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Review

Resealed Erythrocyte Drug Delivery: A Review

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Abstract

Drug delivery is now entering quite an exciting and challenging era. Targeted drug delivery, known as smart drug delivery focuses on targeting the medicaments by increasing the residence time and the concentration of drug at the target site of the body (organs, cells, tissues) thus improving the efficacy of treatment and reducing the side effects. Various carriers that can be used in this type of delivery are liposomes, nanoshells, erythrosomes and resealed erythrocytes. In this Innovation era, resealed erythrocytes have become the choice of drug delivery system because of its excellent biocompatibility, biodegradability and ability to entrap several molecules. The promising aspect of resealed erythrocytes makes them useful as a drug carrier. Because of its capacity to circulate throughout the body, biocompatibility, zero order release kinetics of medicines, repeatability, and convenience of administration, resealed erythrocyte is becoming more popular. Erythrocytes are biocompatible, biodegradable, and have long rotation half-lives, allowing them to be loaded with a variety of physiologically active composites in a number of chemical and physical forms (hypotonic dilution, hypotonic hemolysis, electro- insertion, ruse by endocytosis, hypoosmotic lysis). The method of isolation involves collection of sample from the interest, then separating the plasma and finally resealing it. Erythrocytes are prepared by using methods like hypotonic dilution, hypotonic dialysis, pre-swelling, osmotic lysis, endocytosis and chemical penetration. The tremendous potential to achieve site specific drug delivery makes them as first choice of drugs in areas like enzyme therapy, cancer, hepatic tumours and antiviral. This review focus on various methods of encapsulated erythrocytes preparation, techniques of drug loading such as electro- encapsulation, chemical perturbation, entrapment by endocytosis etc, mechanism of resealed erythrocyte release and evaluation resealed erythrocytes and highlights its applications with particular stress in areas of cancer, hepatic tumours and enzyme therapy.

Keywords: Resealed erythrocytes, controlled drug release, targeted drug delivery.

INTRODUCTION

Generally a drug is administered into the body with a motive to get the required actions of that drug on a specific target. But the fact is that apart from its actual target, the drug also acts on some non-target sites, resulting in adverse drug reactions-which are commonly called as side effects. 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. Target specificity would also reduce the dosage and frequency of administration. Present pharmaceutical scenario is aimed at developing drug delivery systems that maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases. 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. The various drug delivery systems available today aim at the target of making the drug exhibits its pharmacological action only on the specified target site. Targeted drug delivery, sometimes called smart drug delivery, is a method of delivering drug to the patient in a manner that increases the concentration of drug in targeted parts of the body. Targeted drug delivery system is based on a method which delivers a certain amount of a therapeutic agent for a prolonged period of time to a targeted area within the body [1]. Among the various carriers used for targeting drugs to various body tissues, the cellular carriers meet several criteria desirable in clinical applications, among the most important being biocompatibility of carrier and its degradation products. Leucocytes, platelets, erythrocytes, nanoerythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier systems[2,3].

Among all the various types of carrier's used erythrocytes because of its unique nature and property are being used in providing an ideal drug delivery system. Therapeutic uses of a variety of drug carrier systems have significant impact on the treatment and potential cure of many chronic diseases, including cancer, diabetes mellitus, rheumatoid arthritis, HIV infection, and drug addiction.

Blood is the fluid present in the body that delivers necessary substance such as nutrient and oxygen to the cell and transports the metabolic waste away from these cells. Blood contains around 55% of plasma and other 45% is made-up of corpuscles. In Blood the 45% of corpuscles is composed of different type of cells like erythrocytes (RBC), leucocytes (WBC) and platelets that are suspended in the blood plasma that constituent 55% of body fluid. Blood cells are developed in the bone marrow. All blood cells are developed from the same bone marrow i.e. stem cells. Stem cells are immortal that is they don't die. Stem cells are also undifferentiated cells and they have not yet been developed into a particular cell type. Stem cells are pluripotent they have the capability to become any type of blood cell. These immortal, undifferentiated, pluripotent stem cells give birth /rise to erythrocytes, leukocytes and platelets of the entire cell present the most abundant in the blood are the RBC'S. Leukocytes that are also called as white blood cells are group of related cell types that are involved in immune function. Leukocytes include neutrophils, eosinophils, basophils, lymphocytes and monocytes. Red blood cells (RBC, erythrocytes) major function is the transportation of gases for respiratory process. Erythrocytes are developed through a process called as erythropoesis. Whereas platelets are responsible for conversion of fibrogen into fibrin whose major function is to clot the blood.

