ml	Gibco™
Ficoll solution	50ml	Sigma-Aldrich
Hemoglobin	5g	Macklin
Pipettor	10μl/20μl/100μl/1ml	BRAND
toluene	100ml	Macklin
syringes	20ml	D&B
filters	0.22μm	Merck millipore
sodium alginate	100g	Macklin

chitosan	100g	Macklin
acetic acid	50ml	Macklin
pure water	4.5L	Wahaha
BCA protein quantitative kit	/	Servicebio
ethanol	/	Macklin
optical microscope	/	XTALQUEST
ELIASA	/	Leopard

2.2 Red blood cell density gradient centrifugation purification

1) We transfer 1ml of whole blood to a 15ml centrifuge tube, then dilutes it with 10ml of PBS solution and gently mixes thoroughly.

2) We prepare two 15ml centrifuge tubes by adding 5ml of Ficoll solution into each.

3) We carefully layer the diluted blood onto the Ficoll in both tubes, ensuring gentle handling to prevent mixing of the solutions.

4) We dilute 10ml of blood in each centrifuge tube and centrifuge at 800g for 20 minutes, ensuring the deceleration setting is set to no break or only 10-20% braking.

5) Following centrifugation, layers will form as depicted in the diagram, with the RBC-containing cell layer appearing red.

6) At this stage, we aspirate the cells from this layer into a clean 15ml centrifuge tube using a straw. We add 10-15ml of PBS, centrifuge at 800g for 10 minutes, discard the supernatant, and repeat this washing process with culture medium five times.

7) Finally, we resuspend the cells in 5-10ml of PBS and stores them at 4 ℃.

2.3 Erythrocyte fixation

① Procedure for observing red blood cell morphology under optical microscope ② Procedure for counting blood cells plate ③ Procedure for fixing red blood cells: The red blood cells were immobilized using a 4% polyformaldehyde solution prepared in an ice environment.④ Procedure for extracting hemoglobin using toluene and pure water.⑤Configuration of hypotonic 0.75xPBS, configuration of 0.9%NaCl, and calibration and use of pH meters: A solvent with a salt content of 0.9% was prepared, a total of 15 ml, containing about 135 mg of salt. Finally, the pH of the brine in the test tube was measured using a calibrated pH meter.

2.4 Mineralization

1) Washing after fixing the red blood cells: after the fixed red blood cells are centrifuged, the fixed solution is drawn out, leaving only the red blood cells, and then washed with 1xPBS solution for 3 times, and each time the PBS solution is added, it is centrifuged once and then the separated solution is drawn out.

2) For mineralizing red blood cells, we add the prepared 100mMol TMOS solution into the red blood cells test tube and incubate it on the rotating mixing machine.

3) To configure the mother liquor of the layer self-assembly solution, we put sodium alginate and chitosan powder in two separate test tubes and dissolve them with water - for chitosan, we need to add an additional trace of acetic acid.

4) To learn the BCA method for determining hemoglobin, we mix the prepared BCA solution with a small amount of hemoglobin and conduct a controlled experiment to detect the hemoglobin concentration using an enzyme-labeled instrument.

5) To adjust the pH, we add sodium chloride or sodium hydroxide to the 0.9% salt water prepared the previous day to adjust the pH value of the salt water to about 3.

2.5 Red blood cell dehydration

①The oxygen absorption curve of fresh blood was measured by enzyme-labeled instrument. ② Red blood cell morphology was observed by optical microscope after mineralization. ③Washing after strengthening mineralized red blood cells, preparation of ethanol gradient dehydration solution and red blood cell dehydration process operation: First, we prepare five tubes of 25 ml ethanol solution with concentrations of 30 percent, 50 percent, 70 percent, 90 percent, and 100 percent ethanol, respectively. We also prepare two tubes of HDMS solution with concentrations of 50 percent and 100 percent, respectively, and finally, a tube of 15 ml ethanol. Then, we mix the first tube of solution with red blood cells into the centrifuge. After waiting for the centrifuge to complete, we take it out and aspirate the supernatant from the centrifuge tube. We continue by adding the second tube of solution to mix, and repeat this process sequentially with the remaining solutions.

