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RESEALED ERYTHROCYTES AS A NOVEL CARRIER FOR DRUG DELIVERY: A REVIEW

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ABSTRACT: Drug delivery systems including chemical, physical and biological agents that can enhance the bioavailability, improve pharmacokinetics and reduce toxicities of the drugs. Carrier erythrocytes are one of the most promising biological drug delivery systems investigated in recent decades. Resealed Erythrocytes are biocompatible, biodegradable, possess long circulation half-life and can be loaded with variety of active drug substances. Resealed erythrocyte has got several advantages over the other drug delivery system which makes it superior than other systems. Carrier erythrocytes are prepared by collecting blood sample from the organism of interest and separating erythrocytes from plasma. By using various methods, the cells are broken and the drug is entrapped into the erythrocytes, finally they are resealed and the resultant carriers are then called "resealed erythrocytes". Resealed erythrocytes, as a drug delivery system has excellent capacity to enhance the therapeutic index and patient compliance. It has got tremendous potential to achieve site specific drug delivery with least wastage of drugs and it also prolong the release of drug. So many drugs like aspirin, steroid, cancer drug which having many side effects are reduce by resealed erythrocyte. The present review signifies various features, drug loading methods and applications of resealed erythrocytes.

INTRODUCTION: To achieve a required therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a "perfect" drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments.

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The delivery systems currently available enlist carriers that are either simple, soluble macromolecules or more complex multicomponent structures (microcapsules, Microparticles, cells, cell ghosts, lipoproteins, liposomes, erythrocytes) [1].

Erythrocytes are biocompatible, biodegradable, possess very long circulation half-lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods [2].

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 [3].

Targeting of an active biomolecule 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 nanoparticle, micro-spheres, lipid vesicular carrier, microemulsion, aquasomes, pharmacosomes, ethosomes, cellular carrier and macromolecule [4].

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 [5].

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 [6].

Erythrocytes: Red blood cells (also referred to as erythrocytes) are the most common type of blood cells and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system.

They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. These cells' cytoplasm is rich in hemoglobin, an iron-containing bimolecule that can bind oxygen and is responsible for the blood's red color [7].

Resealed Erythrocytes: 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 [8].

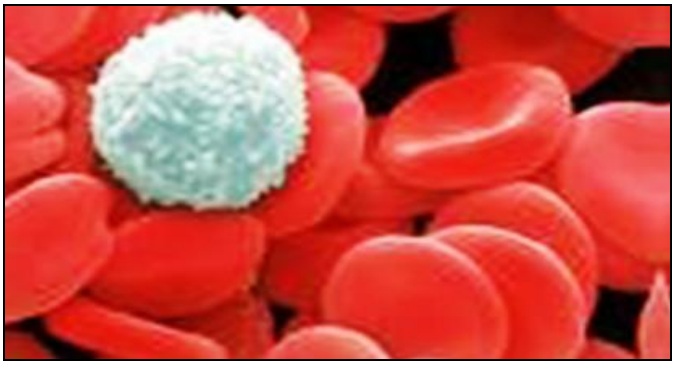

FIG. 1: STRUCTURE OF RESEALED ERYTHROCYTE

Source and isolation of Erythrocytes: Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. To isolate erythrocytes, blood is collected in heparinized tubes by venipuncture. Fresh whole blood is typically used for loading purposes because the encapsulation efficiency of the erythrocytes isolated from fresh blood is higher than that of the aged blood.

Fresh whole blood is the blood that is collected and immediately chilled to 4°C and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed cells are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid-citrate-dextrose buffer at 4°C for as long as 48 h before use [9].

FIG. 2: ISOLATED RBCs

Composition of Erythrocytes [10]:

- Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements.
- Normal blood cells have extensile, elastic, biconcave and non-nucleated configuration with a diameter ranging from 6-9 μ and the thickness is nearly 1-2 μ.

- Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water.

