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Resealed Erythrocytes: A Comprehensive Review

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Abstract **Review Article**

Among the many carriers that are turned down for administering medications to various body nerves, cellular carriers must meet a number of requirements for clinical use, the most crucial of which is the carrier's biocompatibility with its degradation products. Leucocytes, platelets, erythrocytes, Nano erythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier structures. Among these, the erythrocytes have been the most explored and have found to own better potential in drug delivery. Bio-pharmaceuticals, intensely significant peptides and proteins, antigens, anticancer drug as well as vaccines, are among the freshly attentive pharmaceuticals for being transported using carrier erythrocytes. Vaccines, antigens, and anticancer drugs are among the pharmaceuticals that have recently been targeted for delivery via carrier erythrocytes. Red blood cells, or erythrocytes, have been studied in great detail because of their possible use as drug delivery vehicles. They are special and helpful carriers because of their biocompatibility, nonpathogenicity, nonimmunogenicity, and biodegradability. To prepare carrier erythrocytes, draw blood from the target organism and separate the erythrocytes from the plasma. Through a variety of techniques, the drug is entrapped within the broken cells, resulting in the erythrocytes being resealed and referred to as "resealed erythrocytes"—the carriers of the result. Many medications, such as aspirin, steroids, Resealed erythrocytes reduce the side effects of cancer drugs, which are numerous. The current review focuses on the isolation, drug loading techniques, evaluation techniques, and drug delivery applications of sealed erythrocytes.

Keywords: Erythrocytes, Resealed Erythrocytes, Application, Carrier.

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INTRODUCTION

Blood contains different type of cells like erythrocytes (RBC), leucocytes (WBC) and platelets, among them erythrocytes are the most interesting carrier and posses great potential in drug delivery due to their ability to circulate throughout the body, zero order kinetics, reproducibility and ease of preparation [1] primary aim for the development of this drug delivery system is to maximize therapeutic performance, reducing undesirable side effects of drug as well as increase patient compliance [2]. The use of erythrocytes as potential site-targeted delivery systems or slow drug release for a range of bioactive substances from various therapeutic domains has gained [3]. These days, our goal is to create drug delivery systems with high therapeutic benefits and improved drug targeting for the safe and efficient treatment of illnesses. Drug targeting can be accomplished through chemical modification or by using suitable transporter. There are many drug delivery carriers has been investigated presently like nonoparticle, microspheres, lipid vesicular carrier, microemulsion, pharmacosomes, ethosomes, cellular carrier and macromolecule. Many carriers have been used to target drugs, but cellular carriers have the most potential benefits in terms of their biodegradability, biocompatibility, self-degradability, and high drug loading ability [4, 5]. The targeted or site-specific drug delivery is a very promising goal because it provides one of the most effective ways to improve the therapeutic index (TI) of drug deavoiding its potential interaction with non-targeted tissue.

Erythrocytes

Erythrocytes, also known as red blood cells, have been extensively studied for their potential capabilities for the delivery of drugs and drug-loaded microspheres. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately quarter of the cells in the human body are red blood cells

[6]. The most common type of blood cells, red blood cells are essential to vertebrate organisms because they carry oxygen (O_2) to body tissues through blood flow via the circulatory system. As they squeeze through the body's capillaries, they take up oxygen in their lungs or gills and release it. Haemoglobin, an iron-containing bio molecule that can bind oxygen and give blood its red colour, is abundant in the cytoplasm of these cells. Human developed red blood cells are biconcave, flexible disks without a cell nucleus. Most organelles every second, 2.4 million fresh erythrocytes are created [7]. Simply draw blood from the organism of interest, separate the erythrocytes from the plasma, entrap the drug in the erythrocytes, and then seal the resulting cellular carriers to create drug-loaded carrier erythrocytes. These carriers are therefore known as resealed erythrocytes. The way these cells react to osmotic conditions determines the overall process. The drug-loaded erythrocytes act as slow-circulating depots upon reinjection, directing the drugs toward the reticular endothelial system [8-10].