Erythrocytes, are the most plentiful cells in the human body [i.e. RBC- 4.1 to 6.3 cells/mcL, WBC-4,000 to 11,000 cell/ul, platelets-1,50,000 to 4,50,000 cells/ul] that have potential carrier capabilities for the drug delivery. These are biocompatible, biodegradable, possess considerably long circulation half-lives and can be loaded with a range of biologically active compounds using different chemical and physical methods [4].

RBC'S have been extensively studied for their potential carrier capabilities for the drug delivery and drug-loaded microspheres. Such drug-loaded carrier erythrocytes are produced merely by gathering blood samples from the organism of interest, unraveling (i.e. separating) erythrocytes from plasma, thereby entrapping the drug in the erythrocytes and thus resealing the resultant cellular carriers. Therefore they are called resealed erythrocytes. This whole process is based on the response of these cells under osmotic conditions. Upon reinjection, the resealed erythrocytes work as slow circulating depots and thereby targeting the drugs to a reticulo-endothelial (RES) [2].

Erythrocytes are natural products of the body, biodegradable in nature, isolation of these is easy and large amount of drug can be loaded in small volume of cells, non-immunogenic in action and can be targeted to disease tissue or organ, prolong the systemic activity of the drug while residing for a longer time in the body (Lejeune A, 1997), protest the premature degradation, inactivation and excretion of proteins and enzymes, act as a carrier for number of drugs, target the drugs within the reticuloendothelial system (RES) as well non RES organs/sites. Moreover, the possibility of targeting carrier erythrocytes to non-RES organs has been exploited in recent years, e.g., using homing devices such as IgG or IgM. Also these cells are non-immunogenic and biodegradable; they freely circulate throughout the body and offer ease of preparation; they have the capacity to carry large amounts of drug; and can behave as a slow-release long-acting system (Al-Achi A, 1990). Also, lungs to tissues and the CO_2 produced in tissues back to lungs. Thus, erythrocytes are a highly specialized O_2 carrier system in the body. Because as aging erythrocytes are normally phagocytized by cells of the reticuloendothelial system, thus, these cells could serve as a natural target for delivery of their pay load to these

organs. Potential clinical indications for "RES targeting" include iron over-storage diseases, parasitic diseases, hepatic tumors (Jain S, 1997) (Jain S, 1995) (Guyton CA, 1996) and lysosomal storage diseases.

Erythrocytes have been the most interesting carrier and have found to possess great potential in drug targeting. Resealed erythrocytes are gaining more popularity because of their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation.

Most of the resealed erythrocytes used as drug carriers are rapidly taken up from blood by macrophages of reticuloendothelial system (RES), which is present in liver, lung and spleen of the body.

Erythrocytes are probably the most common cells found in the human body. After long research, we found their intense application as a drug carrier in the drug delivery system. The resealed erythrocytes are biodegradable, biocompatible, non- immunogenic, non-pathogenic, self-degradable, reproducible, easy to prepare, possess prolonged circulation half-life and can be used to incorporate a wide variety of active drugs. All such properties make them a revolutionary drug carrier which can efficiently be used to increase the therapeutic effect of the drug as well as to prevent any possible toxic effects [5].

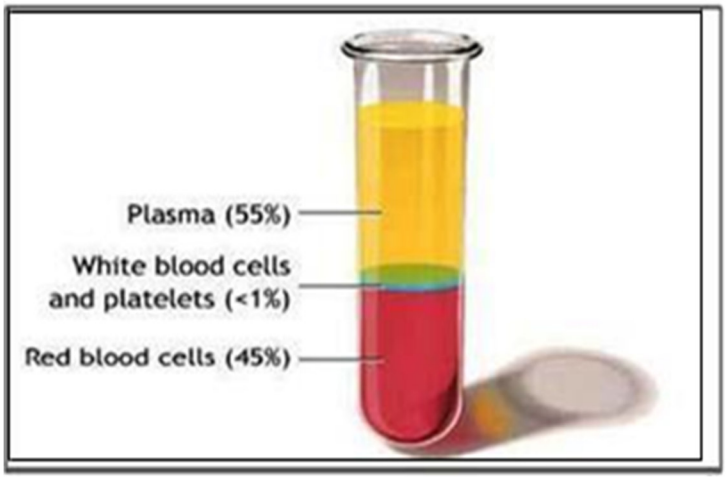

Fig 1: Composition of Blood

Composition of erythrocytes

Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, possess very long circulation half-lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods **[6,7]** . Erythrocytes or Red blood corpuscles (*erythro*- red; *cyte*- cell) are biconcave discs with diameter of 7–8 µm. Erythrocytes contain the oxygen-carrying protein haemoglobin, a pigment that gives whole blood its red colour **[8]**. Erythrocytes are highly specialized for their oxygen transport function. These are produced in the bone marrow by regulatory effect oferythropoietin **[9]**. Around 760g of haemoglobin in total which is about 10% of total protein content of the body is contained in the erythrocytes **[10,11**,12] . The enclosed haemoglobin transport oxygen for prolonged period**[13]**. The sources of energy to the erythrocytes are glycolysis and hexose monophosphate shunt. Erythrocytes travel 250 km throughout the cardiovascular system in their life span of 100 – 120 days **[14**] .The processes of erythrocyte production within the body are called as erythropoiesis; the erythrocytes are produced in red bone marrow under the regulation of a hemopoeitic hormone known as erythroprotein**[8]**.

