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An Overview of Resealed Erythrocyte Drug Delivery

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ABSTRACT

Among the various carriers used for drug delivery, erythrocytes (red blood cells, RBC) constitute potential biocompatible carriers since they possess several properties which make them unique and useful carriers. Erythrocytes are biocompatible, biodegradable, possess long circulation half lives, and can be loaded with a variety of biologically active compounds using various chemical and physical methods. This systemic review deals with advantages, requirement, methods of drug loading, characterizations, and application of erythrocyte mediated drug delivery.

Keywords: Erythrocyte mediated drug delivery, Carriers, Drug loading.

1. INTRODUCTION

Current research is aim at development of drug delivery system with maximum therapeutic benefits for safe and effective management of disease(s). The development of drug delivery system in future will be aimed to maximize therapeutic performance, eliminate undesirable side effect of drug and increase patient compliance. The idea of a drug-carrier system with target specificity has fascinated scientists and tremendous efforts have been made to achieve this goal.[1] Erythrocytes, also known as red blood cell (RBCs), have been extensively studied for their potential carrier capabilities for the delivery of drugs and drug-loaded microspheres.[2-4] Such drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the organisms of interest, separating erythrocytes from plasma, entrapping drug in the erythrocyte and resealing the resultant cellular carriers[1]. Hence these carriers are called as resealed erythrocytes. The overall process is based on the response of these cells under osmotic condition. Upon reinjection, the drug-loaded erythrocytes serve as slow circulating depots and target the drug to a reticuloendothelial system (RES).[5]

2. Anatomy, physiology and composition of RBCs[6]

RBCs have shapes like biconcave discs with a diameter of 7.8 µm and thickness near 2.2 µm. Mature RBCs have a simple structure. It is also in elastic in nature. Their plasma membrane is both strong and flexible, which allows them to deform without rupturing as they squeeze through narrow capillaries. RBCs lack a nucleus and other organelles and can neither reproduce nor carry on extensive metabolic activities. RBCs are highly specialized for their oxygen transport function, because their mature RBCs have no nucleus, all their internal space is available for oxygen transport. Even the shape of RBC facilities it's function. A biconcave disc has a much greater surface area for the diffusion of gas molecules in to and out of the RBC than would; say a sphere or a cube. The red blood cell membrane, a dynamic, semi-permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cations (Na^-, K^+) and anions (Cl^-, HCO_3^-). Each RBC contains about 280 million hemoglobin molecules. A hemoglobin molecules consists of a protein called globin, composed of four polypeptide chains; a ring like non-protein pigment called a heme, is bound to each of the four chains. At the center of the heme ring combine reversibly with one oxygen molecule, allowing each hemoglobin molecule to bind four oxygen molecules. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), methehemoglobin (0.5%), and hemoglobin (33.67%).

3. Advantages: [7-16]

- Biocompatible, particularly when autologous cells are used hence no possibility of triggered immune response.
- Natural product of the body, which are biodegradable in nature.
- Biodegradability with no generation of toxic products.
- Considerable uniform size and shape of carrier.
- Relatively inert intracellular environment can be encapsulated in a small volume of cells.
- Isolation is easy and large amount of drug can be loaded.
- Prevention of degradation of the loaded drug from inactivation by endogenous chemical.
- Entrapment of wide variety of chemicals can be possible.
- Entrapment of drug can be possible without chemical modification of the substance to be entrapped.
- Possible to maintain steady-state plasma concentration, decrease fluctuation in concentration.
- Protection of the organism against toxic effect of drug.
- Targeting to the organ of the RES.
- Ideal zero-order drug release kinetic.
- Prolong the systemic activity of drug by residing for a longer time in the body.
- Carrier for number of drugs.
- A longer life span in circulation as compared to other synthetic carrier

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Table 1: Different types of methods for drug loading in erythrocyte