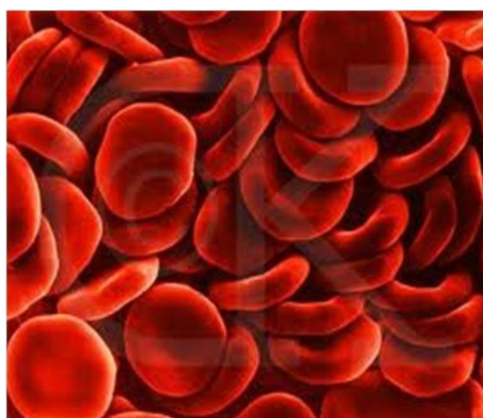

Figure 1: Erythrocytes

Advantage

1) Avoidance of the loaded drug's degradation due to endogenous chemical inactivation.
2) Considerable consistency in the carrier's size and form.
3) Isolation is easy and large amount of drug can be loaded.
4) Biocompatible; therefore, there is no chance of an immune reaction being triggered, especially when autologous cells are used.
5) Biodegradability with no generation of toxic products.
6) Considerable uniform size and shape of carrier.
7) It is possible to contain a relatively inert intracellular environment in a tiny volume of cells.
8) It is feasible to keep the plasma concentration in a steady state and reduce concentration fluctuations.
9) Protection of the organism against toxic effect of drug
10) The prevention of any undesired immune response against the loaded drug.
11) Extend the drug's systemic action by keeping it in the body for a longer period of time.
12) Ideal zero-order drug release kinetic [11-18].

Disadvantage

1) Possibility of clumping of cells and dose dumping may be there.
2) The storage of the loaded erythrocytes is a further problem provided that there are viable cells and need to survive in circulation for a long time upon re-entry to the host body.
3) The main problem with this drug carrier is that they remove in vivo by RES, which limits their usefulness as drug carriers and in some cases it may cause toxicological problems [19- 21].

RESEALED ERYTHROCYTES

Resealed erythrocytes (RBCs) are utilized in parental control release formulations for their potential carrier and delivery capabilities of drug-loaded microspheres [22]. Drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the organism oftenest, separating erythrocytes from plasma, entrapping drug in the erythrocytes resealing the resultant cellular carriers. Hence, these carriers are called resealed erythrocytes. Resealed erythrocytes are the part of parental control release formulation, RBCs have been used extensively studies for their potential carrier & capability for delivery of drug loaded microsphere [23]. In this carrier erythrocytes are prepared and the blood sample is collect from the organism of interest, the erythrocytes are separating from the plasma, then the entrapping drug in the erythrocytes & resealing the cellular carriers. Hence the overall process is based on the response of these cells under osmotic condition. Through the process of reinjection, the drug loaded erythrocytes provide slow circulating depots & target the drug t o a disease tissue organ [24].

Physiology and Morphology of Erythrocytes

Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm3 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. Hope Seyler discovered hemoglobin and its vital function in delivering oxygen to different body parts in the 1800s [25]. RBCs resemble biconcave discs with a thickness of about 2.2 µm and a diameter of 7.8 µm**.** Mature RBCs have a simple structure. It is also in elastic in nature [2]. The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3 m wide. Mature erythrocytes are quite simple in structure. Since mature RBCs lack a nucleus, they are highly specialized for their oxygen transport function. They have Sample internal space for the transportation of oxygen. 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 O2 transport. Also, because mitochondria are absent and because energy is generated an aerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying [26]. Because mature red blood cells lack a nucleus and all of their internal space is available for oxygen transport, red blood cells are highly specialized for oxygen transport. Even the RBC's shape fulfills its purpose. Better still, a biconcave disc has a surface area compared to, say, a sphere or a cube for the diffusion of gas molecules into and out of the RBC. The red blood cell membrane, a dynamic, semi permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cation (Na+, K++) and anions (ClHCO3) [27]. Each RBC contains about 280 million hemoglobin in molecules. A ring-shaped non-protein pigment known as a heme is attached to each of the four polypeptide chains that make up the protein known as globin, which makes up an entire molecule of hemoglobin. One oxygen molecule is combined reversibly at the center of the heme ring permitting four oxygen molecules to be bound by each hemoglobin molecule. RBCs are composed of 63% water, 0.5 lipids, 0.8 percent glucose, 0.7 percent mineral, 0.9 percent non-hemoglobin protein, 0.5 percent meth hemoglobin, and 33.67% hemoglobin [28].

