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Resealed Erythrocytes: A Novel Carrier for Drug Targeting

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ABSTRACT

Erythrocytes, also known as red blood cells, and have been extensively studied for their potential carrier capabilities for the delivery of drugs. Such drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers, these carriers are called resealed erythrocytes. An attempt has been made to make a review on the fabrication, in vitro characterization and biomedical application of Resealed erythrocytes.

Key word: Resealed erythrocytes, Novel carrier, Drug targeting.

INTRODUCTION

Present pharmaceutical scenario is aimed at development of drug delivery systems which maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases [1]. Targeting of an active biomolicule from effective drug delivery where pharmacological agent directed specifically to its target site. Drug targeting can be approaches by either chemical modification or by appropriate carrier. Various drug delivery carriers has been investigated presently like nonoparticle, microspheres, lipid vesicular carrier, microemulsion, aquasomes, pharmacosomes, ethosomes, cellular carrier and macromolecule [2]. The targeted or site-specific delivery of drugs is indeed a very attractive goal because this

provides one of the most potential ways to improve the therapeutic index (TI) of drug whilst devoiding its potential interaction with non-targeted tissue [3]. Various carrier has 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. Leukocytes, platelets and erythrocytes have been proposed as cellular carrier systems. Erythrocytes have been the most interesting carrier and have found to possess great potential in drug targeting. Resealed erythrocytes are gaining more popularity because of their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation. Most of the resealed erythrocytes used as drug carriers are rapidly taken up from blood by macrophages of reticuloendothelial system (RES), which is present in liver, lung, and spleen of the body [4]. The aim of the present review is to focus on the various features, drug loading technology and biomedical application of resealed erythrocytes.

Characteristic of Resealed Erythrocytes

When erythrocytes are suspended in a hypotonic medium they swell to about one and a half times their normal size, and the membrane rupture in the formation of pores with diameters of 200 to 500 A^0. The pores allow equilibration of the intracellular and extracellular solution. If the ionic strength of the medium then is adjusted to isotonic and the cells are incubated at 37^oC, the pores will close and cause the erythrocyte to "Resealed". Using this technique with a drug present in the extracellular solution, it is possible to entrap up to 40 % of the drug inside the resealed erythrocyte and to use this system for targeted delivery via intravenous injection. Normal aging erythrocytes, slightly damaged erythrocytes, slightly damaged erythrocytes and those coated lightly with antibodies are sequestered in the spleen after intravenous vein fusion, but heavily damaged or modified erythrocytes are removed from the circulation by the Liver. This suggests that resealed erythrocytes can be targeted selectively to either the liver or spleen depending on their membrane characteristics. The ability of resealed erythrocyte to deliver drug to the liver or spleen can be viewed as a disadvantage is that other organs and tissues are inaccessible [5].

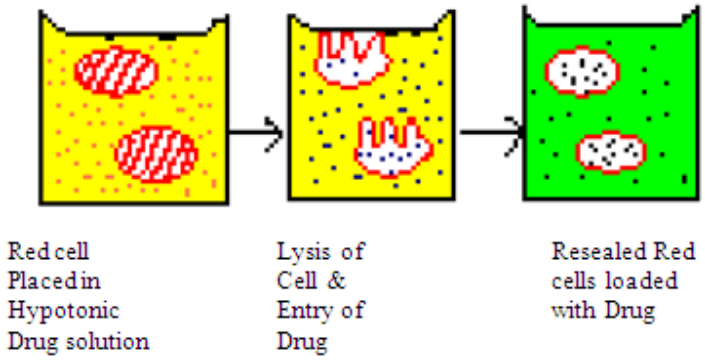

Fig. 1: Showing Drug loading in erythrocytes

Isolation of Erythrocytes

Blood can be isolated by withdrawing from cardiac/splenic puncture (in case of small animals) and through veins (in case of larger animals) into a syringe containing a drop of anticoagulant. The whole blood centrifuged at 2500 rpm for 5 min, at 4 $\pm$ 1 ^oC in a refrigerated centrifuge. The serum and buffy coats are carefully removed and packed cells washed 3 times with phosphate

buffer saline (PBS. 7.4 pH), the washed erythrocytes are diluted with PBS and stored at 4 ^{o}C until used [4, 6].

