Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Review Article

RESEALED ERYTHROCYTES: A PROMISING DRUG CARRIER

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Received: 16 April 2012, Revised and Accepted: 15 May 2012

ABSTRACT

Drug targeting is the delivery of drugs in a site specific and target oriented manner which exhibit maximal therapeutic index with minimum adverse effects. Various carriers have been used for the drug targeting among which cellular carriers (e.g. erythrocytes, leukocytes, platelets, hepatocytes, fibroblasts) offer a greater potential advantages than other system. Resealed erythrocytes as drug delivery system, has tremendous potential to achieve site specificity and prolonged release of drug therapy enhancing therapeutic index and patient compliance. For the preparation of drug loaded carrier erythrocytes, the blood samples are simply collected from the organism of interest, and then erythrocytes are separated from plasma. Various methods like physical, osmosis based systems and chemical methods can be used to load the therapeutic agent. After loading, the erythrocytes are exposed to various physical, chemical and biological evaluations. Resealed erythrocytes have wide applications including drug targeting to RES and non-RES organs, in enzyme therapy, antiviral agent delivery etc.

Keywords: Erythrocyte, Cellular carriers, RES, Osmosis based system.

INTRODUCTION

Drug targeting is the delivery of drugs to the receptors or organs or any other specific part of the body to which one wishes to deliver the drugs exclusively. The drug's therapeutic index, as measured by its pharmacological response and safety, relies in the access and specific introduction of the drug with its candidate receptor, whilst minimizing its introduction with non-target tissue.[1] Therefore the current pharmaceutical scenario is aimed at development of drug delivery systems with maximum therapeutic benefits for safe and effective management of diseases.[2] Drug targeting can be achieved by either chemical modification or by appropriate carrier. The drug delivery systems currently available enlist carriers that are simple soluble macromolecules such as monoclonal antibodies, soluble synthetic polymers, polysaccharides in addition to biodegradable polymers. Moreover they include complex multicomponent structures like microcapsules, microparticles, lipoproteins, liposomes, ghost cells and cells.[3]

Various carriers has been used for the drug targeting among which cellular carrier offer a greater potential advantages related to its biodegradability, non-pathogenecity, non-immunogenicity, biocompatibility, self degradability along with high drug loading efficiency. The cellular carriers have been a useful device as drug delivery system, these carriers including leukocytes, platelets, hepatocytes, fibroblasts and erythrocytes.[4,5] Among these, the erythrocytes have been the most investigated and have found to possess great potential in novel drug delivery. 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.[6]

MORPHOLOGY AND PHYSIOLOGY OF ERYTHROCYTES

Erythrocytes (Fig1) are the most abundant cells in the human body (~5.4million cells/mm^3 blood in healthy male and ~4.8 million cells in a healthy female). These cells were described in human blood samples by Dutch Scientist Lee Van Hock in 1674. In the 19th century, Hope Seyler identified haemoglobin and its crucial role in oxygen delivery to various parts of the body.[7]

Erythrocytes are biconcave discs with an average diameter of 7.8µm, a thickness of 2.5µm in periphery, 1µm in the centre, and a volume of 85–91$µm^3$.[8] The red blood cell membrane is 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^-).[9] The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3m wide. Mature erythrocytes are quite simple in structure. They lack a nucleus and other organelles. Their plasma membrane encloses haemoglobin, a heme-containing protein that is responsible for O_2–CO_2 binding inside the erythrocytes. The main role of erythrocytes is the transport of O_2 from the 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 a nucleus is absent, all the intracellular space is available for O_2 transport. Also, because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying.