Isolation of Erythrocytes

- By venipuncture, blood is drawn into heparin-zed tubes.
- Using a syringe, blood is drawn from a cardiac or splenic puncture in small animals and from veins in large animals with a droplet of anticoagulant inside.
- The whole blood is centrifuged at 2500 rpm for 5 min. at 4 ±1 C in a refrigerated centrifuge.
- After carefully removing the Buffy coats and serum, the packed cells are thoroughly cleaned three times using phosphate buffer saline pH is 7.4.
- The washed erythrocytes are diluted with PBS and stored at 4o C until used**.**
- Many kinds of mammalian erythrocytes, such as those from mice, cattle, pigs, and other animals, have been utilized to deliver drugs dogs, rats, rabbits, chickens, sheep, goats, and monkeys.
- To isolate erythrocytes, blood is collected in heparinized tubes by venipunture because the erythrocytes isolated from fresh blood have higher encapsulation efficiency than those from aged blood, fresh whole blood is usually used for loading purposes.
- Fresh whole blood is collected and immediately chilled to 4 C and stored for less than two days.
- After that, the erythrocytes are collected and centrifuged to clean them. The cleaned cells float in a solutions are frequently kept in acid-citrate-dextrose buffer at 4 0C for up to 48 hours prior to use and can be used at different hematocrit values as needed.
- Jain and Vyas have described a well-established protocol for the isolation of erythrocytes.
- Adenosine triphosphate (ATP) can be obtained from erythrocyte ghost by entangling dextrin (molecular weight 10–250 kDa) and medication loading in erythrocytes were documented independently.[17,29]

Method of Drug Loading in Resealed Erythrocytes

Drugs or other bioactive substances can be loaded into erythrocytes using a variety of techniques, such as physical (e.g., electrical osmosis-based systems, chemical methods (such as chemically perturbing the erythrocyte membrane), and pulse method 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 in contrast to degradation in erythrocytes, lack of physical or chemical interaction with erythrocyte membrane, and precise pharmacokinetic and pharmacodynamic properties [30]. The several methods for loading drug in erythrocytes are giving in follows:

1) Hypo-osmotic lysis
   a. Dilution method
   b. Pre swelling method
   c. Dialysis method
   d. Osmotic lysis method
2) Membrane perturbation method
3) Electro encapsulation method
4) Endocytosis method
5) Lipid fusion method
6) Electric cell fusion method

1. Hypo - Osmosis Lysis Method

The internal and extracellular solute of erythrocytes is exchanged during this step by osmotic lysis and re sealing that drug inside the RBCs will be enclosed by this method [31]. These are divided into four types are as follows-

a. Dilution method
b. Pre swelling method
c. Dialysis method
d. Osmotic lysis method

a) Hypertonic Dilution Method

The simplest and fastest way to encapsulate chemicals into erythrocytes is by hypotonic dilution, which was the initial approach investigated. This method involves diluting a volume of packed erythrocytes with two to twenty volumes of an aqueous drug solution. Next, the tonicity of the solution is restored by a hypertonic buffer is added. a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution tonicity is then restored by addition a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is cast-off, and the pellet is washed by means of isotonic buffer solution. The major drawbacks of this technique include low

entrapment efficacy and a considerable loss of hemoglobin and other cell components. This reduces the circulation half-life of the loaded cells.[32] Enzymes like galactosidase and glycosidase, asparaginase, and arginase as well as bronchodilators like salbutamol are loaded using hypotonic dilution.