Electrolyte composition of Erythrocytes [11]:

- The concentration of K^+ is more in erythrocytes and Na^+ in plasma.
- The osmotic pressure of the interior of the erythrocytes is equal to that of the plasma and termed as isotonic (0.9% NaCl or normal physiological saline.)
- Changes in the osmotic pressure of the medium surrounding the red blood cells changes the morphology of the cells.
- If the medium is Hypotonic water diffuses into the cells and they get swelled and eventually lose all their hemoglobin content and may burst.
- And if the medium is hypertonic, (i.e. higher osmotic pressure than 0.9% NaCl) they will shrink and become irregular in shape.
- Balanced ion solutions like Ringer's and Tyrode's soln. which are not only isotonic but also contain ions in proper quantity are used in erythrocyte related experiments.

Properties of Resealed Erythrocyte as Novel Drug Delivery Carriers [12]:

1) The drug should be released at target site in a controlled manner.
2) It should be biocompatible and should have minimum toxic effect.
3) It should possess specific physicochemical properties by which desired target size could be recognized.
4) The degradation product of the carriers system, after release of the drug at the selected site should be biocompatible. It should be physico-chemically compatible with drug.
5) The carrier system should have an appreciable stability during storage.

Advantages of Resealed Erythrocytes as Drug Carriers: The resealed erythrocytes should have the following advantages.

1. Their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response [13].
2. Their biodegradability with no generation of toxic products [14].
3. The considerably uniform size and shape of the carrier [15].
4. Relatively inert intracellular environment [16].
5. Prevention of degradation of the loaded drug from inactivation by endogenous chemicals [17].
6. The wide variety of chemicals that can be entrapped [18].
7. The modification of pharmacokinetic and pharmacodynamic parameters of drug [19].
8. Attainment of steady-state plasma concentration decreases fluctuations in concentration [20].
9. Protection of the organism against toxic effects of drugs (e.g. antineoplastics) [21].
10. The prevention of any undesired immune response against the loaded drug [22].
11. Their ability to target the organs of the RES [23].
12. The possibility of ideal zero-order drug-release kinetics [24].
13. The lack of occurrence of undesired immune response against encapsulated drug [25].
14. The large quantity of drug that can be encapsulated within a small volume of cells ensures dose sufficiency [26].
15. Easy control during life span ranging from minutes to months [27].
16. A decrease in side effects of drugs [28].
17. A considerable increase in drug dosing interval with drug residing in therapeutic window region for longer time periods [29].

Disadvantages:

1) They have a limited potential as carrier to non-phagocyte target tissue.

2) Possibility of clumping of cells and dose dumping may be there [14].

Methods 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 [30].

The several methods are giving in follows:-

- Hypotonic hemolysis method
- Use of red cell loader method
- Hypotonic dilution method
- Hypotonic preswelling
- Hypotonic dialysis
- Isotonic osmotic lysis
- Chemical perturbation of the membrane
- Electro-insertion or electro encapsulation
- Entrapment by endocytosis

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 mos m/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* [31].

The principle of using these ruptured erythrocytes as drug carriers is based on the fact that the ruptured membranes can be resealed by restoring isotonic conditions. Upon incubation, the cells resume their original biconcave shape and recover original impermeability [32].

Use of Red Cell Loader: Novel method was developed for entrapment of no diffusible drugs into erythrocytes. They developed a piece of equipment called a "red cell loader" [33]. With as little as 50 ml of a blood sample; different biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells.

Hypotonic Dilution: Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes [14] and is the simplest and fastest [34]. 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. The major drawbacks of this method include low entrapment efficiency [35].

Hypotonic Preswelling: This method was developed by Rechsteiner [36] in 1975 and was modified by Jenner *et al*, for drug loading. The technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100-120 liters portions of an aqueous solution of

the drug to be encapsulated. The mixture is centrifuged between the drug-addition steps. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer.

Then, the cell suspension is incubated at 37^{o}C to reanneal the resealed erythrocytes [27, 31]. Such cells have a circulation half-life comparable to that of normal cells. This method is simpler and faster than other methods, causing minimum damage to cells [37].