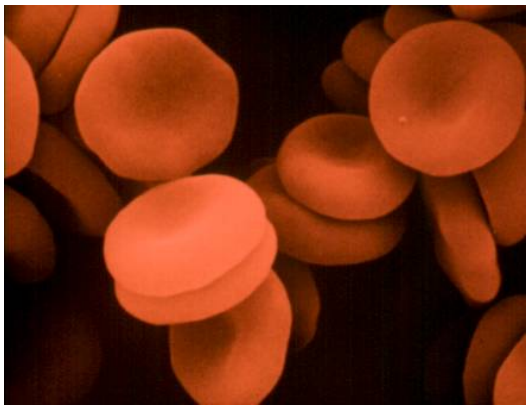

Fig. 1: Erythrocytes

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. The process of erythrocyte formation within the body is known as erythropoiesis. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called erythropoietin.[10]

ISOLATION OF ERYTHROCYTE

Source: Erythrocytes may be prepared as carriers from blood taken from human beings and from different animal species including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats and rabbits.[11-15] To isolate erythrocytes, the blood is collected in heparinised tubes by venipuncture. Freshly collected

blood is centrifuged in a refrigerated centrifuge and washed in order to obtain erythrocyte. The washed cells are suspended in buffer (e.g. acid-citrate-dextrose buffer) at various hematocrit values as desired.

ADVANTAGES OF ERYTHROCYTE AS DRUG CARRIERS[7,8,10,16-35]

- Principle advantage is their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response.
- Complete carrier biodegradability and no generation of toxic products.
- Have a longer life span (120 days) in circulation as compared with other synthetic carriers.
- Uniform size and shape with relatively inert intracellular environment and possibility of entrapment of wide variety of chemicals.
- Degradation of the loaded drug from inactivation by endogeneous chemicals is prevented.
- Due to availability of different techniques and facilities, ease of separation, handling, transfusion and working with erythrocytes.
- Attainment of steady state plasma concentration with possibility of zero order drug release kinetics.
- Modification of pharmacokinetics and pharmacodynamic parameters of drug.
- Significant decrease in side effects.
- Large quantities of drug that can be encapsulated within a small volume of cells ensure dose sufficiency.
- Ability to target the organs of RES.

DRAWBACKS OF ERYTHROCYTES AS DRUG CARRIERS[17,36-40]

- Major problem with this drug carrier is that they are removed in vivo by RES. This seriously limits their useful life as drug carriers and in some cases may pose toxicological problems.
- Rapid leakage of certain encapsulated material from the loaded erythrocytes.
- Liable to biological contamination due to the origin of blood.
- Rigorous and special controls are required for the collection and handling of erythrocytes.
- Several molecules may alter the physiology of erythrocytes.
- Encapsulated erythrocytes may present some inherent variations in their loading and release characteristics compared to other carrier systems.

METHODS OF DRUG LOADING IN ERYTHROCYTES

In general, the potential use of erythrocytes depends on their ability to encapsulate exogenous enzymes or other substances into erythrocytes.[41] 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 erythrocytes membrane, and well-defined pharmacokinetic and pharmacodynamic properties.[4,18] The following methods are used for entrapment of therapeutic agent into erythrocytes:

Osmosis- based methods

Erythrocytes have an ability to undergo reversible swelling in a hypotonic solution and have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. Erythrocyte can increase in volume by 25-50% leading to an initial change in the shape from biconcave to spherical adapt additional volume while keeping the surface area constant.[44,45] This change is due to the absence of superfluous membrane. Therefore, the cells can maintain their integrity up to a tonicity of ~150 mosm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200-500Å are generated on the membrane. The remnant left after cell lysis and depletion of cell component is called an *erythrocyte ghost* which can be resealed by restoring isotonic conditions having the drug inside. The cells resume their original biconcave shape and original impermeability upon incubation.[17,30,43,46,47] Comparison between percent drug loading, advantages as well as disadvantages of different osmosis based system is shown in table 1.

Hypotonic preswelling: In this technique erythrocytes are incubated in a hypotonic buffered solution to produce swelling and centrifuged at low centrifugation values. The supernatant is discarded and the cell fraction is brought to the lysis point (lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation) by adding 100-200µL portions of an aqueous solution of the drug to be encapsulated and centrifugation between the drug addition steps. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer. Then, cell suspension is incubated at 37^{0}C to reanneal the resealed erythrocytes.[30,43,48] This method is simpler and faster than other methods, causing minimum damage to the cell.