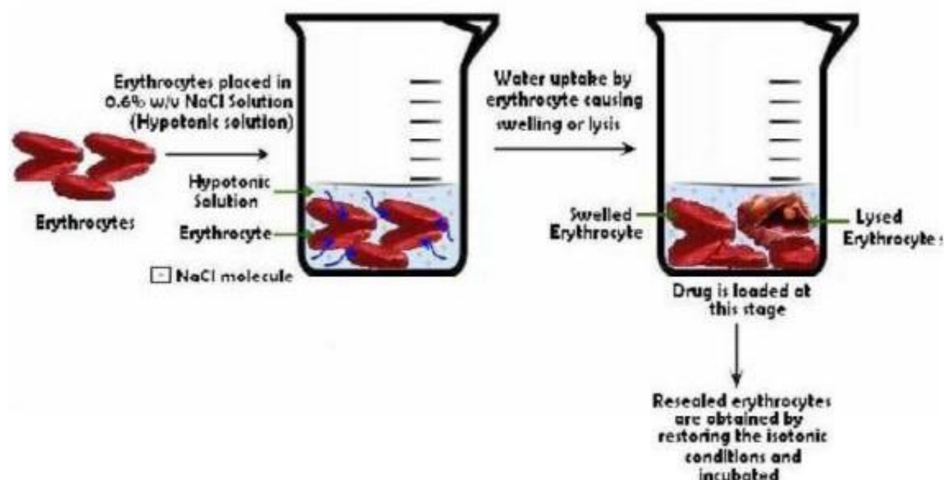

Figure 2: Hypotonic Dilution Method

b. Hypotonic Dialysis Method

In order to load enzymes and lipids, this technique was first described by Klibansky in 1959 and used by De loach, Ihler, and Dale in 1977. The procedure involves an isotonic, buffered erythrocyte suspension with a hematocrit. A prepared and placed value of 70-80 standard dialysis tube submerged in 10–20 a hypotonic buffer's volume. The format is slowly agitated for 2 hours [33]. 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 in which an isotonic, buffered suspension of erythrocytes with a hematocrite 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. One can either replace the surrounding medium with an isotonic buffer or directly add a calculated amount of a hypertonic buffer to it in order to restore the tonicity of the dialysis tube [34]. 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 [35-37] or by adding the drug to a dialysis bag after the stirring is complete.

Using this method, the hypotonic buffer and blood compartment were filled with the erythrocyte suspension and the drug to be loaded was inserted into a receptor chamber. This gave rise to the idea of "continuous flow dialysis," which has been applied by numerous other scientists [38-40]. This method has been used for loading enzymes such as – galactosidase, glucoserebrosidase [41], asparagines [37], inositol hexaphosphatas, as well as drugs such as gentamicin [35], adriamycin [36], pentamidine and furamycin [42], interlukin-2 [43], desferroxamine [44-47], and human recombinant erythropoietin.

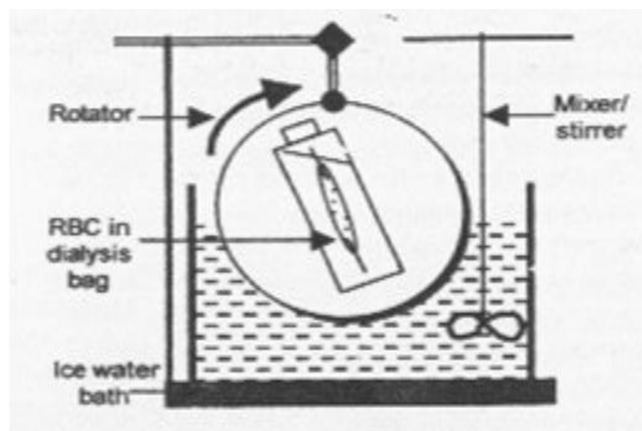

Figure 3: Hypotonic Dialysis Method

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c. Hypotonic Pre Swelling Method

Rechsteiner created this technique in 1975, and Jenner *et al.,* modified it for drug loading. The method is predicated on preliminary regulated edema in a buffered, hypotonic solution [48]. 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. 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 [48]. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer [49]. After that, the cell suspension is incubated at 37 °C to cause the erythrocytes to reseal. These cells have a half-life in circulation that is similar to typical cells. This approach results in the least amount of cell damage and is quicker and easier to use than other approaches. Drugs encapsulated in erythrocytes using this method include propranolol, aspaginase, cyclopohphamide, 1-antitrypsin, methotrexate, insulin, metronidazole, levothyroxine, enalaprilat and ionized [50, 51].