Method	Procedure	% Loading	Advantages	Disadvantages
Dilution method[22]	Based upon hypotonic lysis of cells in a solution containing the drug/enzyme to be entrapped followed by restoration of tonicity to reseal them and also ability of erythrocytes to undergo reversible swelling in a hypotonic solution.	1-8%	Fastest and simplest, especially for low molecular weight drug.	Entrapment capacity low.
Dialysis[23]	It can be carrying out lysis and resealing within a dialysis tube using hypotonic and isotonic solution.	30-45%		Better *in vivo* survival. Time consuming.
Preswell dilution[23]	The technique is based upon initial controlled swelling of erythrocytes without lysis by placing them in slightly hypotonic solution followed by centrifugation at low 'g' to take them up to point of lysis. Finally, the addition of small volume of drug solution to attain drug loaded resealed erythrocytes.	20-70%	Good retention of cytoplasm constituents and good survival *in vivo*.	—
Isotonic osmotic lysis[23]	Resealed erythrocytes were prepared under isotonic conditions. Haemolysis in isotonic solutions can be achieved both by chemical agent and physical methods.	—	Better *in vivo* surveillance. consuming.	Impermeable only to large molecules, process is time

- Easy control during life span ranging from minutes to months.
- A decrease side effect of drugs.
- A considerable increase in drug dosing interval with drug residing in therapeutic window region for longer time periods.

4. Requirements for encapsulation

A wide variety of biologically active substance (5000-600,000 Daltons in size) can be entrapped in erythrocyte. Generally, the molecules should be polar, hydrophilic, non-polar and hydrophobic molecules have also been successfully. Sucrose is routinely used as a marker for encapsulation studies. Non-polar molecules may be entrapped in erythrocyte in their respective salts. Molecules which interact with the membrane and cause deleterious effects on membrane structure are not considered to be appropriate for encapsulation in erythrocyte[17-20].

5. Methods of drug loading

Several methods can be used to load drugs or bioactive compounds in erythrocyte, include physical osmotic based systems and chemical methods. Different types of this methods summarized in table 1.

5.1 Osmotic method

In this process, the intracellular and extracellular solutes of erythrocytes are exchanged by osmotic lysis and resealing. The drug present will be encapsulated within the erythrocytes membrane by this process[21].

5.2 Electro-insertion or Electro-encapsulation

Electrical pulse method is used to encapsulate bioactive molecules. Also known as electroporation, the method is based on the observation that electrical shock brings about irreversible changes in an erythrocyte membrane.

The use of transient electrolysis is to generate desirable membrane permeability for drug loading into red blood cells. The components can be entrapped when an electric pulse of greater than a threshold voltage of 1-10 kV/cm is applied for 20-160 microsec in media and resealed in osmotic medium. The potential difference across the membrane is built up either directly by inter- and intracellular electrodes or indirectly by applying internal electric field to the cells. The electromechanical compression of the membrane after breakdown leads to formation of pores. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of the suspending medium. Once the membrane is perforated, regardless of the size of the pores, ions rapidly distribute between the extra-and intracellular space to attain Donnan equilibrium, however the membrane still remain impermeable to its cytoplasmic macromolecules[24,25].

5.3 Entrapment by Endocytosis

The vesicle membrane separates the endocytosed substance from the cytoplasm, which may shelter drugs prone to inactivation in erythrocytes or alternatively protect the erythrocytes from drug. The resulting erythrocytes contain vacuoles and probably have different *in vivo* survival characteristics from resealed cells, prepared using other methods. The swollen ghosts so prepared exhibit larger (>0.5 μ diameter) endocytic vacuoles. The drug substances are trapped in these endocytic vacuoles. Drug induced endocytosis is quite common and a variety of amphiphilic cations/drugs produce first stomatocytosis and then, mostly at the advancing lip of the stoma, inside-out endocytic vacuole formation. Several classes of drugs as reported by Schrier, 1987 can produce this phenomenon of visible investigation. This method is efficient for loading large particles such as virus (up to 100 nm dia.), enzyme and small molecules.[26]

5.4 Loading by Chemical Perturbation of Membrane (Drug Mediated Loading)

This method is based upon the observation that the permeability of the erythrocytic membrane is increased, when it is exposed to some chemical agents. This allows the low molecular weight substances to get entrapped.[23]

6. Characterizations[23,27]

Table 2: Summary of characterization parameters & their determination for resealed RBCs

