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Review Article

A REVIEW ON RESEALED ERYTHROCYTE AS A NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Cellular carriers possess great potential and merits in various modules of drug delivery. Among such cellular carriers, erythrocytes have been found to possess huge merits for the targeted and controlled drug delivery since they are non-immunogenic, non-toxic and their circulation can be readily controlled and could be manipulated with reference to time. Erythrocytes mediated drug delivery has been reported with therapeutic enzymes and antiviral agents to maximize therapeutic performance, reduce the side effects, as circulating depots for controlled drug release, drug targeting, treatment of parasitic diseases, hepatic tumors, removal of toxic agents etc. In this review, isolation of carrier erythrocytes, methods of drug loading, characterization parameters methods and clinical applications of resealed erythrocytes were presented.

Keywords: Resealed erythrocyte, Drug targeting, Controlled release, Carrier mediated drug delivery system.

INTRODUCTION

Drug delivery is the method of formulation, technology or systems for transporting an active pharmaceutical ingredient to the target receptor at an organ in the body without any loss or compromise in the chemical integrity of the molecule and could be manipulated to effect the desired pharmacological action. Drug delivery technology plays around modification and improvement of various pharmacokinetic parameters such as drug release profile, absorption, distribution, and elimination, etc. The substances that are used to transport the drug to the target site were called as drug carriers and should aid the drug to prolong its *in vivo* actions, decrease metabolism, eliminates toxicity and with tailored pharmacokinetic parameters [1].

Drug carriers are substances that serve as transporters to deliver the drugs to target and improve the effectiveness of drugs. New drug delivery systems have been developed to overcome the limitations associated with the conventional drug delivery systems in order to improve the patient compliance and safety. They may be classified based on their size as (i) microcarriers for example, Liposomes, resealed erythrocytes, microspheres (ii) nanocarriers for example, niosomes, pharmacosome, aquasomes, nanoparticles, solid lipid nanoparticles (SLN), miscelles (iii) variable carriers for example, dendrimers.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Thus, drugs that are encapsulated in a nano cage-functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes.

Resealed erythrocytes are prepared by the delivery of drugs and drug-loaded microspheres into the Erythrocytes, have been extensively studied for their potential carrier capabilities. 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. Hence, these carriers are called resealed erythrocytes.

Microsphere is characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size <200 µm. It is the reliable means to deliver the drug to the target site with specificity if modified, and to maintain the desired concentration at the site of interest.

Pharmacosomes are colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of the drug-lipid complex. It is based on the principle that the drug binds covalently to a lipid where the resulting compound is the carrier and the active compound at the same time.

Aquasomes are one of the most recently developed delivery system for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites. They are spherical in shape with 60-300 nm particles size.

Nanoparticles (including nanospheres and nanocapsules of size 10-200 nm) are in the solid state and are either amorphous or crystalline. They are able to absorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. Biodegradable polymeric nano particles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, in targeting particular organ/or tissue, as carriers of DNA in gene therapy, and in their abilities to deliver proteins peptides and genes through peroral route.

SLN are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research. Due to their unique size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could use for drug targeting.

Micelles formed by self-assembly of amphiphilic block copolymers (5-50 nm) in aqueous solutions are of great interest for drug delivery applications. The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water-solubility.

Dendrimers are nanometer-sized, highly branched, and monodisperse macromolecules with symmetrical architecture. They consist of a central core, branching units, and terminal functional groups. Targeting effectiveness is affected by attaching targeting ligands at the external surface of dendrimers, while their stability and protection

from the mononuclear phagocyte system (MPS) is being achieved by functionalization of the dendrimers with polyethylene glycol chains (PEG).

Colloidal drug carrier systems such as micellar solutions, vesicle, and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10-400 nm diameter, show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity.

CELL BASED DRUG DELIVERY SYSTEM

Among the cell based delivery systems two categories could be identified:

- Transduced cells:

Capable of expressing pharmaceutically relevant agents.

- Cellular carriers:

This could be loaded with drugs or therapeutics. In this category, the carrier cells could release the drug content in circulation or at selected sites or could target the drug to other relevant cells in the body. There are two desirable properties for a drug carrier, to selectively direct a drug to a target tissue and to protect the drug from premature bio inactivation. Amongst the various carriers used for targeting of drugs to various body tissues, the cellular carriers meet several criteria Desirable in clinical applications, among the most important being biocompatibility of carrier and its degradation products.