e. Hypotonic Osmotic Lysis Method

This technique, sometimes referred to as the osmotic pulse method, uses chemical or physical means to achieve isotonic haemolysis. It's possible that the isotonic solutions avoid being isotonic. In1975, Schrieretal reported this technique. This procedure, also referred to as the osmotic pulse technique uses isotonic haemolysis that is accomplished using physical or chemical methods [52]. When erythrocytes are placed in solutions of a high membrane conductivity substance The solute will diffuse into the space due to permeability cells as a result of the gradient in concentration. An influx of water happens after this process to maintain osmotic balance. Chemicals examples include urea solution, polyethylene glycol and the uses of ammonium chloride hemolysis at an isotonic pH. But this technique also is susceptible to membrane changes composition of a structure. Franco and associates paper in 1987 created a procedure that involved suspending Erythrocytes in an isotonic dimethyl sulfoxide DMSO,a sulfoxide [51]. In 1987, Franco *et al.,* developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO). The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 370C.

2. Membrane Perturbation Method

In 1973, Deuticke *et al.,* exposed that the permeability of erythrocytic membrane rises upon exposure to polygene antibiotic such as amphotericin B [53]. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycinin human and mouse erythrocytes used halothane for the same purpose. Kitao and Hattori successfully entrapped the anticancer medication daunomycin in human and mouse erythrocytes in 1980 using this method [54]. Halothane was employed by Lin *et al.,* for a related purpose. But these methodscause cellular membrane damage that is irreversible, which is why they are not very well-liked [55].

3. Electro Encapsulation Method

In 1973, Zimmermann experimented with encapsulating bioactive molecules using an electrical pulse method. Also referred to as electroporation, the process is predicated on the finding that an electrical shock causes permanent alterations to an erythrocyte's membrane. Transient electrolysis was proposed by Tsong and Kinosita in 1977 as a means of producing the appropriate membrane permeability for drug loading [56]. The A breakdown in the dielectric property opens the erythrocyte membrane. The pores can then be sealed again by incubating in an isotonic medium at 370C. It is also recognized as electroporation method, which is based on using transient electrolysis leading to generate pores that produce desirable membrane permeability for drug loading into erythrocytes. It includes suspending of erythrocytes in an isotonic buffer in an electrical discharge chamber. It has a capacitor in an exterior circuit which is charged to a certain voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential. The anticancer medication daunomycin was effectively entrapped in human and mouse erythrocytes in 1980. Additionally, this procedure causes irreversible, damaging alterations to the cell membrane, which is why it is not widely used now [30, 57, 58].

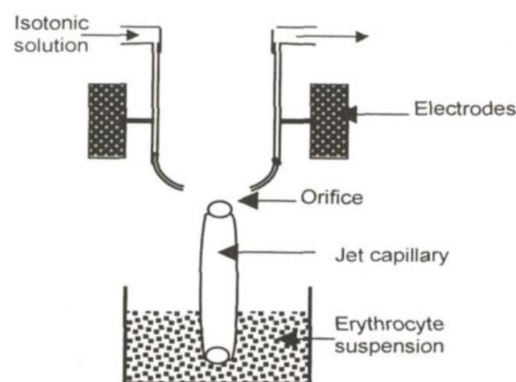

Figure 4: Electro encapsulation Method

1. Hypotonic Endocytosis Method

Schrier *et al.,* published a report on this technique in 1975 [59]. Using this method, one volume of washed and packed erythrocytes is added to nine volumes of buffer containing 2.5MM ATP, 2.5MM mgcl2, and 1MM CaCl2. The mixture is then allowed to sit at room temperature for two minutes. The pores created by this method are resealed by using 154MM of NaCl and incubate at 370C for 2 minute. Several chemicals are entrapped in erythrocytes by this method are primaquine and related 8- aminoquinoline, vinblastin, chlorpromazine, and related phenothiazines, hydrocortisone, tetracaine and vitamin A [60, 61].

