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A Review on Resealed Erythrocytes as a Novel Drug Delivery System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The most prevalent form of blood cell is the red blood cell. RBCs resemble biconcave discs with a 7.8 mm diameter and a thickness of about 2.2 mm. There are two ways that erythrocytes can be used as carriers: Targeting a specific tissue or organ, for continuous or extended medication release. For the delivery of medications, various kinds of mammalian erythrocytes have been employed. There are numerous techniques, including the hypo-osmotic method, the dilution method, the dialysis method, the press well method, the isotonic osmotic lyses, the electrical breakdown method, the endocytosis method, the membrane perturbation method, the normal transport method, and the lipid fusion method. After erythrocytes have been loaded with the therapeutic drug, carrier cells are subjected to physical, cellular, and biological examinations. Several invitro tests have shown the value of carrier RBCs. The RBC mediated microinjection that occurs most frequently invitro. Today, a wide range of uses for resealed erythrocytes as medication carriers, enzyme replacement treatment, etc. have been proposed.

Keywords: RBCs; erythrocytes; resealed erythrocytes; bio carrier.

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1. INTRODUCTION

There are various types of cells in blood, including erythrocytes (RBC), leucocytes (WBC), and platelets. Erythrocytes, are the most interesting carriers and they have the greatest potential and zero order kinetics, because of their capacity to circulate throughout the body, in drug delivery method [1,2]. The creation of this medication delivery method's key goals were repeatability and convenience of use preparation method aims to minimize harmful pharmacological side effects while maximizing therapeutic effectiveness as well as boost patient cooperation [3-10].

2. ERYTHROCYTES

The most common type of blood cell is the red blood cell, which is also the vertebrate organism's primary source of oxygen (O2) delivery to the bodily tissues through blood flow in circulatory system. They take in oxygen through their gills or lungs and release it through their capillaries. The cytoplasm of these cells is enriched in haemoglobin, a biomolecule with iron that can bind oxygen and is in charge of giving blood its red color. Human red blood cells evolve into flexible biconcave discs without a cell nucleus or the majority of organelles. Every second, 2.4 million new erythrocytes are created [11].

3. ANATOMY AND PHYSIOLOGY

RBCs resemble biconcave discs in shape, measuring 7.8 mm in diameter and close to 2.2 mm in width. Erythrocytes that are mature have a simple structure. Its nature is also quite stretchy. Additionally, their plasma membrane is robust. As flexible, allowing them to squish into tiny capillaries without rupturing as they contract. RBCs are unable to multiply or engage in a wide range of metabolic processes because they lack a nucleus and other organelles. Since mature RBCs lack a nucleus and have just a little amount of internal space, they are completely devoted to carrying oxygen. Even the RBC's form serves its purpose A biconcave disc has a substantially greater surface area for the diffusion of gas molecules into and out of the RBC than, a sphere or cube. The dynamic, semipermeable red blood cell membrane, which is connected to energy metabolism in the preservation of different cations' (Na+, K++) and anions' (ClHCO3-) permeability properties that are characteristic of the cell. Hemoglobin molecules make up about 280 million molecules per RBC. A molecule of hemoglobin is made up of the protein globin, which is made up of four polypeptide chains. Each of the four chains is attached to a hem, a non-protein pigment that resembles a ring. Each hemoglobin molecule can bind four oxygen molecules because the center of the hem ring combines reversibly with one oxygen molecule [12,13,14].

4. SOURCE OF ERYTHROCYTES

The use of several mammalian erythrocytes in pharmacological drug delivery system, includes rabbit, rat, and mouse erythrocytes, apes, hens, and sheep.

5. RESEALED ERYTHROCYTES

To prepare erythrocytes for medication loading, blood samples from the appropriate organism were required. Removing the erythrocytes from the plasma, packing the erythrocytes with the medicine, and resealing the cellular carriers. These carriers are hence referred to as resealed erythrocytes. The reaction of these cells under osmotic conditions serves as the foundation for the entire process [15,16].