Present pharmaceutical circumstances are aimed at the improvement of drug delivery system which maximizes the drug targeting along with high therapeutic effect for safe and effective management of disease by reducing the adverse effect of the drug. The substances that serve as mechanisms to improve the delivery and the effectiveness of drugs are called drug carriers [1]. Drug carriers are used in assorted drug delivery systems such as: Controlled-release technology to extend *in vivo* drug actions; diminish drug metabolism, and reduce drug toxicity. These cellular carrier offer a greater prospective advantages related due to their ability to circulate throughout the body, zero order kinetics, reproducibility and ease of preparation primary aim for the development of this drug delivery system is to make best use of therapeutic performance, minimize the unwanted side effects of drug, eco-friendly, non-bug, does not stimulate any immune response, co-exist with living tissues, along with high drug loading efficiency [2].

Erythrocyte

Red blood cells (RBCs) have shapes like biconcave discs with a diameter of 7.8 μm and thickness near 2.2 μm. Mature RBCs have a simple structure. It is also in elastic in nature. Their plasma membrane is both strong and flexible. A biconcave disc has a much greater surface area for the diffusion of gas molecules into and out of the RBC than would; say a sphere or a cube.

Each RBC contains about 280 million hemoglobin molecules. A globin is a protein present in hemoglobin molecules, consist of four polypeptide chains; to each of the four chain, a non-protein pigment called a heme, is bound to it. It combines reversibly with one oxygen molecule, at the center of the heme ring allow each hemoglobin molecule to bind four oxygen molecules. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), meth hemoglobin (0.5%), and hemoglobin (33.67%) [3].

Erythrocytes have been widely used as carrier systems of different drugs, which induce changes in the pharmacokinetics of drug, increase its half-life, reduces its adverse effect, the wide variety of chemicals that can be entrapped into erythrocytes.

Erythrocyte can be used as the carrier in many ways: for targeting particular tissue/organ, for continuous or prolonged release of drug, for targeting reticuloendothelial system (RES), targeting the liver deficiency therapy, for the removal of toxic agents, for the treatment of parasitic disease, treatment of hepatic tumor, for antiviral drug delivery etc., [4].

PROPERTIES ESSENTIAL FOR SUITABILITY OF RBC AS DRUG CARRIER [5,6]

1. Biodegradability: Resealed erythrocytes are biodegradable. Therefore the biodegraded product is of utter importance (90% RBC's are degraded by macrophages present in RES and remaining 10% by hemolysis in circulation)
2. Circulate throughout the circulatory system (On addition of calcium chelating agents or purine nucleosides, the circulation is further enhanced)
3. A large quantity of materials or bioactive agents can be encapsulated within small volumes of cells
4. Can be utilized for organ targeting within RES
5. Erythrocytes are biocompatible provided that compatible cells are used in patients
6. There should be no possibility of triggered immunological reaction
7. The suppleness of RBCs to retain its shape and morphology when placed in isotonic saline also make them appropriate carriers for drugs and enzymes
8. It should have a considerable stability during storage
9. It should have specific physicochemical properties by which a preferred target site could be predicted
10. Reduce the leakage of the drug should take place before target site is reached
11. Prospect of decreasing the side effects of the drug
12. The entrapment of drugs does not require the chemical modification of the substance to be entrapped
13. No possibility of triggered immune response
14. Biodegradability with no generation of toxic or harmful products
15. The considerable uniform size and shape of the carrier.

ADVANTAGES OF RESEALED ERYTHROCYTES [7,8]

1. No chance of triggered immune response
2. Biodegradability with no generation of toxic or harmful products
3. The considerable uniform size and shape of the carrier
4. Relatively inert intracellular environment
5. Prevention of degradation of the loaded drug from inactivation by endogenous chemicals
6. The extensive variety of chemicals and enzymes can be entrapped
7. The alteration of pharmacokinetic and Pharmacodynamics parameters of the drug can be done
8. Attainment of steady-state plasma concentration which decreases fluctuations in the concentration of the drug
9. Protection of the organism against toxic effects of drugs (e.g., antineoplastic)
10. Ease of circulation and ability to target RES organ
11. Prolong systemic activity of the drug while residing for a longer time in the body.