Factors which considering resealed erythrocytes as carrier

- Its shape and size to permit the passage through the capillaries.
- Its specific physico-chemical properties by which a prerequisite site can be recognized.
- Its biocompatible and minimum toxicity character.
- Its degradation product, after release of the drug at the target site, should be biocompatible.
- Low leaching/leakage of drug should take place before target site is reached.
- Its drug released pattern in a controlled manner.
- High drug loading efficiency for broad spectrum of drugs with different properties.
- Physico-chemical compatibility with the drug.
- The carrier system should have an appreciable stability during storage.

Various advantages of resealed erythrocytes

- They are the natural product of the body, which are biodegradable in nature.
- Isolation of erythrocytes is easy and larger amount of drug can be encapsulated in a small volume of cells.
- The entrapment of drug does not require the chemical modification of the substance to be entrapped. This is in contrast with other systems which involve covalent coupling of the drug and carrier which may effect the inherent biological activity of the parent drug.
- They are non-immunogenic in action and can be targeted to disease tissue/organ.
- They prolong the systemic activity of drug while residing for a longer time in the body.
- They protect the premature degradation, inactivation and excretion of proteins and enzymes and act as a carrier for number of drugs.
- They can target the drug within reticuloendothelial system (RES).
- They facilitate incorporation of proteins and nucleic acid in eukaryotic cells by cell infusion with RBC.

Method of drug loading in resealed erythrocytes

Several methods can be used to load drugs or other bioactive compounds in erythrocytes including physical (e.g., electrical-pulse method) osmosis-based systems, and chemical methods (e.g., chemical perturbation of the erythrocytes membrane). Irrespective of the method used, the optimal characteristics for the successful entrapment of the compound requires the drug to have a considerable degree of water solubility, resistance against degradation within erythrocytes, lack of physical or chemical interaction with erythrocyte membrane, and well-defined pharmacokinetic and pharmacodynamic properties [7].

1. Hypo-osmotic lysis 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 [8].

Hypotonic hemolysis

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution. Erythrocytes have an exceptional capability for reversible shape changes with or

without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an initial change in the shape from biconcave to spherical. This change is attributable to the absence of superfluous membrane; hence, the surface area of the cell is fixed. The cells assume a spherical shape to accommodate additional volume while keeping the surface area constant. The volume gain is 25–50%. 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 200–500 Å are generated on the membrane. After cell lysis, cellular contents are depleted. The remnant is called an erythrocyte ghost [9,10,11,12].

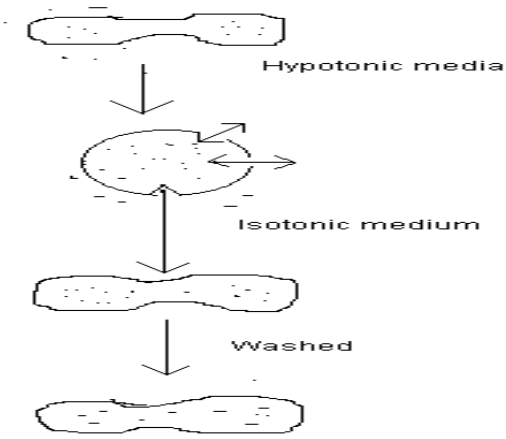

Fig 2: Showing hyposmotic lysis technique

Hypotonic dilution

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes and is the simplest and fastest [13]. 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 [10,13]. The major drawbacks of this method include low entrapment efficiency [14, 15] and a considerable loss of hemoglobin and other cell components. This reduces the circulation half life of the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as -galactosidase and -glucosidase [13], asparginase [16, 17], and arginase [18], as well as bronchodilators such as salbutamol [19].

Hypotonic dialysis

This method was first reported by Klibansky [20] for loading enzymes and lipids. Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In the process, an isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is prepared and placed in a conventional dialysis tube immersed in 10–20 volumes of a hypotonic buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer [21, 22]. The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the

beginning of the experiment or by adding the drug to a dialysis bag after the stirring is complete [23, 21,24, 25].