Parameter	Method / instrument used
I Physical characterization	
Shape and surface morphology	Transmission electron microscopy, Scanning electron microscopy, Phase contrast microscopy, Optical microscopy
Vesicle size and size distribution	Transmission electron microscopy, Optical microscopy
Drug release	Diffusion cell, dialysis
Drug content	Deproteination of cell membrane followed by assay of resealed drug, rediolebelling
Surface electrical potential	Zeta potential measurement
Surface pH	pH sensitive probes
Deformability	Capillary method
II Cellular characterization	
% Hb content	Deproteinization of cell membrane followed by hemoglobin assay
Cell volume	Laser light scattering
% cell recovery	Neubaur's chamber, hematological analyzer
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
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 ml/min flow rate and estimation of residual drug and hemoglobin. Vigorous shaking followed by hemoglobin estimation
Erythromycin sedimentation rate	ESR method
III Biological characterization	
Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test
Animal toxicity	Toxicity tests
IV Miscellaneous	
Cell size, mean cell volume, energy metabolism, lipid composition, membrane fluidity, rheological properties etc, density gradient separation	—

Table 3: Resealed erythrocytes used in RES targeting

Treatment/Diseases	Name of Drug(s)	Purpose	Reference
Treatment of lysosomal storage diseases	Lysosomal enzymes, C-glucuronidase, 13-galactosidase and 6-giucosidase	To deliver lysosomal enzymes and drugs to lysosomes of the erythrophagocytic cells.	30
Treatment of Gaucher's disease	Glucocerebrosidase	Loaded cells survived for 10 days in treated patient and no untoward reactions were found with respect to blood counts, blood pressure and renal functions.	31
Treatment of liver tumors	Anticancer like Bleomycin, Adriamycin, Carboplatin, Gentamycin, etc encapsulated in erythrocytes	Targeting to hepatic carcinomas.	32
Treatment of parasitic diseases	Pentamidine loaded, immunoglobulin-G coated 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.	33
	Glutaraldehyde treated erythrocytes	Liver targeting of an antimalarial agent - primaquine phosphate and an antiamoebic agent, metronidazole.	28
Removal of RES iron overload	Desferoxamine, an iron-chelating drug in erythrocyte ghosts	To promote excretion of iron in patients with excess body stores.	34
Removal of Toxic Agents	Murine carrier erythrocytes containing bovine rhodanese and sodium thiosulphate	Antagonism of cyanide intoxication ORTo antagonize the lethal effects of potassium cyanide in mice.	35

7. Drug release characteristics of loaded drugs

There are mainly three ways for a drug to efflux out from erythrocyte carrier's i. e. phagocytosis, diffusion through the membrane of the cell, and use of specific transport system. The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid bilayer it is greatest for molecule with high lipid solubility and gradually goes down with polarity or charged groups of the molecule. Obviously, considerable control over the rate of drug release is possible by introducing or eliminating polor or charged subsistent. Many substances enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes including both purine and pyrimidine nucleoside is transported extremely rapidly by facilitated diffusion. Prolongation of release could presumably be accomplished by entrapment of potent inhibitors of the appropriate transport protein along with the drug.

8. Toxicological, Immunological and Targeting Potential

The use of erythrocytes circulating blood carrier have shown no toxic effect as evident from animal studies. Resealed erythrocytes could be safely infused into patients and the loading at a high haematocrit value resulted into a long circulation time *in-vivo*. The immunological characteristics of a drug carrier are of two types: the immunogenicity of the carrier itself and the ability of the carrier to protect an entrapped drug from immunological detection.

Talwar & Jain was selectively targeted drug loaded erythrocytes after gluteraldehyde treatment to the liver or spleen.[28] They found that gluteraldehyde fixation of erythrocytes renders them resistant to both osmotic shock and turbulence induced lysis. It is concluded that, at low concentration glutaraldehyde treated cells are removed by spleen whereas at high concentration treated cells are removed by the liver.

9. Applications of Resealed Erythrocytes

Resealed erythrocytes have been proposed for a variety of applications in human and veterinary medicine. Various applications can be summarized as under:

a) *In-Vitro* Applications [29]

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. Enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The biochemical defects such as the glucose-6-phosphate dehydrogenase (G6PD) deficiency can be useful tool for discerning the mechanism that eventually causes these effects.