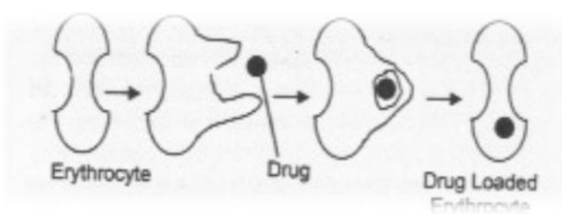

Figure 5: Entrapment by Endocytosis

2. Lipid Fusion Method

Using this method, drug molecules are first loaded into erythrocyte ghosts and then adhere to these cells to the intended 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. Chemical cross-linking of an antibody against a particular target cell surface protein can steer drug-loaded cells toward the desired cells [63, 64]. Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid entrapped drug.

EVALUATION OF RESEALED ERYTHROCYTES

Shape and Surface Morphology

The process of morphologically characterizing erythrocytes involves comparing them to untreated scanning electron microscopy (SEM) or transmission electron microscopy (TEM) to examine erythrocytes. Additional techniques such as phase contrast microscopy may also be employed. The morphology of erythrocytes decides their life span after administration [65].

Cell Counting and Cell Recovery

This entails calculating the quantity of red blood cells in each unit volume of whole blood, typically with an automated device ascertained by calculating the number of intact cells per cubic millimeter of packed erythrocytes both prior to and following drug loading [66].

Drug Content

The drug content of the cells controls the method's entrapment effectiveness. Using 2.0 mL of acetonitrile, packed, loaded cells (0.5mL) are deproteinized during the process and centrifugation for ten minutes at 2500 rpm. Spectro photometric analysis is used to determine the drug content of the clear supernatant [67].

Miscellaneous

Additional characteristics of sealed erythrocytes include mean cell volume, energy metabolism, lipid composition and cell sizes separation of density gradients, membrane fluidity, and rheological characteristics [68].

Osmotic Shock

For osmotic shock study, erythrocytes suspension (1 ml 10% hct) was diluted with distilled water (5ml) and centrifuge at 300 rpm for 15 minutes. An analytical estimate of the supernant's hemoglobin release percentage was made [69].

Turbulence Fragility

The test is conducted by passing cell suspension through needles that have a smaller internal diameter (such as 30 gauges) or shaking the cell suspension ferociously. Haemoglobin and medication released following the procedure are identified in both situations. The erratic It is discovered that resealed cells are more fragile [70].

Parameter	**Techniques**
Physical	
Size, shape, surface morphology	Transition electron microscopy, optical microscopy, Scanning electron microscopy, phase contrast microscopy
Drug release	Diffusion cell dialysis
Drug content	Deproteinization of cell membrane followed by assay of drug, radiolabelling.
Surface electrical potential spectroscopy	Zeta potential determination by photon correlation [PCS]
Vesicle size & size distribution	Transmission electron microscopy, Optical microscopy
Surface pH	pH sensitive probes
2. Biological	
Pyrogenicity	LAL test, Rabbit method
Toxicity	Sterility testing method
Sterility	Toxicity testing method
3. Cellular	
% Hb content	Deproteinization of cell membrane followed by haemoglobin assay.
%Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay.

Application of Resealed Erythrocytes

There are numerous potential uses for sealed erythrocytes in the fields of veterinary and human medicine. These cells could be applied as circulating carriers to spread a medication over an extended period of time in the bloodstream or in organs that are specific to the target, such as the lymph nodes, liver, and spleen. Preclinical research is where most drug delivery

experiments involving erythrocytes loaded with drugs are currently conducted [71-73].

