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An Overview on Resealed Erythrocytes: An Innovative Drug Delivery

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Abstract **Review Article**

An innovative drug delivery system is a brand-new approach that successfully delivers pharmaceutical chemicals throughout the body as needed to produce the desired pharmacological effect by fusing creative formulation, new technology, and inventive methodology. There are many cutting-edge medication delivery methods on the market. Of them, "drug-loaded erythrocytes" represent one of the most promising and rapidly growing delivery systems for drugs and enzymes. Because erythrocytes are naturally occurring, they can be used as drug delivery systems to improve the pharmacokinetics, bioavailability, and numerous other characteristics of various drugs. For precise and regulated medication delivery systems, erythrocytes are particularly helpful. Erythrocytes can be loaded with a variety of physiologically active chemicals and are biocompatible, biodegradable, and have a long circulation half-life.

Keywords: Drug Loading Technique, Drug Targeting, Isolation, Resealed Erythrocytes, Carrier Erythrocytes.

INTRODUCTION

In the seventeenth century, erythrocytes were first characterized as particles "25,000 times smaller than a fine grain of sand." Using the early microscope that was available, Dutch microscopist Leeuwenhoek misidentified them for fat globules [1]. Approximately a century later, Howson discovered that these cells were flat discs rather than globules, providing a more accurate description of these cells. By recognizing hemoglobin's critical function in oxygen supply to many tissues, Hoppe Seyler completed the Hünefeld's discovery of hemoglobin in the 19th century [2]. Until the late twentieth century, reversible oxygenation along with CO2 exchange—was thought to be the red cell's major, if not exclusive, physiological activity. Currently, however, we know more about erythrocyte function, including immunological clearance, the clearance of other soluble blood components including cytokines, and the exchange of O2, CO2, H+, and nitric oxide [1]. Gardos attempted to load the "erythrocyte ghosts" with ATP in 1953, which was one of the earliest attempts to entrap chemicals in erythrocytes [3]. Dextran trapping with molecular weights ranging from 10 to 250 KD was documented by Marsden and Ostling in 1959 [4]. The word "carrier erythrocytes" was originally used to characterize the drug-loaded erythrocytes in 1979 [7]. Fourteen years later, Ihler [5] and Zimmerman [6] independently reported the first reports on loading the erythrocyte ghosts by therapeutic substances for purposes of delivery. The potential carrier properties of erythrocytes, or red blood cells, for the transport of medications and drug-loaded microspheres have been thoroughly investigated [8–10]. Simply take blood samples from the organism of interest, separate the erythrocytes from plasma, entrap the drug in the erythrocytes, and then seal the resulting cellular carriers to create such drug-loaded carrier erythrocytes [8]. These carriers are known as resealed erythrocytes as a result. The reaction of these cells in an osmotic environment is the basis of the entire process. The drug-loaded erythrocytes target the pharmaceuticals to a reticuloendothelial system (RES) upon reinjection, acting as slow circulating depots [9–11].

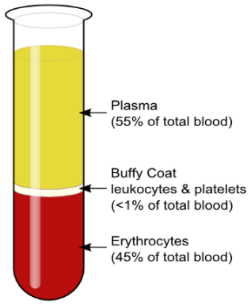

Figure-1: Erthrocytes

ERYTHROCYTES

Erythrocytes, sometimes referred to as red blood cells (RBCs), are the body's highly specialized oxygen carriers that are carried by the circulatory system. As they squeeze through the capillaries, they release the oxygen that has been stored in their lungs or gills. The cytoplasm of these cells is abundant in hemoglobin, an iron-containing biomolecule that binds oxygen and gives blood its red color [12]. The bone marrow produces red blood cells, which circulate throughout the body for 100–120 days until macrophages recycle their constituent parts. Erythropoiesis is the term for the mechanisms by which the body produces red blood cells. These cells are formed in red bone marrow and are controlled by the hemopoeitic hormone erythroprotein [13].

RESEALED ERYTHROCYTES

Controlled release formulations include resealed erythrocytes (RBCs), which have been the subject of substantial research due to their potential as carriers and their capacity to distribute drug-loaded microspheres. Erythrocytes are prepared for this carrier, blood is drawn from the organism of interest, the erythrocytes are separated from the plasma, the medicine is then entrapped in the erythrocytes, and the cellular carriers are sealed once more. Therefore, the reaction of these cells in an osmotic environment forms the basis of the entire process. Drug-loaded erythrocytes deliver slow-circulating depots and direct the medication to a diseased tissue organ through the reinjection procedure [14, 15].