The most frequent *in vitro* application of RBC is that of micro-injection. A protein or nucleic acid was 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 has been used to confirm the site of action of fragment of diphtheria toxin. Antibodies introduced using RBC mediated microinjection is recorded not to enter the nucleus, thus limiting the studies to the cytoplasmic level. Other *in vitro* tests include utilization of erythrocytes carrier to introduce ribosome inactivating proteins into cells by fusion technique.

b) *In – Vivo* Applications

i) Targeting of bioactive agents to RE System

Damaged erythrocytes are rapidly cleared from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. The various approaches to modify the surface characteristics of erythrocytes include surface modification with antibodies, gluteraldehyde, carbohydrates such as sialic acid and sulphydryl.

ii) Targeting to sites other than RES-rich Organs

Resealed erythrocytes have the ability to deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES have been tried recently with resealed erythrocytes. Some of the representative approaches are discussed in brief.

Table 4: Resealed erythrocytes used in other than RES organ targeting

Approaches	Type of drugs	Objective/Purpose	Ref.
Magnet-responsive Erythrocyte Ghosts	encapsulation of small paramagnetic particles into erythrocytes	Localization to a particular location under the influence of external magnetic field.	36, 37
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.	38
Antibody Anchored Erythrocytes (Immunoerythrocytes):	Antibody coating of resealed drug carrier erythrocytes	Drug targeting to the RES.	39
Ultrasound Mediated Delivery of Erythrocytes loaded drug(s):	colloidal particles and red blood cells	Delivery to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound.	40

iii) Erythrocytes as Circulating Bioreactors

Erythrocytes have been realized as carriers for enzymes to serve as circulating bioreactors. Sometimes it is desirable to decrease the level of circulating metabolites that can enter erythrocytes. Erythrocytes have also been used as circulating bioreactors for the controlled delivery of antiviral drugs.

Delivery of Antiviral Agents:

Several reports are available in the literature for the antiviral agents encapsulated in the resealed erythrocytes for effective delivery and targeting.

Table 5: Resealed erythrocytes for delivery of antiviral drugs

Categories of Drugs	Name of drugs	Purpose	Reference
Azidothymidine Derivative	Azidothymidine homodinucleotide-loaded erythrocytes	Slow delivery of the antiretro viral drug azidothymidine	41
Deoxycytidine Derivatives	Antiviral nucleotide analogues	Encapsulated into erythrocytes for targeting to macrophages	42
Azathioprene and Acyclovir Derivatives	Heterodinucleotide of azidothymidine and acyclovir	Selective delivery to macrophage for protection against Human Immunodeficiency Virus or Herpes Simplex Virus	43

iv) Erythrocytes as Carriers for Drugs [29]

Various bioactive agents encapsulated in erythrocytes are developed for the slow and sustained release in circulation to allow effective treatment of parasitic diseases. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drug and vitamins and steroids.

v) Erythrocytes as Carriers for Enzymes

Enzymes can be injected into the blood stream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in the blood due to a disease likewise, environmental, lysosomal storage disorders such as Gaucher's disease, hyperarginiaemia, hyperuricaemia, hyperphenyl- alaninaemia and kidney failure are only few examples of metabolic disorders that can be treated by administration of enzymes.

Table 6: Resealed erythrocytes used in delivery of enzymes

Name of Enzymes	Purpose	Reference
L-Asparaginase	For treatment of leukemia	44
Aminolevulinate dehydratase	To treat adolescent patient suffering from lead poisoning.	45

vi) Erythrocytes as Carriers for Proteins and Macromolecules: [34]

Table 7: Resealed erythrocytes used in delivery of proteins and macromolecules

Name of Proteins	Purpose	Reference
Insulin	For its sustained release	46
Mycotoxins	For specific delivery of this highly toxic proteins to liver macrophages	47
recombinant human erythropoietin (rHuEpo)	A cellular sustained delivery system for *in vivo* administration	48
Aspirin and ferromagnetic colloid compound	Prevention of thromboembolism.	49

10. Novel approaches

a) Nanoerythrosomes [50]

An erythrocyte based new drug carrier, named nanoerythrosome has been developed which is prepared by extrusion of erythrocyte ghosts to produce small vesicles having an average diameter of 100 nm. Daunorubicin (DNR) was covalently conjugated to the nEryt (nEryt-DNR) using glutaraldehyde as homobifunctional linking arm. This led to a complex that is more active than free DNR both *in vitro* and *in vivo*. Daunorubicin (DNR) conjugated to these nanoerythrosomes has a higher antineoplastic index than the free drug. Moreover, since nanoerythrosomes are particles, phagocytosis may be involved in their mechanism of potentiation.

b) Erythrosomes [51]

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.

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