In-Vitro Applications

The biological characteristics of resealed erythrocytes greatly influence their in vivo performance. Therefore, a significant portion of research involving these cellular carriers involves in vitro characterization. Numerous in vitro tests have shown the value of carrier red blood cells. Phagocytosis in vitro cells have been employed to promote the uptake of enzymes by phagolysosomes. An internal analysis of this work demonstrated that the cytochemical technique could be used to visualize the enzyme content within the carrier red blood cells. The most common RBC-mediated microinjection technique used in vitro. A protein or nucleic acid that will be infused through fusion into eukaryotic cells. In a similar vein, when antibody. When molecules are added through the erythrocytic carrier system, the cytoplasm is instantly filled with them. Utilizing auto-injected antibody RBCs into living cells, the site of action of a diphtheria toxin fragment has been verified [74].

In – Vivo Applications
Slow Drug Release

Antineoplastic have been continuously delivered by using erythrocytes as circulating depots cardiovascular medications, vitamins, steroids, anti parasitic and veterinary anti amoebic [75]. The different drug release mechanisms that have been suggested include:

- Diffusion in passive mode.
- Specialized carrier transport associated with membranes.
- RES macrophages phagocytes sealed cells, which leads to drug accumulation inside the macrophage interior and gradual release.
- Erythrocyte accumulation in lymph nodes after subcutaneous injection, which is followed by hemolysis to liberate the medication.

The most popular delivery method is intravenous, which is followed by subcutaneous, intraperitoneal, intranasal, and oral. Published research has examined the enhanced effectiveness of different medications administered in this manner in animal models.

Drug Targeting

Drug delivery should ideally be target and site-specific in order to have the highest possible therapeutic index with the fewest possible side effects. Red blood cells that have been sealed again can serve as both targets and drug carriers. Since macrophages can detect the change in the membrane, surface-modified erythrocytes are used to target organs of the mononuclear phagocyte system, or RES [76].

Treatment of Hepatic Tumor

Damaged red blood cells are quickly removed from the bloodstream by the phagocytic Kupffer cells found in the spleen and liver. Because they have different membranes, resealed erythrocytes can therefore be directed towards the spleen and liver. Among the many methods for altering erythrocyte surface properties are surface modification using antibodies [77]. The surface can be modified using gluteraldehyde, carbohydrates like sialic acid, sulphydryl or surface chemical cross-linking, such as delivering 125I-labeled carbonic anhydrase loaded in erythrocytes and cross-linking them with bis (sulfo succinimidyl) suberate and 3,3 dithio (sulfo succinmidyl propionate] [78].

Carriers for Enzymes

In clinical practice, enzymes are frequently used as replacement therapies to treat disorders linked to their deficiency, such as galactosuria and Gaucher's disease, degradation of poisonous substances secondary to poisoning (cyanide,organophosphorus), as well as pharmaceutical . There have been mentions of the issues with injecting enzymes directly into the body. The use of erythrocytes loaded with enzymes is one way to get around these issues. For the treatment of Gaucher's disease, gluco serebrosidase represents the first successful clinical trial report of resealed erythrocytes loaded with enzymes for replacement therapy [79]. 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. These cells then release enzymes into circulation upon hemolysis 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 for future catalysis [80, 81].

Treatment of Hepatic Tumor

Among the most common forms of cancer are hepatic tumors. Erythrocytes have proven effective in delivering anti-tumor drugs like methotrexate, bleomycin, asparginase, and adriamycin [82]. This problem can be overcome by covalently linking daunorubicin to the erythrocytic membrane using gluteraldehyde or cisaconitic acid as a spacer. Erythrocytes have been successfully used to deliver anti-tumor drugs such as methotrexate, bleomycin, asparginase, and Adriamycin to treat hepatic tumours [83]. A solution to this issue is to covalently attach daunorubicin to the erythrocytic membrane using glutaraldehyde orcisaconitic acid as spacer. Daunorubic in diffuses quickly from the cells upon loading [84].

CONCLUSION

Re-sealed erythrocytes are working very well for the safe and reliable delivery of a variety of medications for both passive and active targeting. The concept needs further optimization to become a routine drug delivery system and we can also use the same concept for extended to the delivery of

biopharmaceuticals and much remains to be explored regarding the potential of resealed erythrocytes. It's very simple to prepare resealed erythrocytes. Nowadays, a number of methods have been discovered that make it simple to ensnare the medication in erythrocytes. It's got both in-vivo and in-vitro application methods.

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