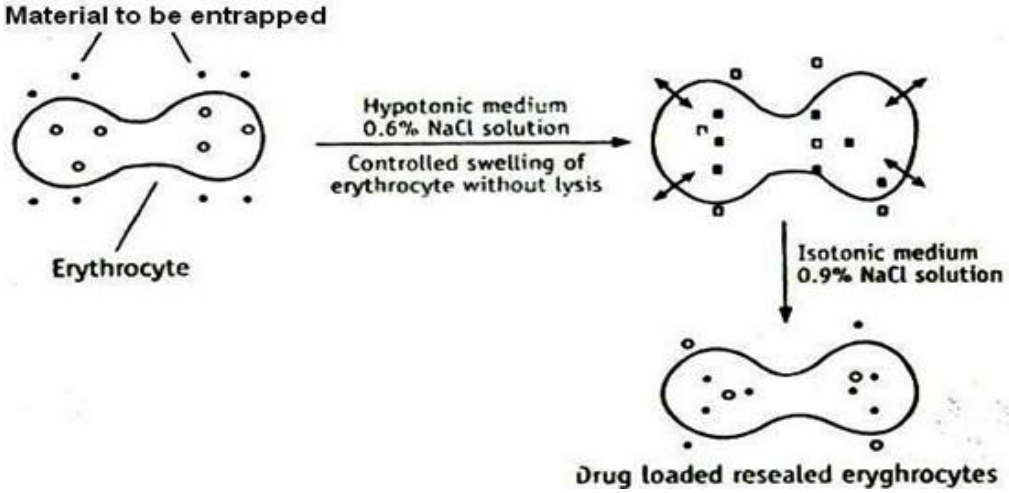

FIG. 3: DRUG LOADED IN ERYTHROCYTE BY HYPOTONIC PRESWELLING METHOD

Hypotonic Dialysis: Several methods are based on the principle that semi permeable 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 [38].

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 [39] or by adding the drug to a dialysis bag after the stirring is complete. The use of standard hemodialysis equipment for loading a drug in erythrocytes was reported by Roper *et al* [40].

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 other researchers [41].

The loaded cells exhibit the same circulation half-life as that of normal cells Also, this method has high entrapment efficiency on the order of 30-50% cell recovery of 70-80%, high-loading capacity [42] and is amenable to automation with control of process variables. The lacks include a long processing time and the need for special equipment [27]. This method has been used for loading enzymes such as galactosidase, glucoserebrosidase, asparginase, inositol hexaphosphatase, as well as drugs such as gentamicin adriamycin, pentamidine and furamycin, interlukin-2, desferroxamine and human recombinant erythropoietin [43].

Isotonic Osmotic Lysis: This method, also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isoionic. 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 [6], polyethylene glycol [44] and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco *et al*., developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO) [45].

The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C.

Chemical Perturbation of the Membrane: This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. Permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B [46]. In 1980, this method was used successfully to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes [47]. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