Hypotonic preswell technique

This method was investigated by Rechsteiner [26] in 1975 and was modified by Jenner *et al*. for drug loading. This method based on the principle of first swelling the erythrocytes without lysis by placing them in slightly hypotonic solution. The swollen cells are recovered by centrifugation at low speed. Then, relatively small volumes of aqueous drug solution are added to the point of lysis. The slow swelling of cells results in good retention of the cytoplasmic constituents and hence good survival in vivo. This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in erythrocytes using this method include propranolol [27], asparginase [28], cyclopohphamide, cortisol-21-phosphate [29, 30], 1-antitrypsin [30], methotrexate, insulin [30, 31], metronidazole [32], levothyroxine [33], enalaprilat [34], and isoniazid [35].

Isotonic osmotic lysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis. Erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. Chemicals such as urea solution [36], polyethylene glycol [37], and ammonium chloride have been used for isotonic hemolysis. In 1987, Franco *et al*. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO) [31].

Membrane perturbation by chemical agent

This method is based upon the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke *et al*. showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B [38]. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes [39]. Lin *et al*. [40] used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

Electro-insertion or electroencapsulation

This method is also known as electroporation, the method consist of creating electrically induced permeability changes at high membrane potential differences. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading [41]. Electrical breakdown is achieved by membrane polarization for microseconds using varied voltage of 2kv/cm is applied for 20 µsec. 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 extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium. Once membrane is perforated, regardless of the size of pores, ions rapidly distribute between the extra and intracellular space to attain Donnan equilibrium, however the membrane still remains impermeable to its cytoplasmic macromolecules. The cell membrane eventually lyses because of the colloidal osmotic pressure of its macromolecular contents. In the case of red blood cells, the colloidal osmotic pressure of haemoglobin is about 30 mOsm. This pressure drives water and ion influx, as a result swelling of

the cells occurs. The membrane is ruptured when the cell volume reaches 155% of its original volume. Thus, cell lysis is a secondary effect of electric modification of the membrane. Since the cell lysis is due to colloidal osmotic swelling, the rational to prevent lysis is to balance the colloidal osmotic pressure of cellular macromolecules. This can be affected by addition of large molecules (like tetrasaccharide stachyose or protein such as bovine serum albumin) and ribonucleases. This helps to counteract the colloidal osmotic swelling of electrically perforated erythrocytes.Under this osmotically balanced condition pores stay open at 4^{o}C for few days. If drug molecules are added at this point, they permeate into red blood cells. A suitable procedure could be subsequently used to reseal these pores [42]. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A [41, 43, 44].

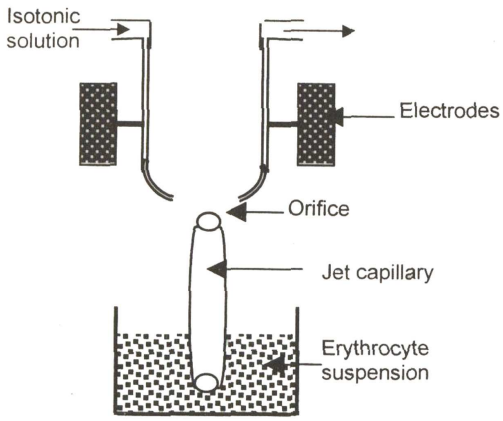

Fig 3. Showing electroencapsulation technique

Entrapment by endocytosis

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 This method was reported by Schrier *et al.* in 1975 [45]. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37 ^{o}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. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A [41, 46, 44].

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 [46, 47]. 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.

Lipid fusion technique

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule. This method provides very low encapsulation efficiency [48].

***In-vitro* Characterization of Resealed Erythrocytes**

Table I summarizes the various evaluation parameters and the techniques applied for their determination.

Drug content quantification

To determine the drug content, packed loaded cells are deproteinized with acetronitrile after cetifugation at 3000 rpm for a fixed time interval. The clear supernatant liquid is analysed spectrophotometrically [48].