2.6 Calcined erythrocyte

1) We use vacuum drying oven to dry red blood cells after ethanol dehydration. 2) We calcined red blood cells by gradient warming method.3) Measurement of hemoglobin dioxy curve: It includes 3 measurements, namely oxygen inhalation, deoxygenation and reoxygenation.

2.7 LBL layer self-assembly

1) We prepare the LBL self-assembled diluent.

2) For the coating of chitosan and sodium alginate: We first mix the chitosan solution into the calcined red blood cells. After centrifugation, we extract the supernatant, then wash with pure water twice. Next, we mix in the sodium alginate solution and extract the supernatant after centrifugation, repeating this process for 3 times.

3. Results

Figure 1 shows the macroscopic state of red blood cells after density gradient centrifugation in Ficol solution. It can be seen that red blood cells are located in the top layer. Then, By repeated PBS washing and centrifugation, we obtained relatively pure red blood cells. We put the red blood cells prepared in experiment 1 on a slide and observed the cells through an optical microscope. In the light microscope, we can observe a round cell with a bright center and a dull periphery(Figure 1b). That is, these cells exhibit a double-concave disc-like structure. This morphological feature can effectively improve its surface area, improve the ability to carry oxygen and material exchange and gives red blood cells a powerful ability to deform, allowing them to pass smoothly through narrow capillaries throughout the body. The silicic acid precursors exchange with water in the hydrogen-bonded interfacial water network around the interfaces of all biomolecules of the red blood cell, and subsequently undergo amphoteric catalysis to form silica through a series of acid and base parts present on the protein surface. This near-end interface catalytic condensation process is naturally self-limiting, resulting in nanoscale (~10 nm thick) silica replicas of all intracellular and extracellular features of red blood cells The silicon template, shown in Figure 1c, replicates the double-concave disc-like structure well. After the preparation of the silicon shell, because the melting point of silicon is much larger than the burning point of red blood cells, we completely remove the red blood cells through high temperature calcination, and the remaining is a relatively pure silicon template. Figure

1d and e are macro pictures before and after calcination respectively. The pure silicon template obtained after calcination is shown in Figure 1f. Then, the skeleton of artificial red blood cells was obtained by self-assembly of sodium alginate layer by layer(Figure 1g).In order to make the artificial red blood cells have the ability of carrying and releasing oxygen, the hemoglobin was loaded on the skeleton, and the function of carrying and releasing oxygen of hemoglobin was detected by enzyme-labeled instrument. As shown in Figure 2, the absorption peak of hemoglobin can be detected in the vicinity of 405~410 after oxygen loading, while the absorption peak is near 430 after oxygen release[8]. This shows that the hemoglobin loaded on the human red blood cell skeleton retains good oxygen carrying and oxygen releasing functions.