Hypotonic dilution: It was the first method investigated for the encapsulation of chemicals into erythrocytes and is simplest and fastest (Fig 2).[17] In this method, a volume of packed erythrocytes is diluted with 2-20 volumes of aqueous solution of a drug. The solution tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded and the pellet is washed with isotonic buffer solution. These cells are rapidly phagocytosed by RES macrophages and hence can be used for targeting RES organs.[30,49]

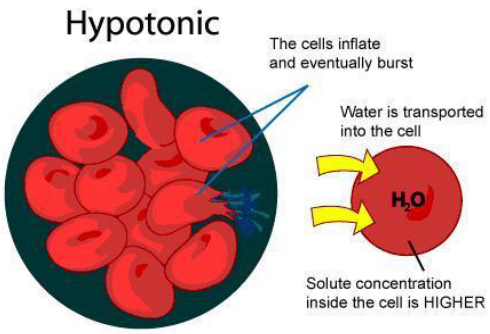

Fig. 2: Hypotonic dilution[50]

Hypotonic dialysis: Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing.[42] In this process, the suspension of erythrocytes with hematocrit 50-90% is placed in a dialysis bag (tube) facing a hypoosmotic buffer at 4^{0}C. The time of dialysis may vary between 20-180min. Subsequently, an annealing process is performed with the loaded erythrocytes in an isoosmotic medium for 10min at 37^{0}C using hyperosmotic buffer (which usually contains glucose, adenosine and magnesium chloride).[51]

The use of standard hemodialysis equipment for loading a drug in erythrocytes was reported in by Roper et al. In this method, the erythrocyte suspension and the drug to be loaded were placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment. This led to the concept of "continuous flow dialysis", which has been used by several researchers.[42]

Isotonic Osmotic Lysis: This method is also known as osmotic pulse method. In which isotonic hemolysis is achieved by physical or chemical means. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals such as urea solution, polyethylene glycol and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. The suspension was diluted with an isotonic buffered drug solution. After the cells were separated, they were resealed at 37^0C.[19]

Table 1: Comparison between percent drug loading, advantages as well as disadvantages of different osmosis based systems[52]

Method	% Loading	Advantages	Disadvantages
Dilution method	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, heterogenous size distribution of resealed erythrocytes
Preswell dilution	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

Chemical perturbation of the membrane

This method is based on the fact that erythrocyte when exposed to certain chemicals like polyene antibiotic such as amphotericin B, halothane etc, and the membrane permeability of erythrocyte increases. The main drawback of this method is that it induces irreversible changes in the cell membrane and hence are not very popular.[42]

Electroporation or electro-insertion or electroencapsulation

This method is based on using transient electrolysis leading to generate pores that produce desirable membrane permeability for drug loading into erythrocytes (Fig 4). 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 components can be entrapped when an electric pulse of greater than threshold voltage of 1-10kV/cm is applied for 20-160µsec in media and resealed in osmotic medium. The characteristic pore diameter created in the membrane depends upon the intensity of electric field, the discharge time, and the ionic strength of suspending medium. Once the membrane is perforated, regardless of the size of the pores, ions rapidly distribute between extra and intracellular space to attain equilibrium, however the membrane still remain impermeable to its cytoplasmic macromolecules.[9,53]

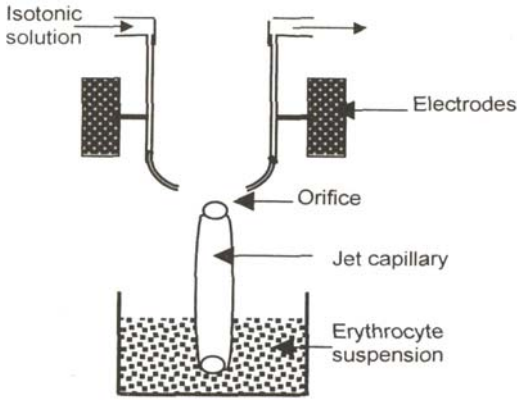

Fig. 3: Electroencapsulation[41]