***In-vitro* drug release and hemoglobin content study**

In-vitro release of drug(s) and hemoglobin are monitored periodically form drug-loaded cells. The cells suspension (5% hematocrit in PBS) are stored at 4 ^{0}C in amber colored glass containers. Periodically the clear supernantent are withdrawn using a hypodermic syrings equipped with 0.45 μ filter, deproteinied using methanol and were estimated for drug content. The supernatent of each sample after centrifugation is collected and assayed, % hemoglobin release may be calculated using the formula.

$$\% \text{ hemoglobin release} = \frac{A_{540} \text{ of sample} - A_{540} \text{ of background}}{A_{540} \text{ of 100\% hemoglobin}}$$

Or

$$\text{Mean corpuscular hemoglobin \{MCH (pg)\}} = \frac{\text{Hemoglobin (g/100ml) x 10}}{\text{Erythrocyte count (millions/cu mm)}}$$

Where a A_{540} refers to absorbance at 540nm [48].

Percent cell recovery and Morphological study

Percent cell recovery may be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug. Phase contrast or electron microscope may be used for normal and drug loaded erythrocytes [48].

Osmotic fragility and Osmotic shock study

When red blood cells are exposed to solution of varying tonicities their shape change due to osmotic imbalance. To study the effect of different tonicities, drug loaded erythrocytes are incubated separately in normal saline solution at 37= ± 2 ^{o}C for 10 minutes, followed by centrifugation at 2000 rpm for 10 min. For osmotic shock study, dispersing the resealed erythrocyte suspension in distilled water and centrifuged at 300 rpm for 15 min. The supernatant was estimated for percent hemoglobin release spectrophotometrically [48].

Turbulence shock study

It is the measure of simulating destruction of loaded cells during injection. Normal and drug loaded cells are passed through a 23 gauge hypodermic needle at a flow rate of 10 ml/min which

is comparable to the flow rate of blood. It is followed by collection of an aliquot and centrifugation at 2000 rpm for 10 minutes. The hemoglobin in withdrawn sample is estimated. Drug loaded erythrocytes appear to be less resistant to turbulence, probably indicating destruction of cells upon shaking [48].

Entrapped magnetite study

The hydrochloric acid is added to a fixed amount of magnetite bearing erythrocytes and contents are heated at 60^0c for 2 hr. Then 20% w/v trichloroacetic acid is added and suparnantant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy [49].

Self life and Stability and Crosslinking of Released Erythrocytes

Glutaraldehyde (0.2%) treated erythrocytes in a sintered glass funnel (G-4) by filtration and dried in vacuum (200mm Hg) for 10 hr. Alternatively the erythrocyte suspension was filled into vials and lyophillized at- 40 ^{0}C to 0.01 torr using a laboratory lyophilizer. The dried powder was filled in amber color glass vials and stored at 4 ^{0}C for month. Improvement in shelf life of the carrier erythrocytes was achieved by storing the cells in powder from, ready for reconstitution at 4 ^{0}C.

Table. 1: Various characterization parameters and method employ for Resealed erythrocytes

PARAMETER	INSTRUMENT / METHOD USED
I. Physical parameter [a] Shape, size, surface morphology	Transmission electron microscopy, Scanning electron microscopy, Optical microscopy, phase contrast microscopy.
[b] Vesicle size & size distribution	Transmission electron microscopy, Optical microscopy.
[c] Drug release	Diffusion cell, Dialysis.
[d] Drug content	Deproteinization of cell membrane followed by assay of drug, radiolabelling.
[e] Surface electrical potential spectroscopy	Zeta potential determination by Photon correlation [PCS]
[f] Surface pH	pH sensitive probes
[g] Deformity	Capillary method
II. Cellular parameter	
[a] % Hb content	Deproteinization of cell membrane followed by hemoglobin assay
[b] Cell volume	Laser light scattering
[c] % Cell recovery	Neubaur's chamber, hematological analyzer
[d] Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
[e] Osmotic shock	Dilution with distilled water & estimation of drug and hemoglobin
[f] Turbulent shock	Passage of cell suspension to 30-gauge hypodermic needle at the rate of 10ml/min flow & estimation of residual drug & hemoglobin, vigorous shaking
[g] Erythrocyte sedimentation rate	Determine by ESR technique.
III. Biological parameter	
[a] Pyrogenicity	LAL test, Rabbit method
[b] Sterility	Sterility testing method
[c] Toxicity	Toxicity test method.

