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## Resealed erythrocytes: A novel carrier

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### Abstract

Carrier erythrocytes have been evaluated in thousands of drug administration in humans proving safety and efficacy of the treatments. Carrier erythrocytes, resealed erythrocytes loaded by a drug or other therapeutic agents. Erythrocytes mediated drug delivery has been reported with therapeutic enzymes and antiviral agents to maximize therapeutic performance, reduce the side effects, as circulating depots for controlled drug release, drug targeting, treatment of parasitic diseases, hepatic tumors, removal of toxic agents etc. Resealed Erythrocytes are biocompatible, biodegradable, possess long circulation half-life and can be loaded with variety of active drug substances.

**Keywords:** Resealed erythrocytes, hepatic tumor, parasitic disease

### Introduction

The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog. Erythrocytes, also known as red blood cells, have been extensively studied for their potential carrier capabilities for the delivery of drugs and drug-loaded microspheres<sup>[1, 2]</sup>.

Application of erythrocytes as promising slow drug release or site-targeted delivery systems for a variety of bioactive agents from different fields of therapy has gained a remarkable degree of interest in recent years.

Technically, there are two main approaches to combine drugs with erythrocytes. The first one is by means of forming transient pores in the erythrocyte membrane, thus allowing for the drug to enter the erythrocyte. This intra-erythrocytic loading method is used with the aim to greatly enhance the half-life of the drug and to avoid hypersensitive reactions in patients by shielding the drug from the immune system. The second strategy consists of coupling the active drug to the erythrocyte membrane, thus exposing it at the surface of the cell. Although this latter method has shown efficiency in the slow release of drugs, it cannot be used to target the RES or to reduce allergenic reactions by protecting the drug from the extracellular environment.

### Basic features of Erythrocytes

Erythrocytes are the most abundant cells in the human body (~5.4 million cells/mm<sup>3</sup> blood in a healthy male and ~4.8 million cells/mm<sup>3</sup> in healthy female). Erythrocytes are biconcave discs with an average diameter of 7.5  $\mu$ m, a thickness of 2.0  $\mu$ m in periphery, 1  $\mu$ m in the center, and a volume of 85–91  $\mu$ m<sup>3</sup> (Fig. 1). The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3  $\mu$ m wide<sup>[3, 4]</sup>.

### Isolation of Erythrocytes

Blood is collected into heparin zed tubes by venipuncture.

Blood is withdrawn from cardiac /splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti-coagulant.

The whole blood is centrifuged at 2500 rpm for 5 min at 4 $\pm$ 10 °C

The serum and buffy coats are carefully removed and packed cells washed three times with phosphate buffer saline (pH=7.4). 4 $\pm$ 10 °C in a refrigerated centrifuge.

The washed erythrocytes are diluted with PBS and stored at 4°C until used<sup>[5, 6]</sup>.

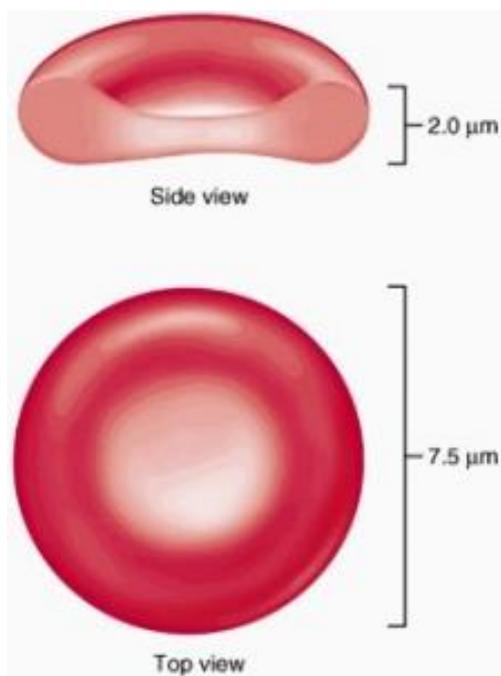
### Source of Erythrocytes

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits<sup>[7]</sup>.