Entrapment by Endocytosis[42]

Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM $MgCl_2$, and 1mM $CaCl_2$, 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 37^0C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa. (Fig 4)

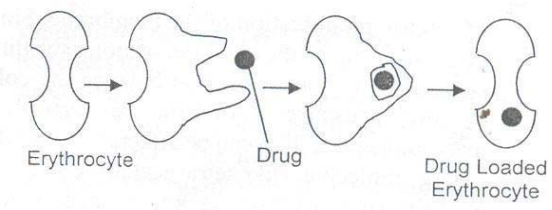

Fig. 4: Entrapment by endocytosis[41]

Loading by Electric cell fusion

This method involves the initial loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The fusion is accentuated by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell-specific monoclonal antibody into an erythrocyte ghost.[54,55] An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells.

Loading by Lipid Fusion

Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid-entrapped drug. This technique was used for entrapping inositol monophosphate to improve the oxygen carrying capacity of cells.[56] However, the entrapment efficiency of this method is very low (~1%).

EVALUATION OF RESEALED ERYTHROCYTES

After loading of therapeutic agent on erythrocytes, the carrier cells are exposed to physical, cellular as well as biological evaluations (Table 2).

Shape and Surface Morphology

The morphology of erythrocytes decides their life span after administration. The morphological characterization of erythrocytes is undertaken by comparison with untreated erythrocytes using either transmission (TEM) or Scanning electron microscopy (SEM). Other methods like phase contrast microscopy can also be used. These techniques are done to detect the morphological changes in the erythrocytes induced by encapsulation methods.[3]

Drug Content

Drug content of the cells determines the entrapment efficiency of the method used. The process involves deproteinization of packed,

loaded cells (0.5 mL) with 2.0 mL acetonitrile and centrifugation at 2500 rpm for 10 min. The clear supernatant is analyzed for the drug content.[19]

Drug Release

The drug loading may produce sustained release of the drug that influences the pharmacokinetic behaviour in vivo of he loaded erythrocytes. In vitro leakage of the drug from loaded erythrocytes is tested using autologous plasma or an isoosmotic buffer at 37^{0}C with a hematocrit adjusted between 0.5% and 50%. The supernatant is removed at previously programmed time intervals and replaced by an equal volume of autologous plasma or buffer.[57] Some authors recommended performing in vitro release studies from loaded erythrocytes using a dialysis bag.

Table 2: Summary of characterization parameters and their determination for resealed erythrocytes[42]

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	Deproteinization of cell membrane followed by assay of resealed drug, radiolabelling
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 haemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubaur's chamber, haematological analyzer
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and haemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and haemoglobin
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 mL/min flow rate and estimation of residual drug and haemoglobin, vigorous shaking followed by haemoglobin estimation
Erythrocyte sedimentation rate	ESR methods
III. Biological characterization	
Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test
Animal toxicity	Toxicity tests

Osmotic Fragility: This test of resealed erythrocytes is an indicator of the possible changes in cell membrane integrity and the resistance of these cells to osmotic pressure of the suspension medium. The test is carried out by suspending cells in media of varying sodium chloride concentration and determining the haemoglobin released. In most cases, osmotic fragility of resealed cells is higher than that of normal cells.[24,34,58]

Turbulence Fragility: The turbulence fragility is yet another characteristic that depends upon changes in the integrity of cellular membrane and reflects resistance of loaded cells against hemolysis resulting from turbulent flow within circulation. It is determined by the passage of cell suspension through needles with smaller internal diameter (e.g., 30 gauge) or vigorously shaking the cell suspension. In both cases, haemoglobin and drug released after the procedure are determined. The turbulent fragility of resealed cells is found to be higher.[24,34,58]