Advantages:

1. Biocompatible, especially when autologous cells are employed, negating the chance of an immune reaction being induced.
2. Biodegradability with no hazardous product formation.
3. A limited volume of cells can enclose a relatively innocuous intracellular environment.
4. Large amounts of drugs can be loaded and isolation is simple.
5. Considerable uniformity in carrier size and shape.
6. Defense of the body against toxic drug effects.
7. Aiming for the RES's organ.
8. Ideal drug release kinetics with zero orders.
9. Extend the drug's systemic activity.
10. Access to information, skills, and resources for working with, manipulating, and transfusing erythrocytes
11. The absence of any unwanted immunological responses to a medication in a capsule.
12. A significant increase in the time between doses of the medicine spending more time in the therapeutic window.

13. Pharmacokinetic and pharmacodynamic adjustments pharmacological parameters.
14. A notable reduction in concentration variations in constant state against the traditional methods of administration of drugs.
15. A reduction in the medications' side effects.
16. Simple control over a life span of minute months [17-23].

Disadvantages:

1. Their capacity as carriers to non-phagocyte target tissues is constrained.
2. There may be a chance for dosage dumping and cell clumping.
3. They only have a small amount of potential as non-phagocyte carriers target organ.
4. Potential contamination because the source was blood, the loading environment and the equipment used.
5. Strict restrictions must be implemented for the gathering and the treatment of the erythrocytes.
6. The main issue arising from the use of natural cells or biodegradable materials as medication carriers is that the RES eliminates them in real time.
7. These are some cases could present toxicological issues.
8. Another step is the storage of the laden erythrocyte's difficulty with carrier erythrocytes for potential therapeutic use [24-26].

6. METHODS OF DRUG LOADING IN ERYTHROCYTES

There are several ways to add medications, enzymes, or other bioactive substances to erythrocytes. Regardless of the technique employed, the ideal characteristics for the compound's successful entrapment include a high degree of water solubility, resistance to erythrocyte degradation, lack of physical or chemical interaction with the membrane, and well-defined pharmacokinetic and pharmacodynamic properties.

Hypo – osmotic method
Dilution method
Dialysis method
Presswell method
Isotonic osmotic lyses
Membrane perturbation
Electro-insertion or electro encapsulation
Entrapment by endocytosis

Hypotonic Hemolytic Method: In this procedure, osmotic lysis and resealing are used to exchange the intracellular and extracellular solutes of erythrocytes. This procedure will encapsulate the medication into the erythrocyte membrane.

Hypotonic Dilution or Dilution Method: This technique involves diluting a volume of packed erythrocytes with 2–20 liters of an aqueous medication solution. After then, a hypertonic buffer is added to restore the solution's tonicity. After centrifuging the resulting mixture, the pellet is washed with isotonic buffer solution after the supernatant has been removed. Low entrapment efficiency and a significant loss of hemoglobin and other cell components are two of this method's main downsides. This shortens the laden cells' circulatory half-life. These cells can be employed to target RES organs since RES macrophages can easily phagocytoze them. Enzymes including B-galactosidase and B-glycosidase, asparagine's, and arginase, as well as bronchodilators like salbutamol, are loaded via hypotonic dilution.

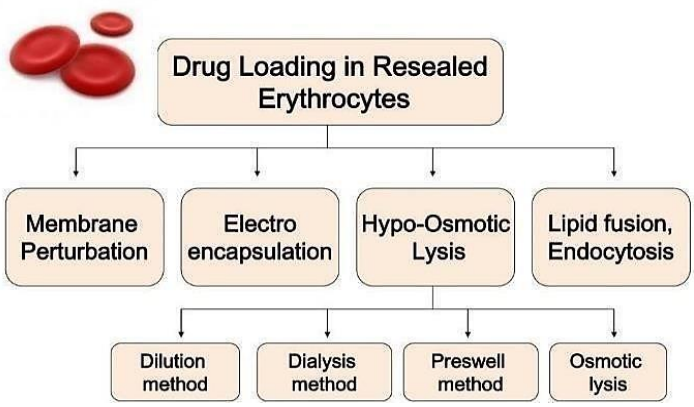

Fig. 1. Classification of drug loading in resealed erythrocyte ()