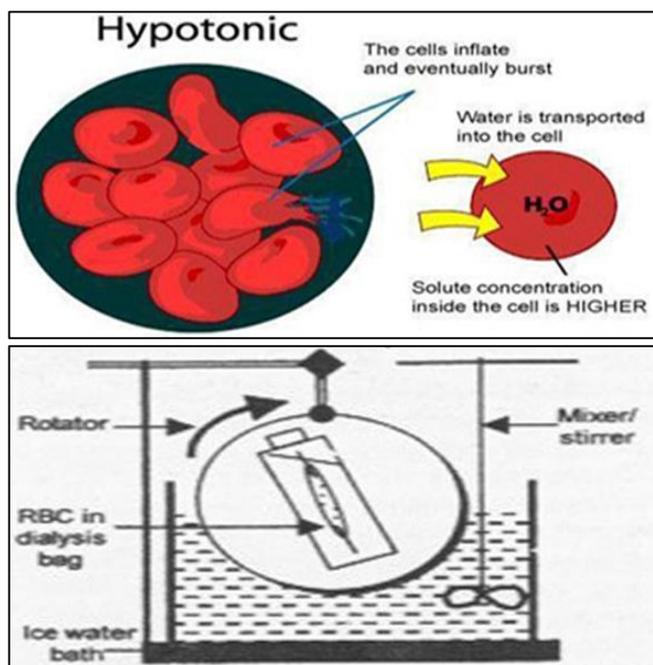
The reasons for this increasing interest in drug delivery are due to the increasing need of safe drugs, capable of reaching the target and with minimal side effects. In fact the main problems associated with systemic drug administration are essentially related to the bio-distribution of pharmaceuticals throughout the body<sup>[8]</sup>.

**Method of drug loading in resealed erythrocytes**

- Hypo-osmotic lysis method
- Hypotonic hemolysis
- Hypotonic dilution
- Hypotonic dialysis
- Hypotonic preswell technique
- Isotonic osmotic lysis
- Electro-insertion or electroencapsulation
- Entrapment by endocytosis<sup>[9]</sup>



**Fig 1:** Structure of resealed erythrocytes



**Fig 2:** Hypotonic Dilution and Hypotonic Dialysis

**Overview of drug encapsulation methods**

The procedure involves suspending erythrocytes in an isotonic buffer in an electrical discharge chamber. A capacitor in an external circuit is charged to a definite voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential. The optimum intensity of an electric field is between 1-10 kW/cm and optimal discharge time is between 20-160<sup>[10]</sup>.

**Release Characteristics of Loaded Drugs**

There are mainly three ways for a drug to efflux out from the erythrocyte carriers: phagocytosis, diffusion through the membrane of the cells and using a specific transport system. RBCs are normally removed from circulation by the process of phagocytosis. The degree of cross linking determines whether liver or spleen will preferentially remove the cells. Carrier erythrocytes following heat treatment or antibody cross-linking are quickly removed from the circulation by phagocytic cells located mainly in liver and spleen. The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid by layer. It is greatest for a molecule with high lipid solubility.

**Drug Delivery Systems**

**Targeted drug delivery**

RES or non-RES ‘targeting’ is another important strategy using erythrocytes as carriers.

**RES targeting**

It is a well-known fact that, in physiologic conditions, as a result of the gradual inactivation of the metabolic pathways of the erythrocyte by aging, the cell membrane loses its natural integrity, flexibility and chemical composition. These changes, in turn, finally result in the destruction of these cells upon passage through the spleen. The other effective site for the destruction of the aged or abnormal erythrocytes is the macrophages of the RES including peritoneal macrophages, hepatic Kupffer cells and alveolar macrophages of the lung, peripheral blood monocytes, and vascular endothelial cells. We know that aging and a series of other factors (e.g., stress during non-gentle loading methods) make the erythrocytes recognizable by the phagocytosing macrophages via changing the chemical composition of the erythrocyte membrane, i.e., the phospholipids component. Therefore, a considerable fraction of carrier erythrocytes that have undergone some degrees of structural changes during the loading procedure will be trapped by the RES organs, mainly the liver and spleen, within a short time period after re-injection<sup>[11]</sup>. A series of approaches have been evaluated to improve RES targeting using carrier erythrocytes. In one of these approaches, the drug-loaded erythrocytes have been exposed to membrane stabilizing agents. This may increase the targeting index of the erythrocytes to RES via decreasing the deformability of these cells<sup>[12]</sup>.

**Non-RES targeting**

Recently, carrier erythrocytes have been used to target organs outside the RES. The various approaches include: Co-encapsulation of paramagnetic particles or photosensitive agents in erythrocytes along with the drug to be targeted, Application of ultrasound waves, Site-specific antibody attachment to erythrocyte membrane.

