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REENGINEERING ERYTHROCYTES A NOVEL DRUG DELIVERY SYSTEM

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Abstract

Drug delivery systems including chemical, physical and biological agents which will enhance the bioavailability, improve pharmacokinetics and reduce toxicities of the drugs. Carrier erythrocytes are one among the foremost promising biological drug delivery systems investigated in recent decades. Resealed erythrocytes are biodegradable biocompatible, possess long circulation half-life and can be loaded with sort of active drug substances. Resealed erythrocyte possesses several advantages over the other drug delivery system which makes it superior than other systems. Carrier erythrocytes are prepared by collecting blood sample from the organism of interest and isolate erythrocytes from plasma. By using various methods, the cells are broken and the drug is entrapped into the erythrocytes, finally they are resealed and therefore the resultant carriers are then called "resealed erythrocytes". Resealed erythrocytes, as a drug delivery system has excellent capacity to reinforce the therapeutic index and patient compliance. It has got tremendous potential to achieve site specific drug delivery with minimum wastage of drugs and it also prolong the discharge of drug. So many drugs like aspirin, steroid, antineoplastic which having many side effects are reduce by resealed erythrocyte. The present review signifies various features, drug loading methods, evaluation, applications and clinical progress of resealed erythrocytes.

Introduction

Present pharmaceutical scenario is focused on advancement of drug delivery systems which maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases. To accomplish a necessary therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a "perfect" drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments Various carriers has been used for the drug targeting among which cellular carrier offer a more prominent and potential advantages related to its biodegradability, non-pathogenicity, non immunogenicity, biocompatibility, self degradability along with high drug loading efficiency.Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, biodegradable, possess very long course half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods.

Erythrocytes

A healthy adult male and female has about 5.4 million RBC per L of blood and 4.8 million RBC per µL of blood respectively. Erythrocytes are biconcave discs with a diameter of 7-8 µm. They contain the O_2 carrying protein Hb, which is a pigment that gives whole blood red colour. Erythrocytes are highly specialized for their O_2-CO_2-transport function. [1]

The erythrocytes have flexible, elastic, biconcave and nucleated structure with mean diameter of 7.3µm and thickness of 2.2µm. The chemical constituents include water (63%), Haemoglobin (33.67%), methemoglobin (0.5%), glucose (0.8%), minerals (0.7%), non- haemoglobin protein (0.9%) and lipids (0.5%). The main aim of

these RBC's is to transport gases for respiratory processes. The production rate of RBC is 2.5 million per second

and life span of 100-120 days. [2]

Figure 1 : Erythrocytes [4]

Resealed Erythrocytes

Such drug-loaded carrier erythrocytes are prepared mainly by gathering blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers. Consequently, these carriers are called resealed erythrocytes. [3]

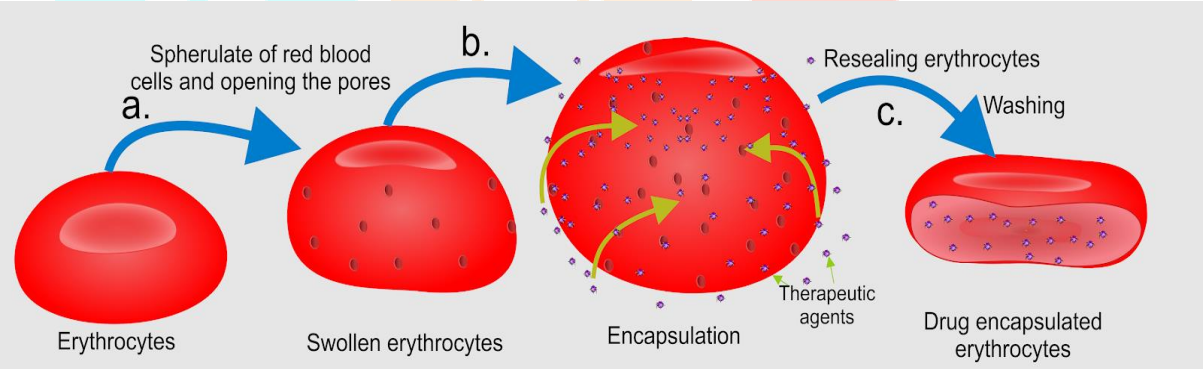


Figure 2: Resealed Erythrocytes [5]