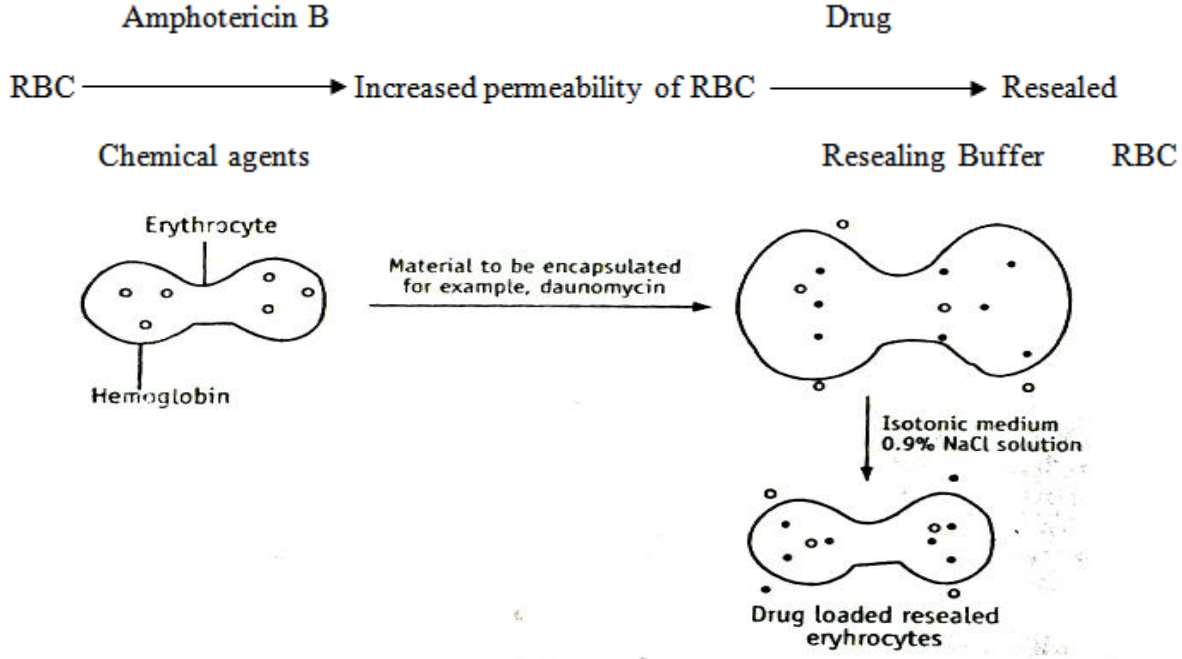

FIG. 4: RESEALING OF RBC BY CHEMICAL PERTURBATION OF THE MEMBRANE METHOD

Electro-Insertion or Electro Encapsulation: In 1973, Zimmermann tried an electrical pulse method to encapsulate bioactive molecules [21]. Also known as electroporation, the method is based on the observation that electrical shock brings about irreversible changes in an erythrocyte membrane. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading [32]. The erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium.

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 [32, 34]. The optimum intensity of an electric field is between 1–10 kW/cm and optimal discharge time is between 20–160 [48].

An inverse relationship exists between the electric-field intensity and the discharge time [32, 34]. The compound to be entrapped is added to the medium in which the cells are suspended from the commencement of the experiment. 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 [49].

The colloidal macromolecules contents of the cell may lead to cell lysis because of the increase in osmotic pressure. This process can be prevented by adding large molecules (e.g., tetrasaccharide stachyose and bovine serum albumin) and ribonucleose. One advantage of this method is a more uniform distribution of loaded cells in comparison with osmotic methods. The main drawbacks are the need for special instrumentation and the sophistication of the process. Entrapment efficiency of this method is 35%, and the life span of the resealed cells in circulation is comparable with that of normal cells.

Various compounds such as sucrose, urease, methotrexate, isoniazid [50], human glycophorin, DNA fragments, and latex particles of diameter 0.2 m can be entrapped within erythrocytes by this method. Mangal and Kaur achieved sustained release of a drug entrapped in erythrocytes with the use of electroporation [51].

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 $MgCl_2$, and 1mM $CaCl_2$, followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mm of NaCl and incubation at 37^oC 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 [52, 53].

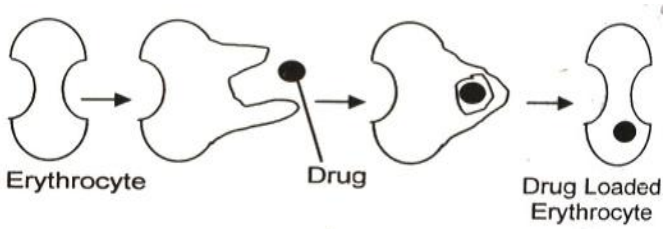

FIG. 5: DRUG ENTRAPMENT BY ENDOCYTOSIS METHOD

Morphology & Physiology of Erythrocytes: The red blood cell membrane is dynamic, semi-permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cations (Na^+, K^+) and anions (Cl^-, HCO_3^-) [54]. Each RBC contains about 280 million hemoglobin molecules.

A hemoglobin molecules consists of a protein called globin, composed of four polypeptide chains; a ring like non-protein pigment called a heme, is bound to each of the four chains. At the center of the heme ring combine reversibly with one oxygen molecule, allowing each hemoglobin molecule to bind four oxygen molecules.

RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), methehemoglobin (0.5%) and hemoglobin (33.67%). Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm^3 blood in a healthy male and 4.8 million cells/mm3 blood 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 hemoglobin and its crucial role in oxygen delivery to various parts of the body.