FORMULATION OF RESEALED ERYTHROCYTES

Isolation of erythrocytes

For drug delivery, mammalian erythrocytes have been used like the erythrocytes of, sheep, goats, monkeys, chicken, rats, rabbits, mice, cattle, pigs, dogs and human. To detach erythrocytes, blood is collected in tubes containing anticoagulant like EDTA, heparin etc., by venipuncture. For loading purposes, Fresh entire blood is characteristically used. Because the encapsulation capability of the erythrocytes isolated from fresh blood is superior to that of the aged blood. Fresh whole blood is collected and immediately chilled to 4°C and stored for less than 2 days. By Centrifuge, erythrocytes are harvested and washed. The washed cells are poised in buffer solutions at various hematocrit values as

desired and are stored in acid–citrate–dextrose buffer at 4°C as long as 48 hrs before use [7,8].

METHODS OF ENCAPSULATION OF ERYTHROCYTES [9,10]

They are various methods for the encapsulation of erythrocyte with drug:

- Hypotonic hemolysis
- Use of red cell loader
- Hypotonic dilution
- Hypotonic preswelling
- Hypotonic dialysis.
- Isotonic osmotic lysis
- Chemical perturbation of the membrane
- Entrapment by endocytosis
- Loading by electric cell fusion
- Loading by lipid fusion.

Hypotonic hemolysis

This method is based on the capacity of erythrocytes to experience reversible swelling in a hypotonic solution. An outstanding capability of erythrocytes for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An initial change in the shape from biconcave to spherical is due to the increase in the volume of the erythrocytes. Hence, the surface area of the cell is fixed. To accommodate the additional volume, the cells assume a spherical shape to keep the surface area constant. The volume gain is ~25-50%. The cells can maintain their integrity up to a tonicity of ~150 million moles/kg, above which the membrane ruptures, releasing the cellular contents. Some transient pores of 200-500 Å are generated on the membrane at this point (just before cell lyses), the cellular contents are depleted after the cell lyses. The residue is called an erythrocyte ghost. The ruptured membranes can be resealed by restoring isotonic conditions are based on the principle of using these ruptured erythrocytes as a carrier. The cells resume their original biconcave shape and recover original impermeability during incubation. Glucose oxidase, glucose 6-phosphatase, anti-hexokinase IgG, ATP was entrapped successfully by above method [10].

Hypotonic dilution

Hypotonic dilution was the simplest and fastest method for the encapsulation of chemicals into erythrocytes. In this method, a 2-20 volume of packed erythrocytes is diluted with aqueous solution of a drug. A hypertonic buffer was added to restore solution tonicity. The consequential mixture is then centrifuged, the pellet is washed with isotonic buffer solution and the supernatant is discarded, the major disadvantage of this method includes low entrapment competency and a significant trouncing of hemoglobin and other cell components. The circulation half-life of the loaded cells is reduced. By RES macrophages, these cells are readily used for targeting RES organ and phagocytosed. The loading of enzymes such as ß-galactosidase and ß-glycosidase, asparagines and arginase, as well as bronchodilators such as salbutamol are done by this method [11].

Hypotonic dialysis

In this method, 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 and the slow agitation of medium is done for 2 h. By adding a calculated amount of a hypertonic buffer to the surrounding medium, the tonicity of dialysis tube is restored. At the beginning of the experiment drug is dissolved in isotonic cell suspending buffer inside a dialysis bag or by adding the drug to a dialysis bag after the stirring is complete. 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 method has been used for loading drugs such as pentamidine furamycin, interlukin-2, desferroxamine, gentamicin, adriamycin and human recombinant erythropoietin as well as enzymes such as ß-galactosidase, glucoserebrosidase, asparginase, and inositol hexaphosphatase [12].

Hypotonic preswell dilution method

This method causes minimum damage to cells, and it is very simple and faster than other methods, to encapsulate a drug in erythrocytes. It was developed by Rechsteiner in1975 and was modified by Jenner *et al*. for drug loading. The technique is based on initial controlled swelling in a hypotonic buffered solution. At low gravitational force, 100-120 µl portions of an aqueous solution of the drug to be encapsulated at the lysis point and the supernatant were discarded. The mixture is centrifuged between the drug-addition steps. The detection of lysis point by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. By adding a calculated amount of hypertonic buffer, the tonicity of a cell mixture is restored at the lyses point. To reanneal the resealed erythrocytes, the cell suspension is incubated at 37°C Such cells have a circulation half-life comparable to that of normal cells. Drugs encapsulated in erythrocytes using this method include Propranolol, levothyroxine, Metronidazole, Levothyroxine, Elaprnailat, and Isoniazid cortisol-21-phsphate, prednisolone-21-sodium, cyclophosphamide, α-1 antitrypsin, interferon alpha-2, insulin [13].