Advantages of Resealed Erythrocytes

- Biocompatibility.
- Biodegradability.
- Incorporation of wide variety of bioactive agents.
- Circulation throughout the circulatory system.
- Encapsulation of large amount of drug in the small volume of cells.
- Targeted specificity within reticuloendothelial system (RES).
- Protection against the premature degradation, inactivation and excretion of protein and enzymes.
- Prolonged systemic activity by long residence time in the body.[6]

Disadvantages of Resealed Erythrocytes

- Possibility of leakage of cell and dose dumping.
- Some molecule alter physiology of cell.[7]

Isolation of Erythrocytes

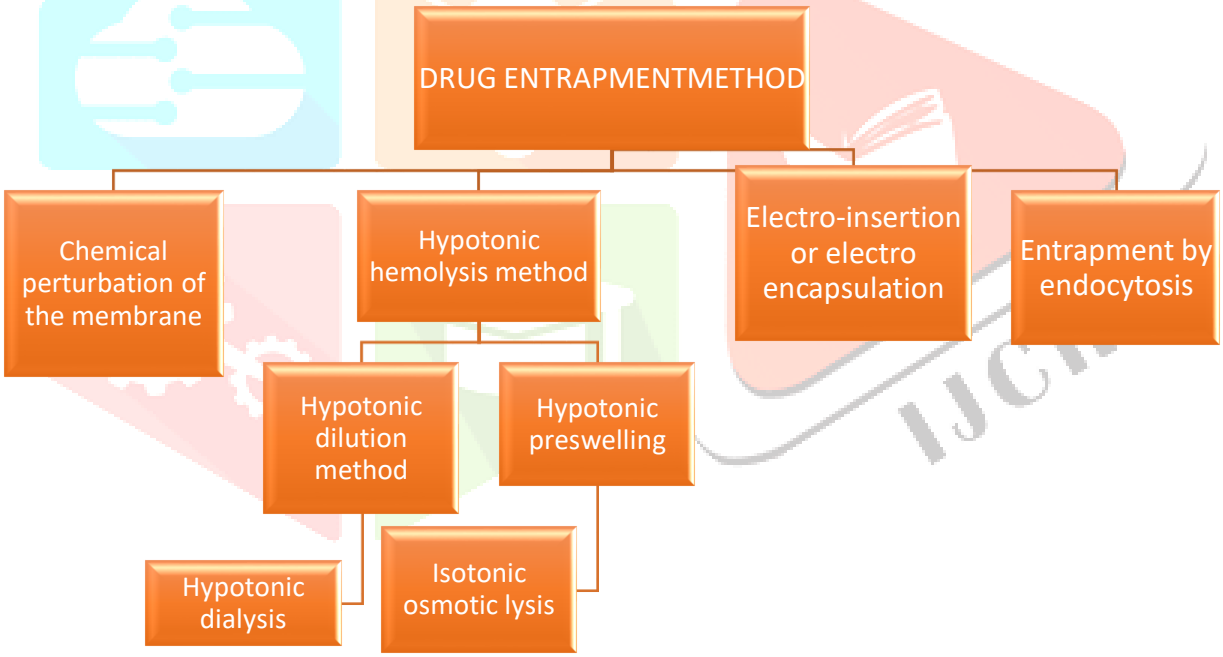
- The blood is collected into heparinised tube by venipuncture.
- Blood is withdrawn from cardiac / splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti coagulant.
- The whole blood is centrifuged at 2500 rpm for 5 mins at 4±1°C in a refrigerated centrifuge.

- The serums and Buffy coats are carefully removed and packed cell wash three times with phosphate buffer saline (ph=7.4).
- The washed erythrocytes are diluted with PBS and stored at 4°celcius until used. [8]

Table 1: Various conditions and centrifugal force used for used for isolation of red blood cells.

