RESEALED ERYTHROCYTES: A PROMISING DRUG CARRIER

PRAGYA', VAIBHAV RASTOGI

Department of Pharmacy, Institute of Foreign Trade and Management (IFTM) University, Moradabad, U.P, India.
Email: ingenious.pragy@gmail.com

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

ABSTRACT

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 on the access and specific introduction of the drug with its candidate receptor, whilst minimizing its introduction with non-target tissue. Therefore the current pharmaceutical scenario is aimed at development of drug delivery systems with maximum therapeutic benefits for safe and effective management of diseases. 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.

Various carriers have been used for the drug targeting among which cellular carrier offer a greater potential advantages related to its biodegradability, non-pathogenicity, 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. 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.

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 on the access and specific introduction of the drug with its candidate receptor, whilst minimizing its introduction with non-target tissue. Therefore the current pharmaceutical scenario is aimed at development of drug delivery systems with maximum therapeutic benefits for safe and effective management of diseases. 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.

Various carriers have been used for the drug targeting among which cellular carrier offer a greater potential advantages related to its biodegradability, non-pathogenicity, 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. 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.

MORPHOLOGY AND PHYSIOLOGY OF ERYTHROCYTES

Erythrocytes (Fig1) are the most abundant cells in the human body (~5.4 million cells/mm³ 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.

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 85–91µm³. 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⁻, HCO₃⁻). The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3µm 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 O₂–CO₂ binding inside the erythrocytes. The main role of erythrocytes is the transport of O₂ from the lungs to tissues and the CO₂ produced in tissues back to lungs. Thus, erythrocytes are a highly specialized O₂ carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O₂ 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](image-url)
In general, the potential use of erythrocytes depends on their ability to encapsulate exogenous enzymes or other substances into erythrocytes. Several methods can be used to load drugs or other endogeneous chemicals into erythrocytes and is simplest and fastest. This method is simpler and faster than other methods, causing minimum damage to the cell.

**ADVANTAGES OF ERYTHROCYTE AS DRUG CARRIERS**

- 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**

- 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. Several methods can be used to load drugs or other bioactive compounds in erythrocytes, including physical (e.g., electrical pulse method), osmotic–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. 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 additional volume while keeping the surface area constant. 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 ressealed by restoring isotonic conditions having the drug inside. The cells resume their original biconcave shape and original impermeability upon incubation. Hypoton 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 disappearence 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 toxicity 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°C to reanneal the resealed erythrocytes. 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). In this method, a volume of packed erythrocytes is diluted with 2-20 volumes of aqueous solution of a drug. The solution toxicity 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.

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.
**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°C.19

<table>
<thead>
<tr>
<th>Method</th>
<th>% Loading</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution method</td>
<td>20-40</td>
<td>Fastest and simplest especially for low molecular weight drugs</td>
<td>Entrapment efficiency is less</td>
</tr>
<tr>
<td>Dialysis</td>
<td>30-45</td>
<td>Better in vivo survival of erythrocytes better structural integrity and membrane</td>
<td>Time consuming, heterogenous size distribution of resealed erythrocytes</td>
</tr>
<tr>
<td>Preswell dilution</td>
<td>30-90</td>
<td>Good retention of cytoplasm and good survival in vivo</td>
<td>-</td>
</tr>
<tr>
<td>Isotonic osmotic lysis</td>
<td>-</td>
<td>Better in vivo survival</td>
<td>Impermeable only large molecules, process is time consuming</td>
</tr>
</tbody>
</table>

**Chemical perturbation of the membrane**

This method is based on the fact that erythrocyte when exposed to certain chemicals like polye Ay 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

**Entrapment by Endocytosis**41

Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM 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 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 the loaded erythrocytes. In vitro leakage of the drug from loaded erythrocytes is tested using autologous plasma or an isoosmotic buffer at 37°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.55 Some authors recommended performing in vitro release studies from loaded erythrocytes using a dialysis bag.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method/instrument used</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Physical characterization</td>
<td></td>
</tr>
<tr>
<td>Shape and surface morphology</td>
<td>Transmission electron microscopy, Scanning electron microscopy, phase contrast microscopy, optical microscopy</td>
</tr>
<tr>
<td>Vesicle size and size distribution</td>
<td>Transmission electron microscopy, optical microscopy</td>
</tr>
<tr>
<td>Drug release</td>
<td>Diffusion cell, dialysis</td>
</tr>
<tr>
<td>Drug content</td>
<td>Deproteinization of cell membrane followed by assay of resealed drug, radiolabelling</td>
</tr>
<tr>
<td>Surface electrical potential</td>
<td>Zeta potential measurement</td>
</tr>
<tr>
<td>Surface pH</td>
<td>pH-sensitive probes</td>
</tr>
<tr>
<td>Deformability</td>
<td>Capillary method</td>
</tr>
<tr>
<td>II. Cellular characterization</td>
<td></td>
</tr>
<tr>
<td>% Hb Content</td>
<td>Deproteinization of cell membrane followed by haemoglobin assay</td>
</tr>
<tr>
<td>Cell volume</td>
<td>Laser light scattering</td>
</tr>
<tr>
<td>% Cell recovery</td>
<td>Neubaur’s chamber, haematological analyzer</td>
</tr>
<tr>
<td>Osmotic fragility</td>
<td>Stepwise incubation with isotonc to hypotonc saline solutions and determination of drug and haemoglobin assay</td>
</tr>
<tr>
<td>Osmotic shock</td>
<td>Dilution with distilled water and estimation of drug and haemoglobin</td>
</tr>
<tr>
<td>Turbulent shock</td>
<td>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</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>ESR methods</td>
</tr>
<tr>
<td>III. Biological characterization</td>
<td></td>
</tr>
<tr>
<td>Sterility</td>
<td>Sterility test</td>
</tr>
<tr>
<td>Pyrogenicity</td>
<td>Rabbit method, LAL test</td>
</tr>
<tr>
<td>Animal toxicity</td>
<td>Toxicity tests</td>
</tr>
</tbody>
</table>