Storage

Stored encapsulated preparation without loss of integrity when suspended in hank's balanced salt solution [HBSS] at 4 ^{0}C for two weeks. Use of group 'O' [universal donor] cells and by using the preswell or dialysis technique, batches of blood for transfusion. Standard blood bag may be used for both encapsulation and storage [50].

Mechanism of Drug Release from Resealed Erythrocyte

There are mainly three ways for a drug to efflux out from erythrocyte carriers.

1. Phagocytosis
2. Diffusion through the membrane of the cell and
3. Using a specific transport system.

Biomedical Applications of Resealed Erythrocytes

Application *in-vitro* as a carrier

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 micro-injection. 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 [51].

Applications in *in-vivo*

(a) Targeting of bioactive agents to RE system

Resealed erythrocytes with modified surface characteristics, as damaged by heat treatment, glutaraldehyde treatment, sulfhydryl reacting agents and antibodies are quickly removed from circulation by phagocytic cells located in liver and spleen, suggesting the possibility lf use of erythrocytes in targeting of bioactive agents to these cells. The drug encapsulated erythrocytes have been used for RBC targeting in the treatment of following diseases.

(I) Treatment of liver tumors

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate [53, 54], bleomycin [55], asparginase [54, 16], and adriamycin [54, 24, 56, 57] have been successfully delivered by erythrocytes. Agents such as daunorubicin diffuse rapidly from the cells upon loading and hence pose a problem. This problem can be overcome by covalently linking daunorubicin to the erythrocytic membrane using gluteraldehyde or cisaconitic acid [58] as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver [59].

(II) Treatment of parasitic diseases

Erythrocytes can be used for targeting of drugs in the treatment of parasitic diseases in which the parasite resides in the organs of RES. Parasitic diseases that involve harboring parasites in the RES organs can be successfully controlled by this method. Results were favorable in studies

involving animal models for erythrocytes loaded with antimalarial [60], antileishmanial [60, 23, 61], and antiamoebic drugs [54, 32].

(III) Removal of toxic agents

Cannon *et al*. reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulfate [62]. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiestrase also has been reported [63].

(IV) Removal of RES iron overload

In iron overload resulting from repeated blood transfusion, reticuloendothelial cells are the primary and the major sites of iron accumulation. Iron chelating drug (desferrioxime) has been entrapped in erythrocytes, for promoting excretion of iron that is present as intracellular ferritin and haemosiderin deposit. The drug forms soluble chelates and depletes the depots [52].

(V) Drug targeting, other than RES

Zimmermann [68] proposed that the entrapment of small paramagnetic particles into erythrocytes might allow their localization to a particular location under the influence of an external magnetic field. The loading of ferrofluids (colloidal suspension of magnetite) has been reported by Sprandel *et al.* [69]. Jain and Vyas [70] reported entrapment of the anti-inflammatory drugs diclofenac sodium and ibuprofen in magnetoresponsive erythrocytes. Photosensitized erythrocytes have been studied as a phototriggered carrier and delivery system for methotrexate in cancer treatment [71]. Chiarantini *et al.* have reported in vitro targeting of erythrocytes to cytotoxic T-cells by coupling of Thy-1.2 monoclonal antibody [72]. Price *et al*. reported delivery of colloidal particles and erythrocytesto tissue through microvessel ruptures created by targeted microbubble destruction with ultrasound [64]. IV fluorescent erythrocytes were delivered to the interstitial of rat skeletal muscle through microvessel ruptures by insonifying microbubbles *in vivo*. This technique provides a noninvasive means for delivering resealed erythrocytes across the endothelial carrier to the target tissue. Other approaches for targeting organs outside the RES include the preparation of carrier erythrocytes fused to thermoresponsive liposomes and their localization using an external thermal source [65], intraperitoneal injection of resealed erythrocytes for drug targeting to peritoneal macrophages [66], and lectin pretreatment of resealed cells loaded with antineoplastic drugs to improve targeting tumor cells [67].