Haemoglobin release: The content of haemoglobin of the erythrocytes may be diminished by the alterations in the permeability of the membrane of the red blood cells during the encapsulation procedure. Furthermore, the relationship between the rate of haemoglobin and rate of drug release of the substance encapsulated from the erythrocytes. The haemoglobin leakage is tested using a red cell suspension by recording absorbance of supernatant at 540nm on a spectrophotometer.[45]

Cell Counting and Cell Recovery: This involves counting the number of red blood cells per unit volume of whole blood, usually by using automated machine. Red blood cell recovery may be calculated on the basis of the differences in the hematocrit and the volume of the suspension of erythrocytes before and after loading. The goal is to minimize the loss during the encapsulation procedure to maximize cell recovery.[43]

Determination of entrapped magnetite: Atomic absorption spectroscopic method is reported for determination of the concentration of particular metal in the sample.

The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60^{o}C for 2hours.then 20%w/v trichloro acetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.[59]

Erythrocyte sedimentation rate (ESR): It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein, especially fibrinogen and α,β globulins.

This test is performed by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.[59]

In vitro stability: The stability of the loaded erythrocytes is assessed by means of the incubation of the cells in the autologous plasma or in an isoosmotic buffer, setting hematocrit between 0.5% and 5% at temperatures of 4 and 37^{0}C.[45]

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.[60] 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.[42]

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.[61]

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 (table3). 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.[62]

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.[11,28,63]

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.[64]

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 (table4).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.[42]

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.[65]

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).

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).[66]

Table 4: Resealed erythrocytes used in other than RES organ targeting[67]

Approaches	Type of drugs	Objective/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 phototriggered carrier/delivery system for methotrexate in tumor therapy
Antibody Anchored Erythrocytes (Immunoerythrocytes):	Antibody coating of resealed drug carrier erythrocytes	Drug targeting to the RES.
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.

Table 3: Resealed erythrocytes used in RES targeting[67]

Treatment/Diseases	Name of Drug(s)	Purpose
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.
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.
Treatment of liver tumors	Anticancer like Bleomycin, Adriamycin, Carboplatin, Gentamycin, etc encapsulated in erythrocytes	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 ghosts	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

Table 5: Resealed erythrocytes for delivery of antiviral drugs [67]

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

Table 6: Resealed erythrocytes used in delivery of enzymes [67]

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

CONCLUSION

The resealed erythrocytes showed promising drug carrier characteristics. Due to the several potential advantages over other, this drug loaded erythrocytes seems to be a promising delivery system for the controlled and site specific delivery of therapeutic agents. The preparation of resealed erythrocytes is very easy and can be easily characterized by different available techniques. However, the concept needs further research and optimization to become a routine drug delivery system. The targeted release of therapeutic agents is among the most attractive applications of erythrocytes carrier which can be extended for the delivery of biopharmaceuticals. Thus the potential of this delivery system need to be explored for management of diseases.