**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 540 nm on a spectrophotometer.65

**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.65

**Determination of entrapped magnetite:** Atomic absorption spectroscopic method is reported for determination of the concentration of particular metal in the sample. The HCI is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60°C for 2 hours, 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°C.65

**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 dipheria toxin. In-vitro tests include utilization of erythrocytes carrier to introduce ribosomes inactivating proteins into cells by fusion technique.41

**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.41

**Drug Targeting**

Ideally, drug delivery should be site-specific and target oriented to exhibit maximum 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 sulphhydryl
- Surface chemical crosslinking
- Surface modification with carbohydrates such as sialic acid.42

**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-glucuronidase, β-galactosidase.11,28,63

**Treatment of hepatic tumors:** Antineoplastic drugs such as metotrexate(MTX), bleomycin, asparaginase 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.44

**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.45

**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).46

| Table 4: Resealed erythrocytes used in other than RES organ targeting45 |
|---------------------------------|-----------------|--------------------------|
| **Approaches**                  | **Type of drugs** | **Objective/Purpose**    |
| Magnet-responsive Erythrocyte   | encapsulation of small paramagnetic particles into erythrocytes | Localization to a particular location under the influence of external magnetic field. |
| Ghosts                           | Methotrexate and photosensitized by subsequent exposure to a haematoporphyrin derivative | A combination of chemotheraphy 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. |
| Photosensitized Erythrocytes     | Antibody coating of resealed drug carrier erythrocytes | Drug targeting to the RES. |
| (Immunoerythrocytes):            | 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  

<table>
<thead>
<tr>
<th>Treatment/Diseases</th>
<th>Name of Drug(s)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage diseases</td>
<td>Lysosomal enzymes, C-glucuronidase, 13-galactosidase and 6-glucosidase</td>
<td>To deliver lysosomal enzymes and drugs to lysosomes of the erythropagocytic cells.</td>
</tr>
<tr>
<td>Gaucher’s Disease</td>
<td>Glucocerebrosidase</td>
<td>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.</td>
</tr>
<tr>
<td>Liver tumors</td>
<td>Anticancer like Bleomycin, Adriamycin, Carbopeplatin, Gentamycin, etc encapsulated in erythrocytes</td>
<td>Targeting to hepatic carcinomas.</td>
</tr>
<tr>
<td>Parasitic Diseases</td>
<td>Pentamidine loaded, immunoglobulin-G coated erythrocytes Glutaraldehyde treated Erythrocytes</td>
<td>Targeting of drugs in the treatment of parasitic diseases in which the parasite resides in the organs of RES, e.g. macrophage-contained leishmania.</td>
</tr>
<tr>
<td>Iron Overload</td>
<td>Desferoxamine, an iron-chelating drug in erythrocyte ghosts</td>
<td>Liver targeting of an antimalarial agent-primaquine phosphate and an antiamoebic agent, metronidazole.</td>
</tr>
<tr>
<td>Toxic Agents</td>
<td>Murine carrier erythrocytes containing bovine rhodanese and sodium thiosulphate</td>
<td>Antagonism of cyanide intoxication or To antagonize the lethal effects of potassium cyanide in mice.</td>
</tr>
</tbody>
</table>

Table 5: Resealed erythrocytes for delivery of antiviral drugs  

<table>
<thead>
<tr>
<th>Categories of Drugs</th>
<th>Name of drugs</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azidothymidine Derivative</td>
<td>Azidothymidine homodinucleotideloaded erythrocytes</td>
<td>Slow delivery of the antiretro-viral drug azidothymidine</td>
</tr>
<tr>
<td>Deoxycytidine Derivatives</td>
<td>Antiviral nucleotide Analouges</td>
<td>Encapsulated into erythrocytes for targeting to macrophages</td>
</tr>
<tr>
<td>Azathioprene and Acylovir Derivatives</td>
<td>Heterodinucleotide of azidothymidine and acylovir</td>
<td>Selective delivery to macrophage for protection against Human Immunodeficiency Virus or Herpes Simplex Virus</td>
</tr>
</tbody>
</table>

Table 6: Resealed erythrocytes used in delivery of enzymes  

<table>
<thead>
<tr>
<th>Name of Enzymes</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Asparaginase</td>
<td>For treatment of leukemia</td>
</tr>
<tr>
<td>Aminolevulinate dehydratase</td>
<td>To treat adolescent patient suffering from lead poisoning</td>
</tr>
</tbody>
</table>

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  


60. Pandey S, Devmurari Viral. Carrier erythrocytes (red blood cells) for delivery of biopharmaceuticals. Der Pharmacia Lettre 2009; (12): 234-244.