STRUCTURE AND ITS PHYSIOLOGY

As the most prevalent cells in the human body, erythrocytes are found in blood at concentrations of about 5.4 million cells/mm3 in healthy males and 4.8 million cells/mm3 in healthy females. With an average diameter of 7.8 μm, a perimeter thickness of 2.5 μm, a center thickness of 1 μm, and a volume of 85–91 μm3, erythrocytes resemble biconcave discs. Erythrocytes are able to pass through capillaries that may only be 3 μm wide because of the flexible biconcave form of these cells [10-12]. This permits them to fit into tiny capillaries because their plasma membrane is robust and flexible, allowing them to deform without rupturing. Replica and significant metabolic activity are not possible for red blood cells (RBCs) since they lack a nucleus and other organelles.

Since adult red blood cells lack a nucleus, all of their internal space is accessible for oxygen transport, which makes RBCs highly specialized for this purpose. The structure of RBCs also helps to facilitate this function. Compared to a sphere or cube, a biconcave disc has a far larger surface area for gas molecules to diffuse into and out of the RBCs. RBCs have 280 millionor more hemoglobin molecules in them. Semipermeable red blood cell membranes are linked to energy metabolism and the preservation of the cell's ability to permeability for different cations (Na+, K++) and anions (Cl-, HCO3-) [16-18].

TWO WAYS IN WHICH ERYTHROCYTES CAN BE USED AS CARRIERS

Focusing on a Certain Organ or Tissue

Only the erythrocyte membrane is utilized for targeting. This is achieved by dividing the cell in a hypotonic solution and letting it reseal into a sphere after the drug has been introduced. Red cell ghosts are the term for these erythrocytes.

Constant or extended medication release

As an alternative, erythrocytes can be employed to deliver a longer-lasting medication activity through a continuous or sustained release mechanism. There are various techniques for encasing medications in erythrocytes. They release the substance that is entrapped slowly and steadily while being in the bloodstream for extended periods of time—up to 120 days [19].

ERYTHROCYTE SOURCES

Mammalian erythrocytes from a variety of species, including mice, cattle, pigs, dogs, sheep, goats, primates, fowl, rats, and rabbits, have been used to deliver drugs [20].

ISOLATION OF ERYTHROCYTES

It is possible to manufacture erythrocytes as carriers using blood obtained from both humans and several animal species, including dogs, rats, mice, and rabbits. Using an appropriate anticoagulant, blood is drawn from the relevant animal species, mouse, rat, or human. Since EDTA is the anticoagulant that best maintains the characteristics of blood cells, it is typically applied. To separate packed erythrocytes, freshly drawn blood is centrifuged in a refrigerator-temperature centrifuge. After that, several washes are carried out. Typically, this procedure entails centrifuging a solution several times in order to extract additional components of blood. An efficient way to wash the erythrocytes is with a capillary hollow fiber plasma separator.

Though working at a 70% hemoglobin level is the most common practice. In 1953, Gardos attempted to use adenosine triphosphate (ATP) to load erythrocyte ghost. Dextran (molecular weight 10–250 kDa) was reported to be entrapped by Marsden and Ostting in 1959. In 1973, Ihler *et al.*, and Zimmermann published different reports on the loading of medicines in erythrocytes. Drug-loaded erythrocytes were referred to as carrier erythrocytes in 1979 [20].

Table 1: Various Condition and Centrifugal Force used for isolation of Erythrocytes

Species	Washing Buffer	Centrifugal force (g)
Rabbit	10mmol KH2PO4/NaHPO4	500-1000
Dog	15mmol KH2PO4/NaHPO4	500-1000
Human	154mmol NaCl	<500
Mouse	10mmol KH2PO4/NaHPO4	100-500
Cow	10-15mmol KH2PO4/NaHPO4	1000
Horse	2mmol MgCl2, 10mmol glucose	1000
Sheep	10mmol KH2PO4/NaHPO4	500-1000
Pig	10mmol KH2PO4/NaHPO4	500-1000

PROPERTIESOFRESEALEDERYTHROCYTES

- ❖ Minimal drug leaching or leakage need to occur prior to the targeted spot.
- ❖ The medication need to be administered steadily at the intended location.
- ❖ The medication ought to possess optimal dimensions and form, enabling it to pass through capillaries with the least amount of drug leakage possible.
- ❖ It should be able to transport a wide range of medications.
- ❖ It need to be blood-compatible and have minimally harmful effects.
- ❖ The carrier system must to possess notable stability during storage and a distinct physiochemical ability to identify the intended target site [21, 22].