REFERENCES

1. Gupta Manish, Sharma Vimukta. Targeted drug delivery system: A review. Res. J. Chem. Sci. 2011; 1(2): 135-138.
2. Gopal V Shavi et al. Erythrocyte as carrier for prednisolon: In vitro and in vivo evaluation. Pak. J. Pharm. Sci. 2010; 23(2): 194-200
3. Pierige F, Serafini S, Rossi L, Magnani M. Cell based drug delivery. Adv. Drug. Deliv. Rev. 2008; 60: 286-295.
4. Hamidi M, Tajerzadeh H. Carrier erythrocytes: An overview. Drug. Deliv. 2003; 10: 9-20.
5. Rossi L, Serafini S, Pierige F, Antonelli A, Cerasi A et al. Erythrocyte-based drug delivery. Expert. Opin. Dug. Deliv. 2005; 2: 311-322.
6. Eichler HG, Ramies H, Bauer K, Korn A, Bacher S, Gasic S. Survival of gentamicin loaded carrier erythrocytes in healthy human volunteers. Eur. J. Clin. Invest. 1986; 16(1): 39-42.
7. Tellen MJ The mature erythrocytes, In: Winthrob's Clinical Hematology. 9th ed. Philadelphia: Lea & Febiger; 1993. p. 101-133.
8. Guyton AC, Hall JE Red blood cells, anaemia and polycytemia, In: Textbook of Medical Physiology. Philadelphia: W. B. Saunders; 1996. p. 425-453.
9. Patel RP. An overview of resealed erythrocyte drug delivery. J. Pharm. Res. 2009; 2: 1008-1012.
10. Tortora GJ, Grabowski SR The Cardiovascular System: The Blood, In: Principles of Anatomy and Physiology. 7th ed. New York: Harper Collins College Publishers; 1993. p. 566-590.
11. Magnani M, Rossi L, Dascenzo M, Panzani I, Bigi L, Zanella A. Erythrocyte engineering for drug delivery and targeting. Biotechnol. Appl. Biochem. 1998; 28: 1-6.
12. Mishra PR, Jain NK. Biotinylated methotrexate loaded erythrocytes for enhanced liver uptake "A study on rat". Int. J. Pharm. 2002; 231(2): 145-153.
13. Kravtzoff R, Ropars C, Laguerre M, Muh JP, Chassaigne M. Erythrocytes as carriers for L-asparaginase. Methodological

and mouse in- vivo studies. J. Pharm. Pharmacaol. 1990; 42(7): 473-476.