The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries. Mature erythrocytes are quite simple in structure. They lack a nucleus and other organelles. Their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O_2–CO_2 binding inside the erythrocytes. The main role of erythrocytes is the transport of O_2 from the lungs to tissues and the CO_2 produced in tissues back to lungs. Thus, erythrocytes are a highly specialized O_2 carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O_2 transport.

Also, because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying. Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from circulation and destroyed in the spleen and liver (RES), and the breakdown products are recycled. The process of erythrocyte formation within the body is known as *erythropoiesis.*

In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called *erythropoietin* [55].

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 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 [56].

Morphological Aspect: The morphological characterization of erythrocytes is undertaken by comparison with untreated erythrocytes using either transmission (TEM) or Scanning Electron Microscopy (SEM). These techniques are done to detect morphological changes in the erythrocytes induced by encapsulation methods. Thus, when erythrocytes are subjected to isotonic solutions (300 mosm kg^{-1}) they reveal the typical morphology of discocyte (biconcave). This evolves to a morphology of stomatocyte (uniconcave) when they are subjected to solutions of 200 mosm kg^{-1}, attaining the spherocytic shape (the most fragile of the three) when the solution is of 150 mosm kg^{-1} [57].

Osmotic Behavior: This test is done to detect the effect of loading process on the fragility of red blood cells to check the status of erythrocytes' membrane. Unloaded and loaded erythrocytes are tested by exposure to different concentration of sodium chloride, making them swell, in order to determine the relative fragility of the red cells Turbulence shock [58].

This test is done to evaluate the stability of the loaded erythrocytes against the turbulence stress exerted by the cells against *in vivo* circulation turbulence. Packed erythrocytes are suspended in 10 mL of PBS in polypropylene test tubes and are shaken vigorously using a multiple test tubes orbital shaker at 2000 rpm for 4 h. To determine the time course of hemoglobin release, 0.5 mL portions of each suspension were withdrawn at 0, 0.5, 1, 2 and 4 h elapsed and after centrifuging at 1000 rpm for 10 min. The absorbances of the supernatants are determined spectrophotometrically at 540 nm.

The percent of hemoglobin release is determined in reference to a completely lysed cell suspension with the same cell fraction (i.e., 0.5 mL packed cells added to 10 mL of distilled water). To compare the turbulence fragilities of the different types of erythrocytes, a turbulence fragility index is defined as the shaking time producing 20% hemoglobin release from erythrocytes [59].

***In-vitro* Drug Release:** The drug loading may produce sustained release of the drug that influences the pharmacokinetic behavior in vivo of the loaded erythrocytes. *In vitro* leakage of the drug from loaded erythrocytes is tested using autologous plasma or an iso-osmotic buffer at 37°C with a hematocrit adjusted between 0.5 and 50%. The supernatant is removed at the time intervals previously programmed and replaced by an equal volume of autologous plasma or buffer [60].

Some authors recommend performing *in vitro* the release studies from loaded erythrocytes using a dialysis bag. The drug release is controlled by molecular weight and liposolubility of the drug. Lipophilic drugs may be released from the red cells by a mechanism of passive diffusion, while hydrophilic drugs need cell lysis to be released [61].

Hemoglobin Release: The content of hemoglobin of the erythrocytes may be diminished by the alterations in the permeability of the membrane of the red cells during the encapsulation procedure. Furthermore, the relationship between the rate of hemoglobin and the rate of drug release contributes to interpreting the mechanisms involved in the release of the substance encapsulated from the erythrocytes. The hemoglobin leakage is tested using a red cell suspension by recording the absorbance of supernatant at 540 nm on a spectrophotometer.

Biological Characterization: Biological characterization of the developed erythrocytes includes sterility test, pyrogenicity test and toxicity tests.

Drug Content Determination: Packed loaded cells are deproteinized with acetonitrile after centrifugation at 3000 rpm far a fixed time interval. The clear supernatant liquid is assayed far drug content.

Turbulence Shock: It is the measure of simulating distribution of loaded cells during injection. In this, drug loaded cells are passed through a 23 gauge hypodermic at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collecting of an aliquot and centrifugation sample is estimated. Drug loaded erythrocytes appears to be less resistant to turbulence, probably indicating destruction of cells upon shaking [62].