ENCAPSULATION REQUIREMENTS

Encapsulation requires the following conditions:

- ❖ Nonpolar molecules can be entrapped in the form of salts in erythrocytes. EX- Bovine red blood cells can be loaded with ex-tetracycline hydrochloride salt.
- ❖ It is possible that the lipohilic molecule absorbs above other molecules and becomes stuck in erythrocytes.
- ❖ When a molecule is bigger than β-galactosidase and smaller than sucrose, the size of the entrapped molecule has a significant role. The charged molecule stays the same longer than the uncharged molecule.
- ❖ One set of pares is present in the dialyzed cell at all times, whereas another set appears and disappears on a regular basis [23, 24]. These two forms of polar are present in the dialyzed erythrocyte.

METHODS FOR DRUG LOADING

1. Hypo-osmotic lysis

a) Dilution method
b) Preswelling method
c) Dialysis method
d) Osmoticlysis method

2. Membrane perturbation method
3. Electro encapsulation method
4. Endocytosis method
5. Lipid fusion method
6. Electric cell fusion method

1. **Hypo-osmotic Lysis Method:** These are classified into four types which are as as follows-

- ❖ Dilution method
- ❖ Preswelling method
- ❖ Dialysis method
- ❖ Osmotic lysis method

Dilution Method: Hypotonic dilution was the earliest, easiest, and fastest way to load compounds into erythrocytes that was studied. This procedure involves diluting a volume of packed erythrocytes with 2–20 volumes of a drug's aqueous solution; the solution's tonicity is then preserved by adding a hypertonic buffer. The final step is to centrifuge the mixture, discard the supernatant, and wash the pellet with an isotonic buffer solution. This method's main drawbacks are its low entrapment efficiency and significant hemoglobin and other cell component loss, which shortens the laden cells' circulation half-life. These can be utilized to target RES organs as RES macrophages phagocytose them. Enzymes such as β-galactosidase, β-glucosidase, and asparginase, arginase & bronchodilators such as salbutamol are loaded using this dilution [25].

Examples of Encapsulated Agents: β-glucosidase, asparaginase, arginase, salbutamol

Preswelling Method: In a hypotonic buffered solution, it is predicated on first regulated swelling. A low g centrifuge value is used for this mixture. By adding 100–120 μL sections of an aqueous solution of the medicine to be encapsulated, the cell fraction is brought to the lysis point and the supernatant is discarded. In between the drug-addition processes, the liquid is centrifuged thereafter. A calculated amount of hypertonic buffer is added to a cell mixture at the lysis point to restore its tonicity. A clear separation between the cell fraction and the supernatant vanishes during centrifugation, indicating the lysis point. The cell suspension is then incubated at 37 0C to cause the resealed erythrocytes to reanneal [26-28].

Examples of Encapsulated Agents: Propranolol, asparginase, methotrexate, isoniazid etc.

Hypotonic Dialysis Method: First proposed by Klibansky in 1959, this technique was applied for the loading of lipids and enzymes by Deloach, Ihler, and Dale in 1977. The idea of a semi-permeable dialysis membrane, which increases the ratio of intracellular to extracellular volume for macromolecules during lysis and resealing, is the foundation of several techniques. In this method, the medication solution and erythrocyte suspension are mixed to obtain the appropriate hemocrit. After the mixture is inserted into the dialysis tubing, the tube's two ends are knotted with thread. There remains an air bubble in the tube that makes up around 25% of its interior capacity. The tube is put inside the bottle that has 100 milliliters of swelling solution in it.

To achieve the desired lysis time, the bottle is kept at 4oC. By shaking the tube with the strings, the contents of the dialysis tubing are combined. A dialysis tube is then submerged in 100 milliliters of sealing solution. Following this, the loaded erythrocytes were collected and subsequently washed at 4°C in cold phosphate buffer. This results in a good entrapped efficiency [29, 30]. β-galactosidase, glucose rebrosidase, asparginase, inositol hexaphosphatase, and medications including gentamicin, adriamycin, pentamidine, uramycin, interlukin-2, desferroxamine, and human recombinant erythropoietin have all been loaded using it [25].

Examples of encapsulated agents: Gentamicin, adriamycine, erythropoietin, furamycin A, IgG etc.

Osmotic lysis method: The osmotic pulse method is another name for it. An influx of water is necessary to maintain osmotic equilibrium when erythrocytes are incubated in solutions containing a material with high membrane permeability. This is because the solute will diffuse into the cells due to the concentration gradient. Isotonic hemolysis has been achieved using chemicals such as ammonium chloride, polyethylene glycol, and urea solution. After the suspension was finally diluted with an isotonic-buffered drug solution, the cells were divided and then sealed again at 370°C [23].