1.1 Isolation of erythrocytes [15]

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.

Isolation of erythrocytes

The cellular content is about 40-50% of the blood volume and contains erythrocytes (red blood cells, RBC), Leukocytes (while blood cells, WBC) and thrombocytes (platelets). The primarily waterr (90 to 92%) and protein(7%).Blood is withdrawn from cardiac/splenic puncture (in case of small animal) and through veins (in case large animals) into a syringe containing drop of anticoagulant .The whole blood is centrifuged at

2500 rpm for 5min at 4±4°c in a refrigerated centrifuge. The serum and Buffy coats are carefully removed and packed cells washed 3 times with phosphate buffer saline (PBS pH 7.4).The washed Erythrocyte are diluted with PBS and Stored at 4°c until used[16] Refer table 1.

Table 1: Isolation of erythrocytes

S.no	Species	Washing buffer	Centrifugal force(g)
1	Rabbit	10mmol KH_2P04 /$NaHPO_4$	500-1000
2	Dog	15mmol KH_2P04 /$NaHPO_4$	500-1000
3	Human	154mmol NaCl	<500
4	Mouse	10mmol KH_2P04 /$NaHPO_4$	100-500
5	Cow	10-15mmol KH_2P04 /$NaHPO_4$	1000
6	Horse	2 mmol $MgCl_2$,10 glucose	1000
7	Sheep	10 mmol KH_2P04 /$NaHPO_4$	500-1000
8	Pig	10 mmol KH_2P04 /$NaHPO_4$	500-1000

1.2 Reasealed erythrocytes [17,18]

Resealed erythrocytes are the part of parental control release formulation, RBCs have been used extensively studies for their potential carrier & capability for delivery of drug loaded microsphere. In this carrier erythrocytes are prepared and the blood sample is collected from the organism of interest, the entrapping drug in the erythrocytes & resealing the cellular carriers. Hence the overall process is based on the response of these cells under osmotic condition.Through the process of reinjection, the drug loaded erythrocytes provide slow circulating depots & target the drug to a disease tissue organ.

Properties of resealed erythrocyte of novel drug delivery carriers [19,18,7]

1) The drug should be released at target site in a controlled manner.
2) It should be appropriate size, shape and should permit the passage through capillaries and minimum leakage of drug should take place.
3) It should be biocompatible and should have minimum toxic effect.
4) It should possess the ability to carry a broad spectrum of drug.
5) It should possess specific physicochemical properties by which desired target size could be recognized.
6) The degradation product of the carriers system, after release of the drug at the selected site should be biocompatible. It should be physicochemically compatible with drug.
7) The carrier system should have an appreciable stability during storage.

Requirement for encapsulation

- Variety of biologically active substance i.e. mol wt ranges from 5000-60,000dalton can be entrapped in erythrocytes.
- Non-polar molecule like tetracycline may be entrapped in bovine RBC in tetracycline HCl.
- Generally, this method used for only polar molecules. And in some cases we can see non polar molecules also entrapped.
- Hydrophobic molecules can be entrapped in erythrocyte by absorbing over other Molecules.
- The size of molecule entrapped is a key factor when the molecule is smaller than sucrose and larger than B-galactosidase.

ERYTHROCYTES CAN BE USED AS CARRIERS IN TWO WAYS

1. Targeting particular tissue/organ

For targeting, only the erythrocyte membrane is used. This is obtained by splitting the cell in hypotonic solution and after introducing the drug into the cells, allowing them to reseal into spheres. Such erythrocytes are called Red cell ghosts.

2. For continuous or prolonged release of drugs

Alternatively, erythrocytes can be used as a continuous or prolonged release system, which provide prolonged drug action. There are different methods for encapsulation of drugs within erythrocytes. They remain in the circulation for prolonged periods of time (up to 120 days) and release the entrapped drug at a slow and steady rate [15].