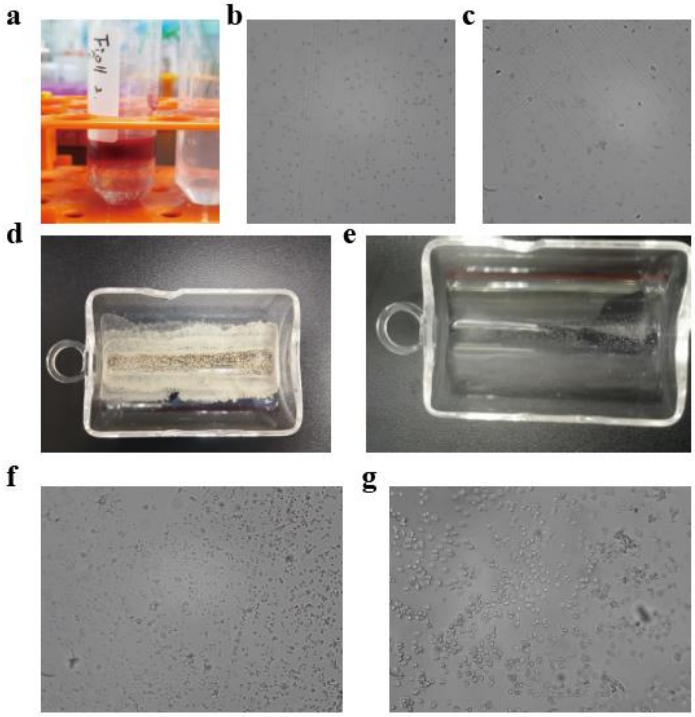

Figure 1: (a)Macroscopic state of red blood cells after density gradient centrifugation. (b)Normal erythrocyte morphology under light microscope. (c)Morphology of red blood cells covered with inorganic silicon under light microscope. (d)Dried silica formwork. (e)Silicic acid template after high temperature calcination, when the organic components are removed by calcination. (f)The light structure of the silicate template after calcination. (g)The morphology of artificial red blood cells coated with sodium alginate by layers of self-assembly under light microscope.

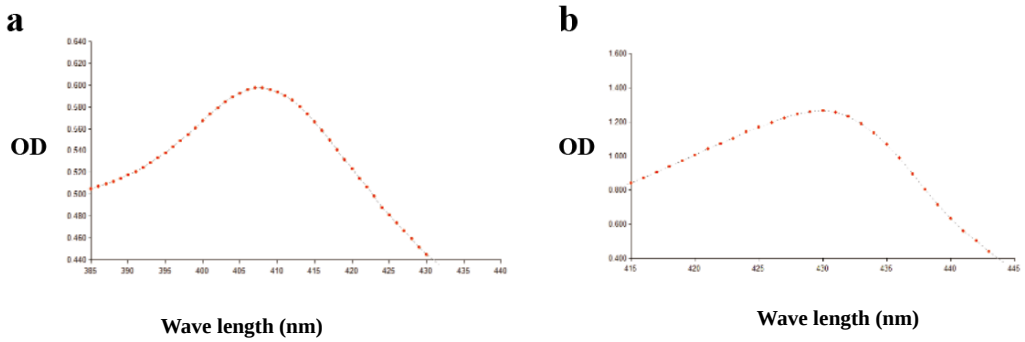

Figure 2: (a)Absorption peak during oxygenation of artificial red blood cells. (b)Absorption peak in deoxygenated artificial red blood cells.

4. Discussion

This paper mainly studies the method of synthetic red blood cell, how to form artificial red blood cell, and its structure and function. The main research methods are as follows: First, fresh blood is purified with ficoll solution and red blood cells are separated. The red blood cells were then immobilized by paraformaldehyde. Then using red blood cells as a template, the double concave disc structure was copied with silicic acid. By high temperature calcination, the organic components were removed, and a double-concave disc template composed of relatively pure silica was obtained. Then, using chitosan and sodium alginate solution, the artificial red blood cell hydrogel skeleton was prepared by layer self-assembly on the basis of silicon template. Finally, by loading hemoglobin on the hydrogel, the artificial red blood cells are endowed with the ability to carry and release oxygen. In conclusion, this study successfully synthesized hydrogel artificial red blood cells with double-concave disc-like structure, similar in size to normal red blood cells, and capable of carrying and releasing oxygen. Compared with ordinary red blood cells, artificial red blood cells are more stable in physical and chemical properties, have a longer storage period, and can overcome the rejection reaction between different blood types, which can greatly reduce the dependence on blood donors. Unfortunately, the preparation of artificial red blood cells is still in the initial stage, and only the oxygen carrying and oxygen releasing functions of red blood cells have been realized. Obviously, red blood cells also have other functions, such as transporting metabolic waste, transporting carbon dioxide, and so on.

5. Conclusion

In this study, artificial red blood cells with oxygen carrying and releasing function were successfully prepared. The size and structure of the normal red blood cells were reduced.

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