Examples of enc apsulated agents: Inositol hexaphosphate.

2. Membrane perturbation method: It focuses on the observation those erythrocytes' membrane permeability increases in response to specific chemical exposures, including polyene antibiotics like amphotericin B. Kitao and Hattori successfully employed this technique in 1980 to entrap the anticancer medication daunomycin in human and animal erythrocytes. Halothane was also utilized for the same reason. However, these techniques are not well-known since they have caused the cell membrane to undergo irreversible, damaging alterations [21].

Examples of encapsulated agents: Daunomycin.

3. **Electro-encapsulation method:** The technique, often referred to as electroporation, is predicated on the use of transient electrolysis to create pores that result in the desired membrane permeability for drug loading into erythrocytes. In an electrical discharge chamber, erythrocytes are suspended in an isotonic buffer. It uses a capacitor in an external circuit that is charged to a specific voltage and discharged through cell suspension within a specific time frame to create a square-wave potential. The anticancer medication daunomycin was effectively entrapped in human and animal erythrocytes in 1980. This approach is not very common because it also causes irreversible, damaging alterations in the cell membrane [31, 32].

Examples of encapsulated agents: Primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, vitamin A.

4. Entrapment by Endocytosis: Schrier reported on it in 1975. In order to initiate endocytosis, one volume of packed, washed erythrocytes is added to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1 mM CaCl2. The mixture is then allowed to sit at room temperature for two minutes. By applying 154 mM of NaCl and incubating at 37°C for two minutes, the pores produced by this technique are shut again. The vesicle membrane keeps the cytoplasm safe from the erythrocytes and divides endocytosed material from it. This method makes use of a number of medications, including vinblastine, hydrocortisone, phenothiazine, vinblastine, eight aminoquinolones, tetracaine, and vitamin A [21].

Examples of encapsulated agents: Hydrocortisone, propranolol, vitamin A Primaquine, vinblastine, chlorpromazine.

5. Lipid fusion method: By directly fusing a drug-containing lipid vesicle to human erythrocytes, a lipid-entrapped drug can be exchanged. This process is known as the lipid fusion method. As a result, this technique is employed to entrap inositol monophosphate, which enhances RBCs' ability to carry oxygen [21].

Examples of encapsulated agents: Inositol monophosphate.

6. **Electric cell fusion method:** The first step is loading drug molecules into erythrocyte ghosts, which are then attached to target cells by these cells. An electric pulse applied to the molecule induces its release, hence promoting the fusion process. A better illustration of this technique is the loading of an erythrocyte ghost with a monoclonal antibody specific to a particular cell. To drive drug-loaded cells to targeted cells, an antibody

against a particular target cell surface protein can be chemically cross-linked to the cells [21].

LOADING PARAMETERS

The loading efficiency of the encapsulation techniques is assessed using three metrics that are often determined. These metrics include cell recovery (the percentage comparing the volume of the first packed cells to that of the final loaded cells), entrapment efficiency (the percent ratio of the loaded amount of the drug to the amount added during the entire loading process), and loaded amount (the total amount of drug encapsulated in the final carrier erythrocytes) [33, 34].

DRUG RELEASE CHARACTERSTICS OF LOADEDERYTHROCYTES

Three primary routes exist for a medication to exit erythrocyte carriers: phagocytosis, diffusion across the cell membrane, and utilization of a particular transport mechanism. Diffusion rate is dependent on how quickly a specific molecule passes through a lipid bilayer; it is highest for molecules with high lipid solubility and progressively decreases with polarity or charged groups in the molecule. It is possible that the medication may be entrapped with strong inhibitors of the relevant transport protein to prolong the release [35].

IN VITRO CHARACTERISATION

The biological characteristics of resealed erythrocytes have a significant impact on how well they function *in vivo*. Therefore, characterisation in vitro plays a crucial role in research involving these types of cellular carriers.

A] Physical Characterization

1] Shape and Surface Morphology

The length of an erythrocyte's life after administration is determined by its shape. Spherical erythrocytes, or spherocytosis, are occasionally seen in light microscopy images, although there is no discernible alteration in resealed cells. According to research using scanning electron microscopy, most of the cells retain their biconcave discoid morphologies during the loading process, whereas a small percentage of stomatocytes develop a kind of spherocytosis that involves an invagination at a single location. Microcytes, or smaller cells, have also been discovered in certain instances [35].