ADVANTAGES OF RESEALED ERYTHROCYTES AS DRUG CARRIERS [20,21,22,23,8]

The resealed erythrocytes should have the following advantages:

- Their biodegradability with no generation of toxic products.
- The considerably uniform size and shape of the carrier.
- Relatively inert intracellular environment.
- Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
- The wide variety of chemicals that can be entrapped.
- The modification of pharmacokinetic and pharmacodynamics parameters of drug.
- Attainment of steady-state plasma concentration decreases fluctuations in concentration.
- Protection of the organism against toxic effects of drugs (e. g. Antineoplastics).
- They are ability to circulate throughout the body and facilities for separation, handling, transfusion, and working with erythrocytes the availability of the techniques
- The prevention of any undesired immune response against the loaded drug
- Their ability to target the organs of the RES.
- The possibility of ideal zero-order drug release kinetics.
- The lack of occurrence of undesired immune response against encapsulated drug.
- The large quantity of drug that can be encapsulated within a small volume of cells ensures dose sufficiency.
- A longer life span in circulation as compared with other synthetic carriers and optimum conditions may result in the life span comparable to that of normal erythrocytes
- Easy control during life span ranging from minutes to months.
- A decrease in side effects of drugs.

Disadvantages

- Limited potential as carrier to non-phagocyte target tissue.
- Possibility of clumping of cells and dose dumping problem may arise.
- Relatively costly.

METHODS OF DRUG LOADING IN ERYTHROCYTES

Several methods can be used to load drugs or other bioactive compounds in erythrocytes, including physical (e.g., electrical pulse method) osmosis-based systems, and chemical methods (E. g. Chemical perturbation of the erythrocytes membrane). Irrespective of the method used, the optimal characteristics for the successful entrapment of the compound requires the drug to have a considerable degree of water solubility, resistance against degradation within erythrocytes, lack of physical or chemical interaction with erythrocyte membrane, and well-defined pharmacokinetic and pharmacodynamics properties.

Types

[A] Osmosis based methods
- 1] Hypotonic Hemolysis
- 2] Hypotonic Dilution
- 3] Hypotonic Dialysis
- 4] Hypotonic Pre-Swelling

B] Chemical perturbation of the membrane

C] Electro-insertion or electro encapsulation

D] Entrapment by endocytosis

[A] OSMOSIS BASED METHODS

1] Hypotonic Hemolysis

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution as shown in figure. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under the cells can maintain their integrity up to a tonicity of150 mosm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 Å are generated on the

membrane Stress.

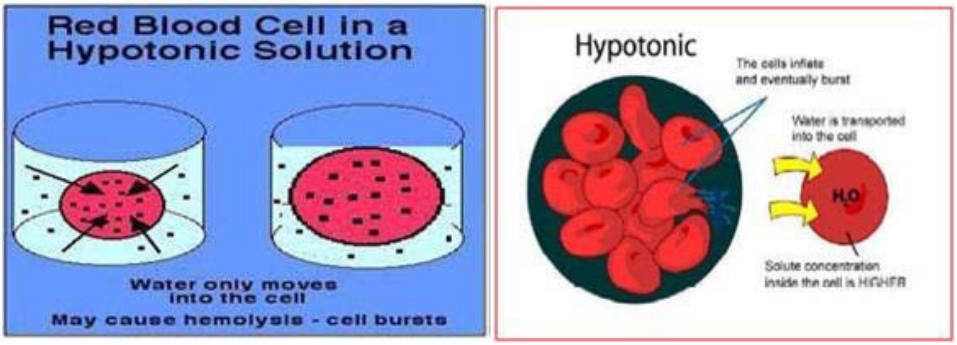

Fig 2: Hypotonic Hemolysis Method and Hypotonic Dilution Method

2] Hypotonic Dilution

The erythrocytes have little capacity to resist volume. This is the simplest and fastest method. In this a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. At an increase in volume above 50-75% of initial volume and in hypotonic solution the erythrocyte membrane ruptures and pores are created to entrapped drug. The solution tonicity is then restored by adding a hypertonic buffer

3] Hypotonic Dialysis

This method was first reported by Klibansky in 1959 and was used in 1977 by Deloach and Ihlerand Dale for loading enzymes and lipids. Several methods are based on the principle that semi permeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In this process a desired hemocrit is achieves by mixing erythrocyte suspension and drug solution. This mixture is placed unto dialysis tubing and then both ends of tube are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. The tube is placed in the bottle containing 100ml of swelling solution. The bottle is placed at 4ºC for the desire lysis time. The contents of the dialysis tubing are mixed intermittently by shaking the tube using the strings. The dialysis tube is then placed in 100 ml of resealing solution. The loaded erythrocytes thus obtained are then washed with cold phosphate buffer at 4°C. In this method a good entrapped efficiency is obtained[24].

4] Hypotonic Pre - Swelling

As shown in Figure- 04 the technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low g values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 L portions of an aqueous solution of the drug to be encapsulated[21].