Isotonic osmotic lysis

This method is achieved by physical or chemical means and it is also known as the osmotic pulse method, Because of the concentration gradient, if erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells. To maintain osmotic equilibrium, this process follows an influx of water. For isotonic hemolysis chemicals such as urea solution, PEG, and ammonium chloride have been used. However, to changes in membrane structure composition, this method is not immune. In 1987, Franco *et al*. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide. The suspension was diluted with an isotonic-buffered drug solution. They were resealed at 37°C, after the cells were separated [14].

Chemical perturbation of the membrane

When the cells are exposed to certain chemicals, to increase its membrane permeability, this method is used. The permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin was shown in this method [15].

Entrapment by endocytosis

In this method, when the one volume of washed erythrocytes was added to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM $MgCl_2$, and 1 mM $CaCl_2$, followed by incubation for 2 minutes at room temperature. The pores were created. Resealing of pores by using 154 mM of NaCl and incubation at 37°C for 2 minutes. By endocytosis the entrapment of material occurs. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and *vice versa*. The various chemicals entrapped by this method include primaquine and related 8-amino-quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propanolol, tetracaine, and vitamin A [9,10].

Loading by electric cell fusion

Initially, loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The release of an entrapped molecule is done by the fusion by which it is accentuated by the application of an electric pulse. Loading a cell-specific monoclonal antibody into an erythrocyte ghost is the best example of this method. Chemical cross-linking of antibody to the specific surface protein of target cells to drug-loaded cells that would direct these cells to desired cells [12].

Loading by lipid fusion

Direct fusion of drug in a lipid vesicle to human erythrocytes, which lead to exchange with a lipid-entrapped drug. To improve the oxygen

carrying capacity of inositol monophosphate, this method was used. However, the efficiency of drug entrapment for this method is very low (~1%) [13].

***In vitro* evaluation of resealed erythrocyte**

Erythrocyte characterization with their quality control assays: Characterization parameters	Analytical methods/ instrumentation [16-23]
Physical characterization	
Shape and surface morphology	TEM, SEM, phase-contrast optical microscopy
Vesicle size and size distribution	TEM, optical microscopy
Drug release	Diffusion cell/dialysis
% Encapsulation	Deproteinization (using methanol or acetonitrile) of cell membrane and assay for released drug or radio-labeled markers
Electrical surface potential and surface pH	Zeta potential measurements and pH-sensitive probes
Cell related characterization	
%Hb content/volume	Deproteinization (using methanol or acetonitrile) of cell membrane and assay for Hb; laser light scattering for cell volume
Mean corpuscular Hb	Laser light scattering
% Cell recovery	Hematological analyzer; Neubaeur's chamber
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and estimation of drug and Hb
Osmotic shock	Dilution with distilled water and estimation of drug and Hb
Turbulent shock	Passing cell suspension through a 23 gauge needle, hypodermic needle (10 ml/minutes), and estimation of residual drug and Hb
ESR	ESR apparatus
Biological characterization	
Sterility	Aerobic or anaerobic cultures
Pyrogenicity	Rabbit fever response test of LAL test
Animal toxicity	Toxicity tests

TEM: Transmission electron microscopy, SEM: Scanning electron microscopy, LAL: Limulus amebocyte lysate, Hb: Hemoglobin, ESR: Erythrocyte sedimentation rate

Shape and surface morphology

The examination of the morphology of these ghost erythrocytes is undertaken to decide the lifespan after administration. The comparison of treated and untreated erythrocytes using transmission electron microscopy, scanning electron microscopy or phase-contrast optical microscopy [16].

Drug content

This method helps in the determination of the entrapment efficiency of the drug. Deprotenization of packed loaded cells (0.5 ml) with 2.0 ml acetonitrile and centrifugation at 2500 rpm for 10 minutes. The clear supernatant is analyzed for the drug content [17].