2] Drug content

The drug content of the cells controls the method's trapping effectiveness. Centrifugation at 2500 rpm for 10 minutes is followed by the deproteinization of packed, loaded cells (0.5 ml) with 2.0 ml acetonitrile. The medication content is examined in the clear supernatant.

3] Deformability

Deformability, or the ability to change shape, is another element that influences how long cells survive. This measure assesses how easily erythrocytes may flow through RES and narrow capillaries. It is dependent on the viscoelasticity of the cell membrane, the viscosity of the cell contents, and the surface-to-volume ratio of the cells and controls the rheological behavior of the cells. The deformability is determined by timing the passage of a specific volume of cells through a 45 μm-average pore-size polycarbonate filter or a capillary with a diameter of 4 μm. A further indirect method is to use turbidimetric analysis to assess shape changes caused by chlorpromazine [35].

4] Drug Release

The drug release pattern is the most significant parameter for assessing resealed erythrocytes. Additionally, medication release always results in hemolysis, or the breakdown of cell membrane integrity, which releases hemoglobin. Based on multiple in vitro release tests conducted on these cells, three overarching drug release patterns are noted:

- Compared to hemoglobin, the rate of drug release is significantly higher. Put differently, the medication diffuses easily. Lipophilic medications such as dexamethasone, methotrexate, phenytoin, primaquin, and vitamin B12 exhibit such a pattern. For the release of such medications, cell lysis is not necessary.
- The pace at which drugs release is similar to that of hemoglobin. This suggests that the drug cannot be released by simple diffusion and that cell lysis is necessary for drug release. Asparginase peptides, which include urogasterone and lysine-l-phenylalanine, as well as polar medications like gentamicin, heparin, and enalapril exhibit this pattern.
- For example, propranolol, isoniazid, metronidazole, and recombinant human erythropoietin have drug release rates that fall between the two extremes indicated above [35].

B] Cellular characterization

1] Osmotic Fragility

The susceptibility of resealed erythrocytes to the osmotic pressure of the suspension medium and potential alterations in the integrity of their cell membrane are indicated by their osmotic fragility. The test involves releasing hemoglobin by suspending cells in medium with different concentrations of sodium chloride. Since intracellular osmotic pressure is higher in sealed cells than in normal cells, resealed cells typically have higher osmotic fragility [35].

2] Turbulent Fragility

The turbulence fragility is yet another characteristic that depends upon changes in the integrity of cellular membrane and reflects resistance of loaded cells against hemolysis resulting from turbulent flow within circulation. It is determined by the passage of cell suspension through needles with smaller internal diameter (e. g., 30 gauges) or vigorously shaking the cell

suspension. In both cases, hemoglobin and drug released after the procedure are determined. The turbulent fragility of resealed cells is found to be higher [35].

3] Percent cell recovery

Before and after loading the medicine, the number of intact cells per cubic millimeter of packed erythrocyte can be counted to determine it.

C] Biological characterization

It can be accomplished by running animal toxicity tests, LAL tests, pyrogen tests utilizing the rabbit technique, and sterility tests.

***IN VIVO* CHARACTERISATION**

The key factor influencing the effectiveness of resealed erythrocytes is how long they remain in the bloodstream after being reinjected. The tagging of cells with 51Cr or fluorescent markers like fluorescin isothiocyanate, or the trapping of 14C sucrose or gentamicin, are among the techniques utilized to calculate the in vivo survival time. Resealed erythrocytes exhibit normal bimodal circulation survival dynamics, with a fast cell loss during the first 24 hours following injection and a steady decline phase with a half-life of a few days or weeks [35].

Route of administration

During experiments, resealed erythrocytes are typically given intravenously via the cardinal vein to laboratory animals. DeLoach used a subcutaneous method to release chemicals that were trapped gradually. He assessed the interleukin-2's behavior in mice that were injected subcutaneously. Propranolol nasal administration based on erythrocytes was recently proposed by Talwar (1993) [36].

***In vitro* storage**

Resealed erythrocytes' in vitro preservation is largely responsible for their efficacy as a drug delivery mechanism. The most popular storage media are acid-citrate-dextrose at 4°C and Hank's balanced salt solution. At this temperature, cells are viable for at least two weeks in terms of their physiologic and carrier features. The circulatory survival period of cells after reinjection is extended by the addition of purine nucleosides or calcium-chelating compounds. It has been observed that resealed erythrocytes are more stable when stored if they are exposed to membrane stabilizing chemicals including dimethyl sulfoxide, dimethyl,3,3-di-thio bispropionamide, gluteraldehyde, and toluene-2-4-disocyanate, followed by lyophilization or sintered glass filtering.