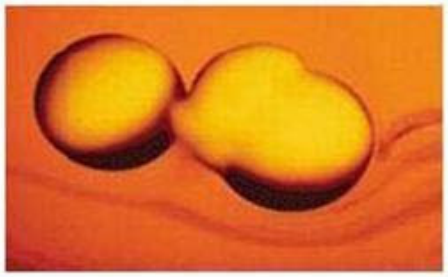

Fig 3: Hypotonic Pre - Swelling Method

Table 2: Comparison between percent drug loading, advantages as well as disadvantages of different osmosis based systems

Method	% Loading	Advantages	Disadvantages
Dilution	20-40	Fastest and simplest especially for low molecular weight Drugs.	Entrapment efficiency is less.
Dialysis	30-45	Better *in vivo* survival of erythrocytes better structural integrity and membrane.	Time consuming, heterogeneous size distribution of resealed erythrocytes.
Presswell	30-90	Good retention of cytoplasm and good survival *in vivo*.	-
Isotonic osmotic Lysis	-	Better in vivo survival.	Impermeable only large molecules, process is time Consuming.

B] CHEMICAL PERTURBATION OF THE MEMBRANE

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke showed that the permeability of erythrocyte membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes these methods induce irreversible destructive changes in the cell membrane and hence are not very popular [25].

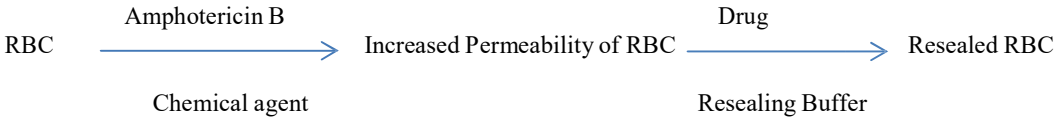

Fig 4: Loading of drug by Chemical Membrane Perturbation Method

C] ELECTRO-INSERTION OR ELECTRO ENCAPSULATION

The method is based on the observation that electrical shock brings about irreversible changes in the erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium. 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 µs. An inverse relationship exists between the electric-field intensity and the discharge time. The compound to be entrapped is added to the medium in which the cells are suspended from the commencement of the experiment [25].

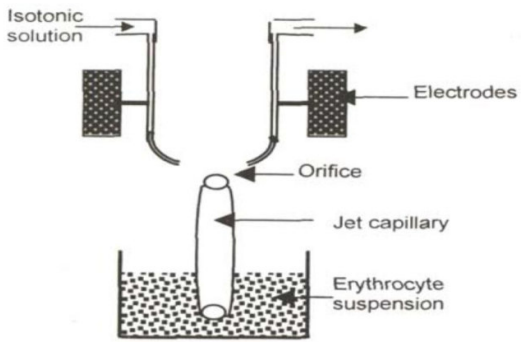

Fig 5: Electro-Insertion or Electro Encapsulation Method

An erythrocyte membrane one advantage of this method is a more uniform distribution of loaded cells in comparison with osmotic methods. The main drawbacks are the need for special instrumentation and the sophistication of the process. Entrapment efficiency of this method is ~35%, and the life span of the resealed cells in circulation is comparable with that of normal cells.

D] ENTRAPMENT BY ENDOCYTOSIS

This method was reported by Schrier in 1975. Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5mM MgCl2 and 1mM CaCl2 followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37 °C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosis material from cytoplasm thus protecting it from the erythrocytes and vice-versa.

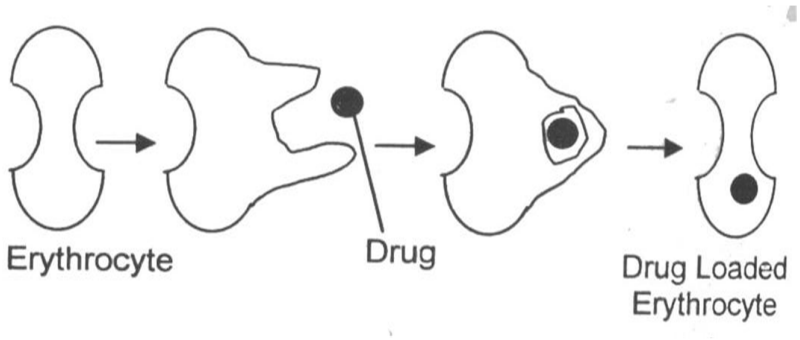

Fig 6: Loading of Drug by Endocytosis Method

EVALUATION OF RESEALED ERYTHROCYTES

After loading of therapeutic agent on erythrocytes, the carrier cells are exposed to physical, cellular as well as biological evaluations [26].

1. Shape and Surface Morphology: The morphological characters such as size, surface area and shape of erythrocytes are must be similar to the normal erythrocytes. The morphological characterization of erythrocytes is undertaken by comparison with untreated erythrocytes by the process such as Ttransmission Electron Microscopy (TEM) or Scanning Electron Microscopy (SEM). Other methods are also used such as like phase contrast microscopy can also be used.