Deformability

The changes in shape (deformability) of erythrocyte are another factor that affects the life span of the cells. The ease of passage of erythrocytes through narrow capillaries and the RES are evaluated by this method. Depending on the visco elasticity of the cell membrane, viscosity of the cell contents, and the cellular surface-to-volume ratio, determines the rheological behavior of the cells and measurement of deformability was done by the passage time of definite volume of cells through capillary of 4 μm diameter or polycarbonate filter with average pore size of 45 μm [18].

In vitro drug release and hemoglobin content study

From the drug-loaded cells, *in vitro* release of drug(s) and hemoglobin are monitored occasionally. Using amber colored glass containers the cell suspension were stored at 4°C, once in a while the clear supernatant are withdrawn using a hypodermic syringes equipped with 0.45 μ filter, deprotenized using methanol and were estimated for drug content. After centrifugation the supernatant of each sample is collected and assayed, % hemoglobin release may be calculated using the formula.

$$\frac{\text{A540 of sample} - \text{A540 of background}}{100\%\text{Hbof A540}} = \%\text{Hbrelease}$$

(or)

$$\frac{\text{Hb}\left(\text{g}/100\text{ ml}\right) \times 10}{\text{Erythrocyte count}\ \left(\text{millions}/\text{cumm}\right)} = \text{Mean corpuscular Hb (MCH)}$$

Where A540 refers to absorbance at 540nm

A sustained release of the drug that influences the pharmacokinetic behavior *in vivo* of the loaded erythrocytes. leakage of the drug from loaded erythrocytes by *in vitro* is tested at 37°C with a hematocrit adjusted between 0.5% and 50% using autologous plasma or an isoosmotic buffer. The supernatant is removed and replaced by an equal volume of autologous plasma or buffer. Certain authors recommend performing *in vitro* the release studies from loaded erythrocytes using a dialysis bag. The factors that have a decisive bearing on the release profile of the active principle from the loaded erythrocytes are the molecular weight and liposolubility of the substance. By passive diffusion, liposoluble drugs may be released from the red cells. Proteins like Band 3 and glycophorin A present in high density on the extra-cellular surface of erythrocytes and which may act as potential targets for anchoring via covalent bond formation with different substances. An important role of Band 3 to act as a carrier protein for anions [19].

Entrapped magnetite study

The hydrochloric acid is added to a preset amount of magnetite-bearing erythrocytes and contents are heated at 600c for 2 hrs. Then, the addition of 20% w/v trichloroacetic acid and after centrifugation, the supernatant obtained is used to decide magnetite concentration using atomic absorption spectroscopy [20].

Shelf life, stability and cross-linking of released erythrocytes

The shelf life of the carrier erythrocytes is improved by storing the cells in powder form. It was then filled in an amber color vial. It was done by filling the erythrocytes suspension in a vial and lyophilized at −40°C to 0.01 torr using a laboratory lyophilizer [21].

Erythrocyte sedimentation rate (ESR)

The stability of erythrocyte suspension in plasma, related to the number and size of the red cells and to the relative concentration of plasma protein is estimated by the rate of sedimentation of blood cells in a standard tube especially, fibrinogen and α, β globulins. This test is performed by determining. 0-15 mm/hr is the normal blood ESR level. The ESR rate is higher indicates that it is active but vague disease processes [22].

Particle size and zeta potential analysis

Using the particle size and zeta analyzer, the Size distribution of the sample was estimated. It was set with a dry accessory system in which a drop of sample is diluted with ten times of double distilled water, and the sample is taken in cuvettes and was analyzed [23].

Osmotic fragility
A test to detect abnormal fragility of RBCs, by exposing the untreated and loaded erythrocytes to the hypotonic solutions and making them swell, so as to determine the relative fragility of the erythrocytes [24].

Osmotic shock
Diluting the Erythrocyte suspension (1 ml, 10%) with H_2O (5 ml) and centrifuge at 3000 rpm for 15 minutes. %Hb release in supernatant was estimated using spectrophotometer [25].

Turbulence shock

Stability of loaded erythrocytes was evaluated by this method. By creating the turbulence stress against the cells was exerted by the *in vivo* circulation turbulence. Through a 22-gauge needle the suspension of cells is passed several times [26].