Route of Administration: Intra peritoneal injection reported that survival of cells in circulation was equivalent to the cells administered by i.v. injection. They reported that 25% of resealed cell remained in circulation for 14 days they also proposed this method of injection as a

method for extra vascular targeting of RBCs to peritoneal macrophages. Sub cutaneous route for slow release of entrapped agents. They reported that the loaded cell released encapsulated molecules at the injection site [63].

***In-vitro* Storage as Carrier Erythrocytes:** Preparing drug-loaded erythrocytes on a large scale and maintaining their survival and drug content can be achieved by using suitable storage methods. The most common storage media include Hank's balanced salt solution and acid-citrate-dextrose at 4°C. Cells remain viable in terms of their physiologic and carrier characteristics for at least 2 weeks at this temperature. The addition of calcium-chelating agents or the purine nucleosides improve circulation survival time of cells upon reinjection [64].

Safety Consideration in Carrier Erythrocytes: The use of erythrocytes as a drug carrier in human has the inherited problems of transfusion of blood from one to another. If two different blood types are mixed together, the blood cells may begin to clump together in the blood vessels, causing a potentially fatal situation. Therefore, it is important to identify the blood type of the acceptor and the type of erythrocyte carrier to minimize mismatching before the administration of drug-loaded erythrocytes takes place. Another inherited problem is the risk of transmitting diseases. Therefore, screening of this carrier for the absence of diseases is important to eliminate any risk of contamination.

Utilization of erythrocyte as a drug carrier raises another potential concern due to the changes in their biochemical nature. In some instances such changes created therapeutic benefits whereas in other cases they yielded unwanted results. For example, conducted a study on erythrocytes loaded with enalaprilat [43].

The process produced erythrocytes that were more rigid, less deformed and more therapeutically efficacious than unloaded erythrocytes. The modification of erythrocytes with proteins such as streptavidin, however, elicited some negative results. The attachment of streptavidin to biotinylated red blood cells caused these cells to be lysed, rapidly cleared from the circulation thereby reducing their biocompatibility [48].

In vivo studies involving humans and animals have also been conducted on biotinylated red blood cells.

Extensive biotinylation severely altered the biocompatibility of these cells causing rapid elimination whereas moderate biotinylation generated stable erythrocytes that circulated for several hours [65].

Applications of Resealed Erythrocytes: Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cells 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. In a few clinical studies, successful results were obtained [66, 67].

Slow Drug Release: Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics [68], antiparasitics, veterinary, anti-amoebics, vitamins, steroids, antibiotics and cardiovascular drugs [69].

The various mechanisms proposed for drug release include:

- Passive diffusion
- Specialized membrane associated carrier transport
- Phagocytosis of resealed cells by macrophages of RES, subsequent accumulation of drug into the macrophage interior, followed by slow release [16, 22].

Routes of administration include intravenous, which is the most common, followed by subcutaneous, intraperitoneal, intranasal, and oral. Studies regarding the improved efficacy of various drugs given in this form in animal models have been published. Examples include an enhancement in anti-inflammatory effect of corticosteroids in experimentally inflamed rats [30, 36], increase in half-life of isoniazid [29], levothyroxine, cytosine arabinoside [70], prolongation of plasma half-life of erythropoietin from 30 min to 35 h in mice [71], and can increase in mean survival time of mice with experimental hepatoma after injecting methotrexate loaded erythrocytes [72].

Thalasemic patients, because of multiple blood transfusions, are prone to hemosydrosis, a disease state associated with an excess storage of iron. This state is treated using SC or IV injections of iron-chelating compound desferrioxamine, which causes severe adverse effects in case of multiple injections. This agent was loaded on to erythrocytes and the performance of these cells upon reinjection was observed and found to be promising [67, 68].

This therapeutic method is approved in the United States as regular management tool of hemosydrosis since 1984 [20].

Drug Targeting: Ideally, drug delivery should be site-specific and target-oriented to exhibit maximal therapeutic index with minimum adverse effects. Resealed erythrocytes can act as drug carriers and targeting tools as well. Surface-modified erythrocytes are used to target organs of mononuclear phagocytic system/ reticulo-endothelial system because the changes in the membrane are recognized by macrophages [73].