2. Drug Content: Drug content of the cells determines the entrapment efficiency of the cells. Where of packed, loaded cells (0.5 mL) with 2.0 mL acetonitrile and centrifugation at 2500 rpm for 10 min. The process involves deproteinization. The clear supernatant is analyzed for the drug content by spectrophotometrically.

3. Cell Counting and Cell Recovery: It was used to count the number cells are present in unit volume of the blood. Now days the automated machines are used to determine or count the number of cells are intact with in cubic mm of blood. They are collected and packed with drug [27].

4. Turbulence Fragility: It is determined by the passage of RBCs in small blood vessels for e.g: suspen- sion passed through needles with smaller internal diameter was about 30 gauges and vigorously shaking the suspension. From these, the release of haemoglobin and drug can be determined [28].

5. Erythrocyte Sedimentation Rate (ESR): From these we estimate the suspension stability of RBC in plasma and determine the number and size of the red blood cells and concentration of plasma protein, such as fibrinogen and α, β globulins. Rate of sedimentation can be determined in standard tube. Normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.

6. Determination of entrapped magnetite: Atomic absorption spectroscopic method is used to esti- mate the concentration of particular metal ions in the sample. Then add small amount of HCl to a fixed amount of magnetite bearing erythrocytes and content were heated at 600^0C for 2 hours, and add 20 %w/v tri chloro acetic acid and supernatant liquid is obtained by the application of centrifugal forces. From these liquid we estimate the metal ion concentration by using Atomic absorption spectroscopy [29,30].

7. In vitro stability: The stability of the loaded erythrocytes is assessed by means of the incubation of the loaded erythrocytes in the autoclave with plasma or isoosmotic buffer, having different haematocrit values is between0.5% and 5% at temperatures of 40^0C and 37^0C.

8. Haemoglobin release: The content of haemoglobin from the erythrocytes may be eliminated or removed by the alterations in the permeability of the membrane of the RBCs during the entrapment procedure. From these, the relationship between the rate of haemoglobin and rate of drug release of the substance encapsulated

from the erythrocytes. The haemoglobin or drug leakage is tested using a red blood cell suspension by recording absorbance of supernatant at 540nm on a UV/ visible spectropho- tometer [31].

9. In-vitro drug release and Hb content: The 5% cell suspensions were stored at 40^0C and placed in ambered colour glass container. Supernant liquid was cleared by using a hypodermic syringe equipped with filters having pore size 0.45, if any proteins are present can be de proteined by using methanol and were estimated for entrapment efficacy. The supernatant liquid was collected from each sample after centrifugation assayed it, calculate the %Hb release by using formula, **% Hb release=A540**

10. Osmotic shock: In osmotic shock studies, 1 ml erythrocytes suspension (10%) was diluted with distilled water (5 ml) and placed in centrifuge at 300 rpm for 15 minutes. It forms two layers. The supernant liquid (plasma) was separated and sediment layer (serum) was used to estimate for the % haemoglobin release analytically.

Route of administration

Intra peritoneal injection administered through I.V injection. As results 25% of resealed cell remained in circulation for 14 days. The extra vascular targeting of RBCs to peritoneal macrophages. Subcutaneous route for slow release of entrapped agents. They results the loaded cell released encapsulated molecules at the injection site.

APPLICATIONS OF RESEALED ERYTHROCYTES

The potential therapeutic applications of carrier erythrocytes as a drug delivery system cover a wide spectrum of pharmacological as well as therapeutic targets mainly based on the intravenous slow drug release as well as the targeted drug delivery [32]. Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cell could be used as circulating carriers to disseminate a drug within a prolonged period of time in circulation or in target-specific organs, including the liver, spleen, and lymph nodes. A majority of the drug delivery studies using drug loaded erythrocytes are in the preclinical phase. However in some cases the successful clinical trials on this delivery system have been reported[18] .

In Vitro Application

Carrier RBCs have proved to be useful for a variety of in vitro tests. For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes by phagolysosomes. An inside to this study showed that enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The most frequent in vitro application of RBC mediated microinjection. A protein or nucleic acid to be injected into eukaryotic cells by fusion process. Similarly, when antibody molecules are introduced using erythrocytic carrier system, they immediately diffuse throughout the cytoplasm. Antibody RBC auto injected into living cells have been used to confirm the site of action of fragment of diptheria toxin. *In-vitro* tests include utilization of erythrocytes carrier to introduce ribosomes inactivating proteins into cells by fusion technique.