Hemoglobin release
By altering the permeability of the erythrocyte membrane, the content of hemoglobin of the erythrocytes may be diminished throughout the encapsulation procedure. Besides, the affiliation between the rate of hemoglobin and the velocity of drug release contributes to interpreting the mechanisms involved in the release of the substance encapsulated from the erythrocytes. Using a red cell suspension the hemoglobin leakage is tested. The absorbance of the supernatant at 540 nm on a spectrophotometer was recorded [27].

Compatibility study
IR spectral analysis of pure drugs and polymers was carried out and observation was made whether changes in the chemical constitution of the drug after combining it with the polymers occurred [28].

***In vivo* evaluation of resealed erythrocytes**

Stability studies

Resealed erythrocytes were tested for stability. All the preparations were divided into three sets and were stored at 5±3°C, 30±2°C/65%+5% RH and at room temperature, in thermostatic damp control over. After 2 weeks and 1-month, drug release of the optimized formulation and the percentage of drug content were determined for all the formulations [29].

***In vivo* tissue distribution studies**
It is one of the *in vivo* study was carried out for distribution of drug loaded resealed erythrocytes to various organs of RES like, kidney and spleen liver, lungs. Nine healthy adult rats/mice weighing 200-240 g were chosen. A steady day and night cycle was maintained and they were fasted for overnight. The animals were divided into three groups, in which two groups each containing four rats and one group containing one rat and the different formulation of resealed erythrocyte was given to each rat/mice. At 1, 2, 4, and 6 h, the rats/mice were sacrificed, and their liver, lungs, spleen and kidney were isolated. Homogenization of The individual organs of each rat/mice and were digested to precipitate the protein with 1ml of 70% v/v of acetonitrile for 2h. Using 25 ml of methylene, the drug was extracted and washed with 2 ml of 0.1 N NaOH, followed by 0.1 N acetic acid as well as finally with water. The extract was evaporated to dryness and redissolved in 5 ml of methanol. The drug content was estimated using UV-spectrophotometer at 239nm. The typical amount of drug was taken throughout the study [29-34].

APPLICATION OF RESEALED ERYTHROCYTES IN DRUG DELIVERY

The *in vitro* and *in vivo* application of resealed erythrocyte in drug delivery includes:

***In vitro* application**
For *in vitro* phagocytosed cells have been used to facilitate the uptake of enzymes by phagolysosomes. RBC mediated microinjection was the most frequent *in vitro* application, by fusion process a protein or nucleic acid to be injected into eukaryotic cells. Likewise, when antibody molecules are introduced using erythrocytic carrier system, they immediately disperse throughout the cytoplasm. Antibody RBC auto injected into living cells be conventional the site of action of a fragment of diphtheria toxin [35-37].

***In vivo* application**

Slow drug release

The sustained delivery of antineoplastic, antiparasitics, veterinary antiamoebics, vitamins, steroids, antibiotics, and cardiovascular drugs was done using erythrocyte as the circulating depot [37].

Drug targeting

Preferably to exhibit maximal therapeutic index with minimum adverse effects drug delivery should be site specific and target oriented. Resealed erythrocytes not only act as drug carriers and also as targeting tool [38].

Targeting RES organs

MPS/RES are targeted by surface modified erythrocytes. Since the change in the membrane is renowned by macrophages. The various approaches used include: Surface alteration with antibodies (coating of loaded erythrocytes by anti-Rh or other types of antibodies), glutaraldehyde, sulfhydryl, surface chemical cross-linking, carbohydrates such as sialic acid [39].

Removal of toxic agents

Reserve of cyanide intoxication with murine carrier erythrocyte containing bovine rhodanese and sodium thiosulfate. Antagonization of organ phosphorus intoxication by released erythrocyte containing a recombinant phosphodiesterase has been reported [40].

Delivery of therapeutic agents

Erythrocytes have been used for release of so many therapeutic agents from several curative groups, both registered and in advance ones. As a basic approach in this review, all studied drugs will be classified into two main groups named pharmaceuticals and biopharmaceuticals [41].