Sr. no.	Species	Washing Buffer	Centrifugal Force (g)
1.	Rabbit	10mmol KH_2PO_4/$NaHPO_4$	500-1000
2.	Dog	15mmol KH_2PO_4/$NaHPO_4$	500-1000
3.	Human	154mmol NaCl	<500
4.	Mouse	10mmol KH_2PO_4/$NaHPO_4$	100-500
5.	Cow	10-15mmol KH_2PO_4/$NaHPO_4$	1000
6.	Horse	2mmol $MgCl_2$,10mmol glucose	1000
7.	Ship	10mmol KH_2PO_4/$NaHPO_4$	500-1000
8.	Pig	10mmol KH_2PO_4/$NaHPO_4$	500-1000

Drug Entrapment Method



Various Evaluation Parameters and Their Determination Methods for Resealed Erythrocytes:

Table 2: Physical Evaluation

Parameter	**Method /Instrument Used**
Shape and surface morphology	Transmission electron microscopy, scanning electron microscopy
Vesicle size and size distribution	Transmission electron microscopy, optical microscopy Diffusion cell, dialysis
Drug release	Deproteinization of cell membrane followed

Table 3 : Cellular Characterization

Parameter	**Method /Instrument Used**
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and hemoglobin
Erythrocyte sedimentation rate	ESR methods
% Cell recovery	Neubaur's chamber, hematologicalanalyzer
% Hb content	Deproteinization of cell membrane followed by haemoglobin assay

Table 4: Biological Characterization

Parameter	**Method /Instrument Used**
Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test
Animal toxicity	Toxicity tests

Application of Resealed Erythrocytes

For Drug Targeting:
Resealed erythrocytes can be used for site-directed and target oriented drug delivery of loaded drugs.

❖ **To The RES Organs**

Resealederythrocytes have been proposed for passive targeting to MPS/RESsystem where modified surface characteristics improved there selectivityand specificity towards target cells. The various approaches tomodify the surface characteristics of erythrocytes include,

• Surface modification with antibodies
• Surface modification with glutaraldehyde
• Surface modification with carbohydrates such as sialic acid
• Surface modification with sulphydryl
• Surface chemical cross-linking e.g. delivery of 125I-labeled carbonic anhydrase loaded in erythrocytes cross-linked with bisulfosuccinimidylsuberate and 3,3- dithiosulfosuccinimidyl propionate.

❖ **To the Liver**

Enzyme Deficiency/Replacement Therapy

Enzymes can be infused into blood stream to supplant the missingor deficient enzymes in metabolic disorders. Exogenous enzymetherapy is complicated by the short half-life of enzymes in blood. Drug Loaded Erythrocytesstream, intolerance and occasionally toxicity against normaltissues. A strategy to eliminate or minimize the problems of immunologicalnature and toxicity, enzymes loaded erythrocytes havebeen employed .The enzymes used include ß-glycosidase, ß-glucoronidase, ß-galactosidase .Use of glucocerebrosides encapsulated erythrocytes in the disease caused by accumulation of the glucocerebrosides in the liver and spleen.

Treatment of Hepatic Tumors

Antineoplastic agents encapsulated in erythrocytes can be usedfor targeting to hepatic carcinoma. Various agents like bleomycin, methotrexate, andriamycin and asparagines have been successfully delivered by erythrocytes.

❖ **Removal of RES Iron Overload**

RES cells are the primary and the major sites for iron accumulationhas been entrapped in erythrocytes, for promising excretion of iron overload in the RES organs

❖ **As Circulatory Bioreactors**

Erythrocytes act as carriers for enzymes to serveas circulatory bioreactors. This immobilization of enzymes which decreases the level of circulating metabolite can be used as bioreactors.

❖ **Delivery of Antiviral Agents**

Antiviral agents are entrapped in resealed erythrocytes for effective delivery and targeting. Resealed erythrocytes have been used to deliverdeoxycytidine derivatives, recombinant herpes simplex virus

Type 1 (HSV-1) glycoprotein B, azidothymidine derivatives, fludarabine phosphate and azathioprene and acyclovir.