***In Vivo* Application**

This includes the following:

Slow drug release

Slow release dosage forms are designed to obtain a prolonged therapeutic effect by continuously releasing the medication over an extended period of time after administration of single dose. Due to the long life span of carrier erythrocyte in the circulation, they can be used as circulating depots for antitumor, antiparasitic, antibiotics as well as cardiovascular drugs. This happened only when the drug and the selected method for the drug loading don't change the morphological and physiological parameters of erythrocytes. Various bioactive agents encapsulated in erythrocytes are developed for the sustained release in the circulation to allow effective treatment of diseases. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drugs, vitamins and steroids.[45]

Drug Targeting

Ideally, drug delivery should be site-specific and target oriented to exhibit maximal therapeutic index with minimum adverse effects. Resealed erythrocytes can act as drug carriers and targeting tools as well. They can be used to target RES organs as well as non RES organs.

Targeting RES organs: Surface modified erythrocytes are used to target organs of mononuclear phagocytic systems/ reticuloendothelial system because the changes in membrane are recognized by macrophages (table 3). The various approaches used include:

- Surface modification with antibodies (coating of loaded erythrocytes by anti-Rh or other types of antibodies)
- Surface modification with glutaraldehyde.

- Surface modification with sulphydryl
- Surface chemical crosslinking
- Surface modification with carbohydrates such as sialic acid[33].

Table 3: Resealed erythrocytes used in RES targeting[34]

Treatment /	Name of Drug(s)	Purpose
Treatment of lysosomal storage diseases	Lysosomal enzymes, C-glucuronidase, cells, 13- galactosidase and 6-glucosidase	To deliver lysosomal enzymes and drugs to lysosomes of the erythrophagocytic cells
Treatment of Gaucher's diseases	Glucocerebrosidase	Loaded cells survived for 10 days in treated patient and no untoward reactions. Disease was found with respect to blood counts, blood pressure and renal functions.
Treatment of liver tumour	Anticancer like Bleomycin Adriamycin, Carboplatin, Gentamycin. Etc.	Targeting to hepatic carcinomas.
Treatment of parasitic diseases	Pentamidine loaded immunoglobulin G coated erythrocytes, Glutaraldehyde treated Erythrocytes	Targeting of drugs in the treatment of parasitic diseases in which the parasite resides in the organs of RES e.g. macrophage-contained leishmania.
Removal of RES Iron Overload	Desferoxamine, an iron chelating drug in erythrocyte agentghosts	Liver targeting of an antimalarial agent-primaquine phosphate and an antiamoebic agent, metronidazole.
Removal of Toxic Agents	Murine carrier erythrocytes containing bovine rhodanese and sodium thiosulphate	Antagonism of cyanide intoxication or To antagonize the lethal effects of potassium cyanide in mice

Liver Targeting
Nowadays this delivery system is used to target the liver for the following reasons:

Enzyme deficiency/replacement therapy: Many metabolic disorders related to deficient or missing enzymes can be treated by administering these enzymes as resealed erythrocytes. E.g. β- glucoside, β-glucouronidase, β-galactosidase [35].

Treatment of hepatic tumors: Antineoplastic drugs such as metotrexate(MTX), bleomycin, asparginase and adiramycin have been successfully delivered by erythrocytes. E.g. in a study, the MTX showed a preferential drug targeting to liver followed by lungs, kidney and spleen.

Treatment of parasitic diseases: Parasitic diseases that involve harbouring parasites in the RES organ can be successfully controlled by this method because of the ability of resealed erythrocytes to selectively accumulate within RES organ and deliver the antineoplastic agent.

Others include removal of RES iron overload, removal toxic agents.

Targeting Non-RES organ: Erythrocytes loaded with drugs have also been used to target organs outside the RES. The various approaches for targeting non-RES organs include:

- Entrapment of paramagnetic particles along with the drug.
- Entrapment of photosensitive material
- Use of ultrasound waves.
- Antibody attachment to erythrocytes membrane to get specificity of action.
- Other approaches include fusion with liposome, lectin pre-treatment of resealed cells etc.[18]

The magnetic erythrocytes, resulting from the co-encapsulation of the drugs with some ferrous fluids such as cobalt-ferrite and magnetite, have been reported to direct the encapsulated drug predominantly to the desired sites of the body by means of external magnetic field. The magnetically guided erythrocytes have been tested successfully for targeting anti-inflammatory drugs to inflamed tissues[35].