Anticancer agents

Usually loading anticancer drugs into carriers reins their toxicity to the body in addition of improving their delivery to tumors relies via several mechanisms, including both specific (i.e., active targeting for example antibody targeting) and less specific (i.e., passive targeting for example enhanced permeation and retention effect, EPR, typical of solid tumors). It has been evaluated as carriers of chemotherapeutic agents for targeting the RES for about 30 years. From another point of view, carrier erythrocytes may find a niche in tumor therapy, for instance, by providing formulations with prolonged circulation [42].

Anti-infective agents

For the delivery of three main anti-infective groups including anti-parasitic, antibiotic, antifungal and anti babesiosis (in veterinary) agents carrier erythrocytes have been used [29,30,43,44].

Corticosteroids

Prolonged delivery of dexamethasone which has been tested *in vivo* in rabbits and humans is the most developed application of carrier erythrocyte. Dexamethasone is a glucocorticoid analog. Corticosteroides (glucocorticoides) are powerful though non-specific, anti-inflammatory agents which have been widely used in a variety of inflammatory disorders. Complete adverse effects are associated with both dose and duration of treatment [31].

Cardiovascular drugs

Using a hypotonic preswelling dilution method, have shown that human erythrocytes loaded by enalaprilat release the drug *in vitro* according to

zero-order kinetics. The *in vivo* results in rabbit model have indicated that the area under the ACE inhibition curve versus time over the entire course of study was appreciably greater following the administration of the erythrocyte-encapsulated drug compared with the free drug. In accumulation, encapsulated drug inhibited the serum ACE with a slow trend, more competently, over a considerably longer time and in a more reproducible manner, than the free drug, thereby emphasizing the role of carrier erythrocytes as slow-release systemic drug delivery system for this ACE inhibitor [32].

Iron chelators

For treatment of iron over accumulation in the thalasemic patients and other forms of anemia that require regular transfusions for that carrier erythrocytes, encapsulated with desferrioxamin (DF), have been studied widely As discussed above, the RES is the main site of destruction of old erythrocytes and, consequently, of iron over-accumulation in these patients. A remarkable degree of targeting DF to RES using carrier erythrocytes has been reported [33].

Therapeutic peptides and proteins

A summary of these amino acid-based biopharmaceuticals (i.e., therapeutic peptides and proteins) that have been delivered using carrier erythrocytes [34,45,46].

Therapeutic enzymes

Unfortunately, some serious problems dispirit the direct injection of the enzymes into blood circulation, including the tiny plasma half-lives of enzymes, toxicity for some tissues, occurrence of some immunologic and allergic reactions, and the need for multiple injections that in turn has the risk of injection-related problems.

Various therapeutic enzymes that have been delivered using erythrocytes are Amyloid β-degrading peptidases, adenosine deaminase and pegademase, alcohol dehydrogenase and aldehyde dehydrogenase, alcohol oxidase, alglucerase, arginase, beta-glucocerebrosidase, brinase, catalase and PEG-catalase, delta-aminolevulinate dehydratase, glutamate dehydrogenase, glutamine synthetase, hexokinase and glucose oxidase, lactate-catabolizing enzymes, l-asparaginase, phosphptriesterase, rhodanase (thiosulfate: cyanate transferase) [47-49].

ANTIGEN DELIVERY FOR VACCINATION [26,50-53]

Aside from the general advantages of erythrocytes for using them as a suitable drug delivery system, they have three other remarkable potentials for being used as a vaccine delivery system including:

1. Used for controlled release of vaccines with an aim to reduce the number of doses for primary immunization or to develop single dose vaccines;
2. They can act as a vehicle to target antigen to antigen-presenting cells, not only macrophages but also dendritic cells (DCs). Depending on the extent of changes occurred in cell physiology and/or morphology during antigen loading procedure, one can prepare erythrocytes loaded by antigens and be capable of serving as controlled antigen release (in the case of minor cell modifications) or antigen targeting (in the case of major cell modifications) vehicles;
3. The intrinsic adjuvancy of loaded erythrocytes due to loading procedure.

CONCLUSION

This review focuses on appropriateness of erythrocytes as biological carriers. It shows that some of the various latent biomedical applications of RBCs based drug delivery systems opening new perspectives to the vision of using our cells for salutary purposes. Thus, the resealed erythrocyte is the promising carrier for various drugs, therapeutic proteins, a vaccine for both targeting and delivery. However, the concept needs further optimization to become a custom drug delivery system. Most of the studies in this area are in the *in vitro* phase and the ongoing projects worldwide stay behind to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

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