Additionally, cells can be maintained by suspending them in 1% soft gelatin-containing oxygenated HBBS. After liquefying the gel by centrifuging the tube in a water bath at 37°C, the cells are well recovered. Cryopreservation of RBCS in liquid nitrogen has been used as an additional storage technique [36].

Table: 2 Various Parameters and Techniques used for Characterization [37]

PARAMETER	TECHNIQUES USED
Physical	
Size, shape, surfacemorphology	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
Biological	
Pyrogenicity	LAL test, Rabbit method
Sterility	Sterility testing method
Toxicity	Toxicity testing method
Cellular	
% Hb content	Deproteinization of cell membrane followed by haemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubaur chamber, hematological analyzer
% Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutiond and determination of drug and hemoglobin assay
Turbulent shock	Dilution with distilled water & estimation of drug and hemoglobin
Erythrocyte sedimentation rate	Determine ESR technique

DRUG RELEASE

When these microspheres are reinjected, the plasma concentration profile of the drug is significantly influenced by the kinetic behavior of drug efflux from the carrier erythrocytes. Drawing from in vitro release experiments conducted on erythrocytes loaded with

various medicines, three distinct release patterns may be identified:

1. There is a significant difference in the rate of drug release compared to the rate of hemoglobin release, which indicates the degree of hemolysis. Stated differently, the medication can easily permeate through the intact cells. Relatively lipophilic medications such phenytoin, primaquin, methotrexate, vitamin B12, and dexamethasone have been shown to have this kind of release pattern [38].
2. The rate at which the medication releases is similar to that of hemoglobin. This indicates that the drug is released only after total cell lysis and is not released as a result of diffusion out of intact cells. Indeed, hemolysis is a necessary condition for the drug release for this class of medications. Polar medications including heparin, gentamicin, and enalaprilat, as well as enzymes and peptides such asparaginase, L-lysine-L-phenyl alanine, and urogasterone, have all been shown to exhibit this kind of release kinetics [38].
3. The two aforementioned extremes are where the drug release rate is located. Certain medications, including isoniazid, metronidazole, propranolol, and recombinant human erythropoietin, have demonstrated this kind of release kinetics. The pattern of drug release from the carrier erythrocytes is mostly determined by the size and polarity of the drug molecule. The greatest candidates for encapsulation in erythrocytes for controlled release and medication targeting to the RES organs, in our opinion, are the second category of medicines (i.e., no release from intact cells) [38].

Numerous techniques have been employed to alter the release of drugs from carrier erythrocytes; however, the most encouraging outcomes were shown when glutharaldehyde was used to cross-link the erythrocyte membrane, thereby reducing the rate of drug release. For the same reason, loading the erythrocytes with a biodegradable esteric prodrug has also been used. These prodrugs can be controlled to become the parent drug inside the erythrocytes after encapsulation, which regulates the release of the drug that is readily diffusible. The parent drug's rate is augmented by cortisol-21-phosphate, prednisolon-21-sodium succinate, cytosine arabinoside monophosphate, O-acetyl and O-pivaloyl propranolol, and some thiamin prodrugs. Certain publications describe how drug complexes and macromolecules including albumin, dextran, and polynucleotides are encapsulated in erythrocytes as separate sources of the drug. The rate of drug release in this instance is determined by the complex's dissociation rate [38].

ROUTES OF ADMINISTRATION

According to a report, intraperitoneal injection-given cells have a comparable chance of surviving to those administered via intravenous injection. The loaded cell released encapsulated molecules at the injection site, according to the subcutaneous method for the gradual release of the entrapped substance. During experimentation, resealed erythrocytes are typically given intravenously via the cardinal vein to laboratory animals. Scientist De Loach assessed the behavior of the interleukin-2 in mice undergoing a subcutaneous injection and used the subcutaneous route for the gradual release of entrapped substances. Propranolol nasal administration based on erythrocytes has been suggested by Talwar (1993) [29].