Table 4: Resealed erythrocytes used in targeting non RES organs [34]

Approaches	Types of Drugs	Objectives / Purpose
Magnet-responsive erythrocyte Ghosts	Encapsulation of small paramagnetic particles into erythrocytes	Localization to a particular location under the influence of external magnetic field.
Photosensitized Erythrocytes	Methotrexate and photosensitized by subsequent exposure to a haematoporphyrin derivative.	A combination of chemotherapy and photodynamic therapy could be a useful modality in the treatment of tumors of body located at site other than RES predominant organs. OR As a photo triggered carrier/delivery system for methotrexate in tumor therapy.
Antibody Anchored Erythrocytes (lmmunoerythrocytes)	Antibody coating of resealed drug carrier erythrocytes	Drug targeting to the RES
Ultrasound mediated delivery of erythrocytes loaded drugs	Colloidal particles and red blood cells	Delivery to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound.

Delivery of antiviral drugs

Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting. Because most antiviral drugs are nucleotides or nucleoside analogs, their entrapment and exit through the membrane needs careful consideration (table5).

Table 5: Resealed erythrocytes for delivery of antiviral drugs [34]

Categories of	Name of drugs	Purpose
Azidothymidine	Azidothymidinehomodinucletide loaded erythrocytes	Slow delivery of the antiretro-viral drug Azidothymidine
Deoxycytidine	Antiviral nucleotide analogue	Encapsulated Into erythrocytes for targeting to macrophages
Azathioprene and AcycIovir Derivatives	Heterodinucleotide of azidothymidine and AcycIovir	Selective delivery to macrophage for protection against Human immunodeficiency Virus or herpes simplex virus.

Enzyme therapy

Enzyme therapy offers considerable promise for the long term treatment of inherited metabolic diseases. For enzyme therapy the selected carrier must have a long circulatory life, although specific ultimate uptake would also be advantageous. For all these, purposes and as a more general carrier of the other therapeutic agents, the erythrocytes offer the greatest potential, being a natural carrier of endogeneous substrates, non - toxic, non - immunogenic, biodegradable and easy to obtain (table6)[31].

Table 6: Resealed erythrocytes used in delivery of enzymes[33]

Name of Enzymes	Purpose
L-asparaginase Aminolevulinate dehydratase	For treatment of leukemia. To treat adolescent patient suffering from lead poisoning.

Advances in Resealed Erythrocytes [36,37,38]

Erythrosomes: These are specially engineered vesicular systems that are chemically cross-linked to human erythrocytes support upon which a lipid bilayer is coated. This process is achieved by modifying a reverse-phase evaporation technique. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs

Nanoerythrosomes: These cellbased carrier systems were derived from erythrocytes after complete haemolysis and carefully engineered to produce nanoerythrosomes. Nanoerythrosomes are vesicles formed by the extrusion of red blood cell ghosts and the average diameter is 0.1µm.

FUTURE PERSPECTIVES

- The concept of employing erythrocytes as drug or bioactive carrier still needs further optimization.
- A large amount of valuable work is needed so as to utilize the potentials of erythrocytes in passive as well as active targeting of drugs.
- The resealed erythrocytes can be utilized for in humans as carriers for drugs.
- Scientists have demonstrated that such engineered red blood cells are suitable for blood transfusion.

Future studies would concentrate on the following

1. Manipulation of autologous properties of erythrocytes, improved understanding of the biology of the red cells and its membrane development of pulsatile and feedback control system, selective drug delivery to CNS and delivery peptide and protein drugs.
2. Technical improvement in the procedure for preparing resealed erythrocytes, routes of administration, stability, crosslinking of resealed erythrocytes, aseptic and sterile processing, optimization techniques, pilot-plant scale up studies and innovative ideas for the application of resealed erythrocytes. Either as carriers or as cellular bioreactors would pave the way for automation and commercialization of this novel drug delivery system.
3. With the availability of technology to clone human DNA prokaryotes and the potential to produce large quantity of human enzymes, the possibility of enzyme replacement therapy targeting and the use of RBC carrier reservoirs should become more of realties.
4. In future greatest interest seems to be related to the targeting of immune-modulators on the phagocytic system anticancer drugs.
5. Drug loaded magnetite bearing cells serve as a promising carrier for delivering the drug to specific site.

CONCLUSION

Now a days there are numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. Until other carrier systems come of age, resealed erythrocytes technology will remain an active field for the further research. The use of resealed erythrocytes shows potential for a safe and effective delivery of various bioactive molecules for effective targeting. In coming future, erythrocytebased drug delivery system with their capability to afford controlled and site specific drug delivery have been developed for disease management. Erythrocyte carriers are “Nano evices in the field of Nanotechnology”. A large amount of valuable work is needed so as to utilize the potentials of erythrocytes in passive as well as active targeting of drugs in diseases like cancer. At present erythrocytes are most effective carriers in novel drugdelivery systems considering their tremendous potential. Genetic engineering aspects can be coupled to give a newer dimension to the existing cellular drug carrier concept. Using RBC’S we can transplant steroids and hormones to the targeting site by reducing their side effects. Erythrocytes are “Golden eggs in novel drug delivery systems”.

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