(VI) Delivery of antiviral agents

Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting [12]. Because most antiviral drugs are either nucleotides or nucleoside analogs, their entrapment and exit through the membrane needs careful consideration. Nucleosides are rapidly transported across the membrane whereas nucleotides are not and thus exhibiting prolonged release profiles. The release of nucleotides requires conversion of these moieties to purine or pyrimidine bases. Resealed erythrocytes have been used to deliver deoxycytidine derivatives [12], recombinant herpes simplex virus type 1 (HSV-1) glycoprotein B [72], azidothymidine derivatives [73], azathioprene, acyclovir [74] and fludarabine phosphate [75].

(VII) Delivery of oxygen to tissue

Resealed erythrocytes are also used in cases of oxygen deficiency where an improved oxygen supply is Required under normal conditions, 95% of hemoglobin is saturated with oxygen in the lungs, whereas under physiologic conditions in peripheral blood stream only 25% of oxygenated hemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to hemoglobin is recirculated with venous blood to the lungs. The use of this bound fraction has been suggested for the treatment of oxygen deficiency. 2, 3-Diphosphoglycerate (2, 3-DPG) is a natural effector of hemoglobin. The binding affinity of hemoglobin for oxygen changes reversibly with changes in intracellular concentration of 2, 3-DPG. This compensates for changes in the oxygen pressure outside of the body, as the affinity of 2, 3-DPG to oxygen is much higher than that of hemoglobin [76]. Other organic polyphosphates can serve as allosteric effectors of hemoglobin with binding affinities higher than those of 2, 3-DPG and can compete with 2,3-DPG for binding to hemoglobin [77]. Inositol hexophosphate (IHP) is one of the strongest effectors of this type [78]. However, because of its ionization at physiologic pH, it cannot enter erythrocytes [79, 78]. Hence, it is entrapped by the electroporation process. Upon encapsulation, IHP irreversibly binds to hemoglobin, thereby decreasing the oxygen affinity to hemoglobin and subsequent shift of oxygen binding isotherm to the right [53]. As a result, the oxygen pressure corresponding to 50% of the total binding capacity of hemoglobin to oxygen (P50 value) increases from 26–27 mm Hg to 50 mm Hg [79]. In the presence of IHP encapsulated in erythrocytes, the difference between the oxygen bound fraction of hemoglobin in lungs and tissues increases, thereby increasing the oxygen concentration in tissues. Also, the extent of carbamate formed in the N-terminal amine group of chain of hemoglobin decreases, which is compensated by an uptake of H and CO2 that leads to increased formation of bicarbonate ion. IV injection of IHP-loaded erythrocytes to piglets led to a decrease in cardiac output with a constant oxygen consumption by animals [79, 80]. This indicates that because of an increased extraction ratio of oxygen by tissues, a given amount of oxygen can be delivered in lower blood flow. In addition, these erythrocytes reduce ejection fraction, left ventricular diastolic volume, and heart rate [81].An isolated perfused-heart model showed reduction in coronary blood flow with increased oxygen consumption by myocardium upon administration of IHP-loaded erythrocytes [82, 83]. The same results are reported when intact animal models were used [84]. An application of resealed erythrocytes for oxygen supply under the following conditions [85] :

- High altitude conditions.
- Small number of alveoli.
- Increased resistance to oxygen diffusion in the lungs.
- Reduction in oxygen transport capacity.
- Liver mediated detoxification processes.

CONCLUSION

The use of resealed erythrocytes extended promising for a safe and effective delivery of various bioactive molecules for effective targeting. However, the concept needs further optimization to become a routine drug delivery system. The same concept also can be extended to the delivery of biopharmaceuticals and much remains to be explored regarding the potential of resealed erythrocytes. Until other carrier systems come of age, resealed erythrocytes technology will remain an active arena for the further research. In future a critical time in this field as

commercial applications are explored. In near future, erythrocytes based delivery system with their ability to provide controlled and site specific drug delivery will revolutionize in effective treatment of various disease. For the present, it is concluded that erythrocyte carriers are "nano device in field of nanotechnology" considering their tremendous potential.

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