APPLICATIONS

1. *In-vivo* Applications

a. Slow Drug Release

Vitamins, hormones, antibiotics, anti-cancer, anti-parasitic, veterinary, anti-amoebic, and cardiovascular medications have all been stored in erythrocytes for long-term delivery [39].

b. Drug Targeting

Drug distribution should ideally be target and site-specific in order to achieve the highest therapeutic index with the fewest side effects. Red blood cells that have been sealed again can serve as both targets and medication carriers. Because macrophages are aware of the alterations in the membrane, surface-tailored erythrocytes are employed to target organs of the reticuloendothelial system and mononuclear phagocytic system [39].

c. Targeting RES organs

Damaged red blood cells are rapidly removed from the bloodstream by phagocytic Kupffer cells found in the spleen and liver. Because their membranes have grown, resealed erythrocytes can be used to specifically target the spleen and liver. Several methods exist for altering the surface properties of erythrocytes, such as:

- Surface modification using antibodies
- Surface modification using gluteraldehyde.
- Surface modification using carbohydrates such as sialic acid
- Surface modification using sulphydryl
- Surface cross-linking, such as the delivery of 125i-labeled carbonic anhydrase loaded in erythrocytes cross-linked with bis (sulfosuccinimidyl) suberate and 3, 3-dithio (sulfosuccinmidyl propionate) [39].

d. Targeting the liver, Enzyme deficiency/ replacement therapy

Injecting these enzymes can treat a variety of metabolic diseases associated with absent or insufficient enzymes. Exogenous enzyme therapy can have several drawbacks, though, such as allergic reactions, toxic symptoms, and a shorter half-life for enzymes in

circulation. Giving the enzymes as resealed erythrocytes is an efficient way to solve these issues. Among the enzymes utilized are galactosidase, glucoseronidase, and glucosesidase. Glucocerebrosidase loaded erythrocytes are a potential treatment for the condition induced by an accumulation of glucocerebrosidase in the liver and spleen [39].

e. Treatment of Hepatic Tumors

One of the most common forms of cancer is hepatic tumors. Erythrocytes have proven to be an effective delivery system for anti-cancer medications such methotrexate, bleomycin, asparginase, and Adriamycin. Agents like daunorubicin cause issues because they quickly diffuse out of the cells when they are loaded. This issue can be resolved by employing glutaraldehyde or cis-aconitic acid as a spacer to covalently connect daunorubicin to the erythrocytic membrane. The liver is where the resealed erythrocytes with carboplatin are localized [39].

f. Treatment of parasitic diseases

Resealed erythrocytes are a useful tool for the delivery of antiparasitic drugs because of their capacity to preferentially mount up into organs of the reticule endothelium system. This strategy can effectively limit parasitic illnesses that involve harbouring parasites in the RES organs. Studies using animal models for erythrocytes laden with antimalarial, antileishmanial, and antiamoebic medications showed promising results [39].

g. Removal of reticule endothelial system iron overload

Erythrocytes laden with desferrioxamine, an iron-chelating drug, have been used to treat individuals with thalassemia who have received multiple transfusions and have excess iron deposited. Because the RES organs' elderly erythrocytes deteriorate and produce an accumulation of iron, it is particularly helpful to target the RES with this medication.

h. Removal of Toxic Agents

Bovine rhodanase and sodium thiosulphate containing mouse carrier erythrocytes were shown by Cannon *et al.*, to suppress cyanide poisoning. It has also been observed that resealed erythrocytes with a recombinant phosphodiestrase can counteract organ phosphorus poisoning [39].

i. Targeting organs other than those of RES

The many techniques consist of:

- ❖ Encasing the medication in paramagnetic particles
- ❖ Photosensitive substance is entangled.
- ❖ Using ultrasonic waves
- ❖ Attaching an antibody to the membrane of an erythrocyte to achieve specificity of action

j. Delivered antiviral agents

Numerous studies on antiviral drugs ensnared in resealed erythrocytes for efficient administration and targeting have been referenced in the literature. Since nucleotides or nucleoside analogs make up the majority of antiviral medications, attention must be taken when entrapment and membrane escape [39].

k. Enzyme Therapy

In clinical practice, enzymes are frequently utilized as medications, replacement therapies for disorders linked to their deficiency (such as galactosuria and Gaucher's disease), and for the breakdown of poisonous substances secondary to poisoning (such as organophosphorus and cyanide). There have been mentioned issues with injecting enzymes into the body. Using erythrocytes laden with enzymes is one way to overcome these issues. Following hemolysis, these cells release enzymes into the bloodstream and function as "circulating bioreactors," where substrates enter the cell, interact with the enzymes, and either produce products or accumulate enzymes in RES for subsequent catalysis.

For the treatment of Gaucher's illness, glucosesere brosidase is the first enzyme loaded resealed erythrocyte to be successfully tested in clinical trials. The condition is defined by an inherited lack of lysosomal-glucosidase in RES cells, which causes glucosere brosides to build up in RES macrophages. Aminolevulinic acid increases as a result in tissues, blood, and urine. This condition results in CNS-related difficulties and delicate porphyria [39].

l. **Improvement in oxygen delivery to tissues**

The protein that gives erythrocytes their ability to carry oxygen is called hemoglobin. In the lungs, 95% of hemoglobin is saturated with oxygen under normal circumstances, whereas in the peripheral blood stream, only 25% of hemoglobin that has been oxygenated deoxygenates under physiological conditions. As a result, venous blood is circulated to the lungs along with the majority of oxygen linked to hemoglobin [39].