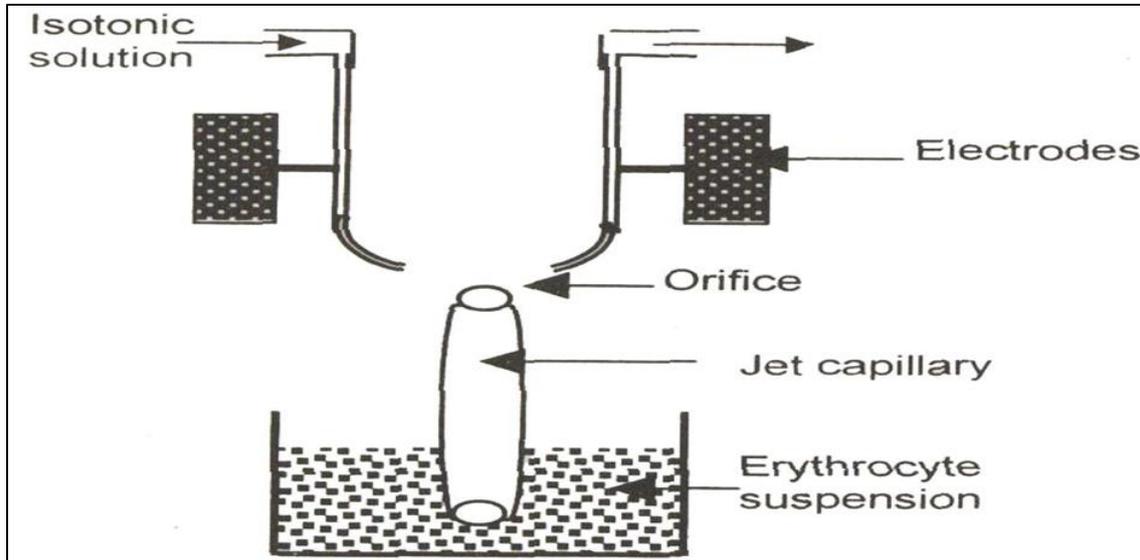
*In vitro* targeting of erythrocytes to cytotoxic T-cells by coupling them to Thy-1.2 monoclonal antibody<sup>[13]</sup>. Delivery

of colloidal particles and erythrocytes to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound. The differential response of photosensitized young and old erythrocytes to photodynamic activation has been studied [14].

**Applications of Resealed Erythrocytes**

- Slow drug release
- Drug targeting
- Targeting RES organs

- Targeting the liver, Enzyme deficiency/ replacement therapy
- Treatment of hepatic tumors
- Treatment of parasitic diseases
- Removal of RES iron overload
- Removal of toxic agents
- Targeting organs other than those of RES
- Delivery of antiviral agents
- Enzyme therapy
- Improvement in oxygen delivery to tissues [15, 16]



**Fig 3:** Showing electro encapsulation technique

**Table 1:** Various characterization parameters and method employ for resealed erythrocytes [17]

PARAMETER	INSTRUMENT / METHOD USED
<b>I. Physical parameter</b>	
[a] Shape, size, surface morphology	Transmission electron microscopy, Scanning electron microscopy, Optical microscopy, phase contrast microscopy.
[b] Vesticle size & size distribution	Transmission electron microscopy, Optical microscopy.
[c] Drug release	Diffusion cell, Dialysis.
[d] Drug content	Deproteinization of cell membrane followed by assay of drug, radiolabelling.
[e] Surface electrical potential spectroscopy	Zeta potential determination by Photon correlation [PCS]
[f] Surface pH	pH sensitive probes
[g] Deformity	Capillary method
<b>II. Cellular parameter</b>	
[a] % Hb content	Deproteinization of cell membrane followed by hemoglobin assay
[b] Cell volume	Laser light scattering
[c] % Cell recovery	Neubaur's chamber, hematological analyzer
[d] Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
[e] Osmotic shock	Dilution with distilled water & estimation of drug and hemoglobin
[f] Turbulent shock	Passage of cell suspension to 30-gauge hypodermic needle at the rate of 10ml/min flow & estimation of residual drug & hemoglobin, vigorous shaking
[g] Erythrocyte sedimentation rate	Determine by ESR technique.
<b>III. Biological parameter</b>	
[a] Pyrogenicity	LAL test, Rabbit method
[b] Sterility	Sterility testing method
[c] Toxicity	Toxicity test method.

### Future Scope

- Treatment of liver tumors
- Treatment of parasitic diseases

- Delivery of antiviral agents
- Targeting of bioactive agents to RE system

**Table 2:** Comparison of various hypo osmotic lysis methods

Methods	% Loading	Advantage	Disadvantage
Dilution method	1-8%	Fastest and simplest for low molecular weight drugs	Entrapment efficiency is very less
Dialysis	30-45%	better <i>in vivo</i> survival of RBCs	Time consuming heterogeneous size distribution
Preswell dilution	20-70 %	Good retention of cytoplasm <i>in vivo</i>	-
Isotonic osmotic lysis	-	Better <i>in vivo</i> surveillance	Impermeable only to large molecule, time consuming

### Conclusion

The use of resealed erythrocytes extended promising for a safe and effective delivery of various bioactive molecules for effective targeting. However, the concept needs further optimization to become a routine drug delivery system. The same concept also can be extended to the delivery of biopharmaceuticals and much remains to be explored regarding the potential of resealed erythrocytes. Until other carrier systems come of age, resealed erythrocytes technology will remain an active arena for the further research. In future a critical time in this field as commercial applications are explored. In near future, erythrocytes based delivery system with their ability to provide controlled and site specific drug delivery will revolutionize in effective treatment of various disease. For the present, it is concluded that erythrocyte carriers are “nano device in field of nanotechnology” considering their tremendous potential.

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