14. Hamidi M, Tajerzadeh H, Dehpour AR. Inhibition of serum angiotensin-converting enzyme in rabbits after intravenous administration of enalaprilate loaded intact erythrocytes. J. Pharm. Pharmacol. 2001; 53(9): 1281-1286.
15. Tonetti M, Astroff AB, Satterfield W, De Flora A, Benatti U, DeLoach JR. Pharmacokinetics properties of doxorubicin encapsulated in glutaraldehyde-treated canine erythrocytes. Am. J. Vet. Res. 1991; 52: 1630-1635.
16. Lewis DA, Alpar HO. Therapeutic possibilities of drugs encapsulated in erythrocytes. Int. J. Pharm. 1984; 22: 137-146.
17. Jain S. Jain NK. Engineered erythrocytes as a drug delivery system. Indian J. Pharm. Sci. 1997; 59: 275-281.
18. Vyas SP, Khar RK Resealed Erythrocytes. In: Targeted and Controlled drug Delivery: Novel carrier systems. India: CBS Publishers and distributors; 2002. p. 387-416.
19. Jaitely V et al. Resealed erythrocytes: Drug carrier potentials and biomedical applications. Indian Drugs 1996; 33: 589–594.
20. Updike SJ, Wakamiya RT. Infusion of red blood cell-loaded asparaginase in monkey. J. Lab. Clin.Med. 1983; 101: 679–691.
21. Alpar HO, Irwin WJ. Some unique applications of erythrocytes as carrier systems. Adv. Biosci. 1987; 67**:** 1–9.
22. Eichler HC et al. In vitro drug release from human carrier erythrocytes. Adv. Biosci. 1987; 67: 11–15.
23. Summers MP. Recent advances in drug delivery. Pharm. J. 1983; 230: 643–645.
24. Talwar N, Jain NK. Erythrocytes as carriers of primaquin preparation, characterization and evaluation. J. Control Release 1992; 20: 133–142.
25. Lewis DA. Red blood cells for drug delivery. Pharm. J. 1984; 233: 384–385.
26. Adriaenssens K et al. Use of enzyme-loaded erythrocytes in in vitro correction of arginase deficient erythrocytes in familiar hyperargininemia. Clin. Chem. 1976; 22: 323–326.
27. Sprandel U. Towards cellular drug targeting and controlled release of drugs by magnetic fields. Adv. Biosci. 1987; 67**:** 243–250.
28. Eichler HG et al. In vivo clearance of antibody-sensitized human drug carrier erythrocytes. Clin. Pharmacol. Ther. 1986; 40: 300–303 (1986).
29. Baker R. Entry of ferritin into human red cells during hypotonic hemolysis. Nature 1967; 215: 424–425.
30. Ihler GM, Tsang HCW. Hypotonic hemolysis methods for entrapment of agents in resealed erythrocytes. Methods Enzymol. 1987; 149: 221– 229.
31. Schlegel RA et al. Phospholipid organization as a determinant of red cell recognition by the reticuloendothelial system. Adv. Biosci.1987; 67: 173–181.
32. Jenner DJ et al. The effect of the intravenous administration of corticosteroids encapsulated in intact erythrocytes on adjuvant arthritis in the rat. Brit. J. Pharmacol. 1981; 73: 212P– 213P.
33. Kinosita K, Tsong TY. Survival of Sucrose-Loaded Erythrocytes in the Circulation. Nature 1978; 272: 258–260.
34. Jain S, Jain SK, Dixit VK. Erythrocytes based delivery of isoniazid: Preparation and in vitro characterization. Indian Drugs 1995; 32: 471–476.
35. Pitt E, Lewis DA, Offord R. The use of corticosteroids encapsulated in erythrocytes in the treatment of adjuvant induced arthritis in the rat. Biochem. Pharmacol. 1983; 132: 3355–3358.
36. Lynch WE, Sartiano GP, Chaffar A. Erythrocytes as carriers of chemotherapeutic agents for targeting the reticuloendothelial system. Am. J. Hematol. 1980; 9 (3): 249– 259.
37. Moss HA, Tebbs SE, Faroqui MH, Herbst T, Isaac JL, Brown J, Elliott TS. A central venous catheter coated with benzalkonium chloride for the prevention of catheter-related microbial colonization. Eur. J. Anaesthesiol. 2000; 17 (11): 680– 687.
38. Valbonesi M, Bruni R, Florio G, Zanella A, Bunkens H. Cellular contamination of plasma collected with various apheresis systems. Transfus. Apher. Sci. 2001; 24: 91–94.
39. Sugai Y, Sugai K, Fuse A. Current status of bacterial contamination of autologous blood for transfusion. Transfus. Apher. Sci. 2001; 24: 255– 259.
40. Alvarez FE, Lichtiger B. Bacterial contamination of cellular blood components. Curr. Issues in Transfus. Med. 1995; 3 (3): 46.
41. Jagadale VL, Aloorkar NH, Dohe GH, Gehlot MV. Resealed erythrocytes: an effective tool in drug targeting. Indian J.Pharm. Educ. Res. 2009; 43(4): 375-383.