2. In-vitro Applications

The most significant in-vitro use is for macromolecule microinjection. There are several uses for proteins, RNA, and DNA in cell biology. As a result, several techniques—such as microinjection—are employed to ensnare these macromolecules in cultivated cells.

3. In microinjection

Erythrocytes are injected into host cells using micro-syringes in the process of microinjection. The microinjection technique entails the in vitro culture of host eukaryotic cells. After coating the cells with a fusogenic agent, the cells are suspended in an isotonic solution containing erythrocytes that contain the molecule of interest. Fusogenic substances such as polyethylene glycol or the glycoproteins of the Sendai

virus have been employed. Co-suspended erythrocytes and eukaryotic cells fuse together due to the fusogen [39].

NOVEL APPROCHES (Recent Developments)

Erythrosomes

These are specially designed vesicular systems that are covered with a lipid bilayer and chemically cross-linked to the support of human erythrocytes. This procedure is accomplished through the modification of a reverse-phase evaporation method. It has been suggested that these vesicles could be an effective way to encapsulate macromolecular medications [39].

Nanoerythrosomes

Erythrocyte ghosts are extruded to create tiny vesicles with an average diameter of 100 nm, which are used to prepare these. Using a gluteraldehyde spacer, daunorubicin was covalently attached to nanoerythrosomes. Daunorubicin that was free on its own was not as active as this combination [39].

ADVANTAGES [40]

- Biocompatible, particularly when utilizing autologous cells, to minimize the possibility of inciting an immunological response.
- It is biodegradable and does not produce any harmful products.
- The carrier's dimensions and form exhibit remarkable uniformity.
- A comparatively inert intracellular environment can be enclosed by a small volume of cells.
- It is easy to isolate, and large volumes of chemicals can be loaded.
- Preventing the degradation of the loaded drug due to endogenous chemical inactivation.
- There are numerous chemical entrapments that could occur.
- Drug entrapment is possible without causing a chemical change to the drug to be trapped.
- It is feasible to decrease concentration volatility and maintain steady plasma concentrations.
- The defense of the body against the toxicity of medications.
- Fixing attention on the organ of the RES.
- Zero-order medication release kinetics is optimal.
- Increase the length of time the medication remains active throughout the body.
- Reaching a stable plasma concentration that may lead to drug release kinetics at zero order.
- Modifying the pharmacokinetic and pharmacodynamic properties of the medication.
- There is a significant decrease in adverse consequences.
- Dose sufficiency is ensured by large volumes of drug that can fit into tiny volumes of cells.
- The ability to focus on RES organs.

DISADVANTAGES [40]

- They are limited in their capacity to act as transporters of target tissues that are not phagocytes.
- Cell clumping and dose dumping could be possible.
- The encapsulated substance leaks quickly from the laden erythrocytes.
- Several chemicals have the ability to alter the erythrocyte physiology.
- Direct injection into the cell nucleus is not feasible.
- A two-week shelf life in storage.
- The economical approach.

FUTURE PROSPECTIVE

The concept of using erythrocytes as drug or bioactive carriers has to be improved, and their potential for both passive and active medicine targeting needs to be fully realized. Without a doubt, illnesses like cancer and others would be healed. Combining genetic engineering components can add a new dimension to the current concept of cellular drugs [40].

Here are some thoughts about the future of sealed erythrocytes:

- To fully use erythrocyte potential for both passive and active medication targeting, a significant amount of valuable work need to be done.
- It is possible for diseases like cancer to be cured.
- It is possible to combine components of genetic engineering to offer the current concept of cellular drug carriers a fresh perspective.

CONCLUSION

The distribution of many medications for both passive and active targeting appears to be improved by the use of resealed erythrocytes. To turn the concept into a regular medication delivery system, though, more refinement is required. As various researchers have demonstrated, sealed erythrocytes can also be used to effectively administer a wide range of medications for the treatment of cancer, tumours, arthritis, and toxicity. But in the near future, erythrocyte-based delivery systems which offer precise, regulated drug distribution will transform the way that many diseases are effectively treated. Given their enormous potential and future, it is currently determined that erythrocyte carriers are "Nano Devices in the field of Nanotechnology."

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