Targeting Reticulo Endothelial System (RES) Organs: Damaged erythrocytes are rapidly cleared from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. The various approaches to modify the surface characteristics of erythrocytes include

- Surface modification with antibodies
- Surface modification with gluteraldehyde
- Surface modification with carbohydrates such as sialic acid [74].
- Surface modification with sulphydryl
- Surface chemical cross-linking e.g. delivery of 125I-labeled carbonic anhydrase loaded in erythrocytes cross-linked with *bis* (sulfosuccinimidyl) suberate and 3, 3 -dithio (sulfosuccinmidyl propionate) [75].

Targeting the Liver Enzyme Deficiency/ Replacement Therapy: Many metabolic disorders related to deficient or missing enzymes can be treated by injecting these enzymes. However, the problems of exogenous enzyme therapy include a shorter circulation half-life of enzymes, allergic reactions, and toxic manifestations. These problems can be successfully overcome by administering the enzymes as resealed erythrocytes.

The enzymes used include-glucosidase, gluco-ronidase, galactosidase. The disease caused by an accumulation of glucocerebrosides in the liver and spleen can be treated by glucocerebrosidase- loaded erythrocytes [76].

Treatment of Hepatic Tumors: Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate, bleomycin, asparginase, and adriamycin [74] 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 [75] as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver [76].

Removal of Reticuloendothelial System (RES) Iron overload: Desferrioxamine-loaded erythro-cytes have been used to treat excess iron accumulated because of multiple transfusions to thalassemic patients [18]. Targeting this drug to the RES is very beneficial because the aged erythrocytes are destroyed in RES organs, which results in an accumulation of iron in these organs.

Removal of Toxic Agents: Cannon et al. reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulfate. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phospho-diestrase also has been reported [77].

Delivery of Antiviral Agents: Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting [39]. Because most antiviral drugs are 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 [39], recombinant herpes simplex virus type 1 (HSV-1) glycoprotein B, azidothymidine derivatives, azathioprene, acyclovir [40], and fludarabine phosphate [78].

Enzyme Therapy: Enzymes are widely used in clinical practice as replacement therapies to treat diseases associated with their deficiency (e.g., Gaucher's disease, galactosuria), degradation of toxic compounds secondary to some kind of poisoning (cyanide, organophosphorus), and as drugs [45]. The problems involved in the direct injection of enzymes into the body have been cited. One method to overcome these problems is the use of enzyme-loaded erythrocytes [15, 33].

These cells then release enzymes into circulation upon hemolysis [33, 35] act as a "circulating bioreactors" in which substrates enter into the cell, interact with enzymes, and generate products or accumulate enzymes in RES upon hemolysis [15, 17, 41] for future catalysis.

The first report of successful clinical trials of the resealed erythrocytes loaded with enzymes for replacement therapy is that of glucoserebrosidase for the treatment of Gaucher's disease [19]. The disease is characterized by inborn deficiency of lysosomal-glucoserebrosidase in cells of RES thereby leading to accumulation of gluco-serebrosides in macrophages of the RES. The most important application of resealed erythrocytes in enzyme therapy is that of asparginase loading for the treatment of pediatric neoplasms. This enzyme degrades aspargine, an amino acid vital for cells. This treatment prevents remission of pediatric acute lymphocytic leukemia [71].

Improvement in Oxygen Delivery to Tissues: Hemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. 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 [11].

CONCLUSION: The use of resealed erythrocytes looks promising for a safe and sure delivery of various drugs for passive and active 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. During the past decade, numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. Until other carrier systems come of age, resealed erythrocytes technology will remain an active arena for the further research.

The commercial medical applications of carrier erythrocytes are currently being tested by a recently formed company that is developing products for human use. The coming years represent 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 disease management.

The International Society for the use of Resealed Erythrocytes (ISURE) through its biannual meetings provides an excellent forum for exchange of information to the scientist in this exciting and rewarding field of research.

CONCLUSION: For the present, it is concluded that erythrocyte carriers are "golden eggs in novel drug delivery systems" considering their tremendous potential.

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