42. Gothoskar AV. Resealed erythrocytes: a review. Pharmaceutical Technology 2004; 1: 140-158.
43. Gamaledin I Harisa, Mohamed F Ibrahim, Fars K Alanazi, Ibrahim A Alsarra. Application and safety of erythrocytes as a novel drug delivery system. Asian J. Biochem. 2011; 6(4): 309-321.
44. Agnihotri J, Gajbhiye V, Jain NK. Engineered cellular carrier nanoerythrosomes as potential targeting vectors for antimalarial drugs. Asian J. Pharm. 2010; 4: 275-282.
45. Gupta A et al. Cell based drug delivery system through resealed erythrocyte: A review. Int. J. Pharm. 2010; 2: 23-30.
46. Dehloach JR, Haris RL, Ihler GM. An erythrocyte encapsulator dialyzer used in preparing large quantities of erythrocyte ghosts and encapsulation of a pesticide in erythrocyte ghosts. Anal. Biochem. 1980; 102: 220-227.
47. Ihler GM. Erythrocyte carriers. Pharmacol. Ther. 1983; 20: 151-169.
48. Pitt E et al. Encapsulation of drugs in intact erythrocytes: An intravenous delivery system. Biochem. Pharmacol. 1983; 22: 3359-3368.
49. Ihler GM, Glew RM, Schnure FW. Enzyme loading of erythrocytes. Proc. Natl. Acad. Sci. USA 1973; 70: 2663-2666.
50. Panchal Rushi, Patel Ankit, Patel Maulik, Jain Hitesh. Resealed erythrocytes: A novel drug delivery system. IJPI's J. of Pharm. and Cosmetology 2011; 1: 20-29.
51. Millan CG, Castaneda AZ, Marinero MLS, Lanao JM. Factors associated with the performance of carrier erythrocytes obtained by hypotonic dialysis. Blood. Cells. Mol. Dis. 2004; 33: 132-140.
52. Gopal VS, Kumar AR, Usha NA, Karthik A, Udupa N. Effective drug targeting by erythrocytes as carrier systems. Curr. Trends Biotechnol. Pharm. 2007; 1: 18-33.
53. Hamidi M, Zarei N, Foroozesh M, Mohammadi Samani S. Applications of carrier erythrocytes in delivery of biopharmaceuticals. J. Control Release 2007; 118: 145-160.
54. Tsong TY, Kinosita K. Use of voltage pulses for the pore opening and drug loading, and the subsequent resealing of red blood cells. Bibl. Haemato. 1985; 51: 108–114.
55. Li LH et al. Electrofusion between heterogeneous-sized mammalian cells in a pellet: Potential applications in drug delivery and hybridoma formation. Biophys. J. 1996; 71 (1): 479–486.
56. Nicolau C, Gersonde K. Incorporation of inositol hexaphosphate into intact red blood cells. I. Fusion of effector-containing lipid vesicles with erythrocytes. Naturwissenschaften 1979; 66 (11): 563–566.
57. Magnani M, DeLoach JR. The use of resealed erythrocytes as carriers and bioreactors. Advances in Experimental medicine and Biology. Vol. 326. Plenum Press, New York: 221-225.
58. Hamidi M et al. In vitro characterization of human intact erythrocytes loaded by enalaprilat. Drug Delivery 2001; 8: 231–237.
59. Shah Shashank. Novel drug delivery carrier: Resealed erythrocytes. Int. J. of Pharma and Biosciences 2011; 2(1): 394-406.
60. Pandey S, Devmurari Viral. Carrier erythrocytes (red blood cells) for delivery of biopharmaceuticals. Der Pharmacia Lettre 2009; 1(2): 234-244.
61. Sah Abhishek Kumar, Rambhade Ashish. Ram Alpana, Jain SK. Resealed erythrocytes: A novel carrier for drug targeting. J. Chem. Res. 2011; 3(2): 550-565.
62. Alvarez FJ et al. Cross linking treatment of loaded erythrocytes increases delivery of encapsulated substance to macrophages. Biotechnol. Appl. Biochem. 1998; 27: 139-143.
63. Deloach JR, Ihler GM. A dialysis procedure for loading of erythrocytes with enzymes and lipids. Biochem. Biophys. Acta. 1977; 496: 136-145.

64. Doijad RC, Deshmukh NV, Bhambere DS, Joseph Rony, Manvi FV. Design and characterization of anticancer engineered resealed erythrocytes. Int. J. Pharm. Sci. and Nanotech. 2008; 1(3): 243-250.
65. Markov DE, Boeve H, Gleich B, Borget J, Antonelli A, Sfara C, Magnani M. Human erythrocytes as nanoparticle carriers for magnetic particle imaging. Phys. Med. Biol. 2010; 55: 6461
66. Sprandel U, Hubbard AR, Chalmers RA. Work in progress towards enzyme therapy using carrier erythrocytes. J. Inher. Metab. Dis. 1981; 4(1): 99-100.
67. Patel RP, Patel MJ, Patel NA. An overview of resealed erythrocyte drug delivery. J. Pharm. Research 2009; 29(6): 1008-1012.