❖ **Thrombotic Therapy**

Anti-thrombotic agents loaded into the resealed erythrocyteshave been proved effective as thrombolytic therapy. Several compoundshave been reported for loading into the resealed erythrocytes includes aspirin and brinase and heparin.

❖ **In Oxygen Deficiency Therapy**

An application of Inositol hexaphosphate-loaded erythrocytes for improved oxygen

Supply is beneficial under the following conditions:

• High altitude conditions where the partial pressure of oxygen islow.
• Reduction in the number of alveoli, where interchange surface ofthe lungs is decreased.
• Increased resistance to oxygen diffusion in the lungs.
• Reduction in oxygen transport capacity.
• Mutation or chemical modification, which involves a decreasein oxygen affinity for haemoglobin.
• Increased radio sensitivity of radiation-sensitive tumours.
• Restoration of oxygen-delivery capacity of stored blood.
• Ischemia of myocardium, brain, or other tissues.

❖ **As Drug/Enzyme Carriers**

Erythrocytes can be used as a carrier for delivery of bioactivecompounds as circulatory depots. They can also be used as carriersfor targeting drugs to liver, spleen and lymph nodes.

Erythrocytes as Carriers for Proteins and Macromolecules

Erythrocytes have been used for delivery of various proteinsand macromolecules which includes insulin, recombinant human erythropoietin (rHuEpo), and mycotoxinand recombinant interleukin-2 (rIL-2).

❖ **Microinjection of macromolecules**

Biological functions of macromolecules such as DNA, RNA, and andproteins are used for various cell biological applications.Hence, various methods are used to entrap these macromoleculesinto cultured cells (e.g., microinjection). A relatively simple structure and a need of complex cellular components (e.g., nucleus) in erythrocytes make them good candidatesfor the entrapment of macromolecules. In microinjection, erythrocytes are used as micro syringes for injection to the host cells.

The microinjection process involves culturing host eukaryoticcells in vitro. The cells are coated with fusogenic agent and then suspended with erythrocytes loaded with the compound of interest in an isotonic medium. Sendai virus (hemagglutinatingvirus of Japan, HVJ) or its glycoproteins or polyethyleneglycol have been used as fusogenicagents . Thefusogen causes fusion of cosuspended erythrocytes and eukaryotic cells. Thus, the contents of resealed erythrocytes andthe compound of interest are transferred to host cell. This procedurehas been used to micoroinject DNA fragments arginase, proteins, nucleic acids, ferritin, latex particles, bovine and human serum albumin, and enzyme thymidinekinase to various eukaryotic cells.

Clinical Progress

The clinical developments reported in this section have received approval by international regulatory agencies; many have also received the designation of 'orphan drug', and the most advanced are based on solid preclinical and/ or phase I/II clinical investigations.

Table 5 Therapeutic goals and drugs considered in this article related to the use of drug-loaded red blood cells as therapeutic agents[22]

Conditions Treated	Drug	Company
Ataxia telangiectasia	Dexamethasone 21-phosphate	EryDel Italy & USA http://www.eryde l.com
Mitochondrial NeurogastrointestinalEncephalomyopathy (MNGIE)	Thymidine phosphorylase (TP)	St George's, University of London UK The Clinical Trial Company UK Orphan Technologies Ltd CH
Phenylketonuria	RTX-134	Rubius USA http://www.rubiu stx.com
Acute lymphoblastic leukaemia/pancreatic cancer	Asparagines	ERYtech Pharma France & USA http://www.eryte ch.com

Conclusion

The use of the erythrocyte as drug carrier is a fundamental advancement in the current exploitation of blood and may be applicable in medical fields for which no effective therapy is currently available .Some companies are leading the clinical applications with products currently in phase III trials and robust pipelines .The commercial medical applications of carrier erythrocytes are currently being tested by a newly formed company that is developing products for human use .The coming years represent a critical time in this field as commercial applications are explored .In near future, erythrocytes based delivery system with their ability to deliver controlled and site specific drug delivery will revolutionize disease management.

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