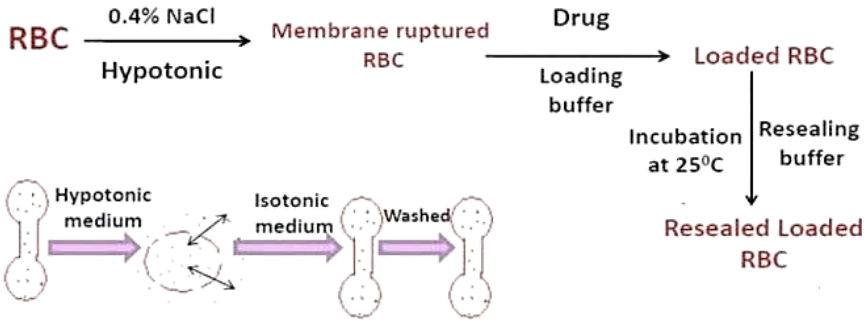

Fig. 2. Hypnotic hemolytic method ()

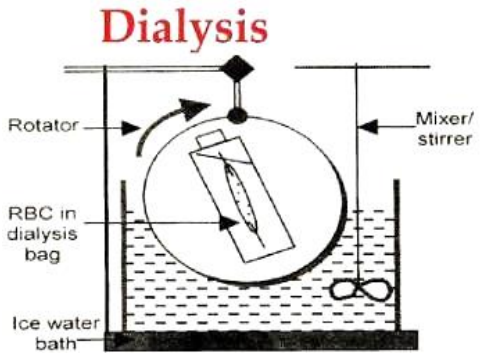

Fig. 3. Hypnotic dialysis ()

Hypnotic Dialysis Method: An isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is made as part of the procedure and put in a standard dialysis tube that has been submerged in 10–20 liters of a hypotonic buffer. For two hours, the medium is slowly stirred. By either directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by substituting isotonic buffer for the surrounding medium, the tonicity of the dialysis tube is restored. When starting the experiment, the medication to be loaded can be dissolved in isotonic cell suspending buffer inside a dialysis bag. Alternatively, the drug can be put to a dialysis bag after the stirring is finished.

Hypotonic Preswell Technique: Rechsteiner investigated this approach in 1975, and Jenner et al. improved it for drug loading. The steps that make up this approach are as follows.

1. Swelling: When erythrocyte cells are placed in a mildly hypotonic solution, such as 0.6% w/v solution, they swell without being lysed.
2. Drug Loading: The site of lysis is loaded with aqueous drug solutions in relatively tiny amounts after swelling (filled buffer). When cells slowly swelled, the cytoplasmic components were well retained, and the experiment was incubated for five (5) minutes at 0 C.
3. Resealing: An erythrocyte that has been loaded with medication is put in resealed buffer and heated to 250 C. This process is straightforward, quicker than others, and causes the least amount of cell damage. medications such as propranolol, asparagine's, and methotrexate, insulin, isoniazid, encapsulated by using this method. This method is 72% efficient.

Isotonic Osmotic Lysis: This technique, often referred to as the osmotic pulse technique, uses chemical or physical techniques to generate isotonic hemolysis. It's possible that the isotonic solutions are not isotonic. The solute will diffuse into the cells due to the concentration gradient if erythrocytes are incubated in solutions of a material with strong trans erythrocytic membrane permeability. Water enters the process after this to keep the osmotic balance. For isotonic hemolysis, substances including urea solution, polyethylene glycol, and ammonium chloride have been employed Electro- insertion or electro encapsulation.

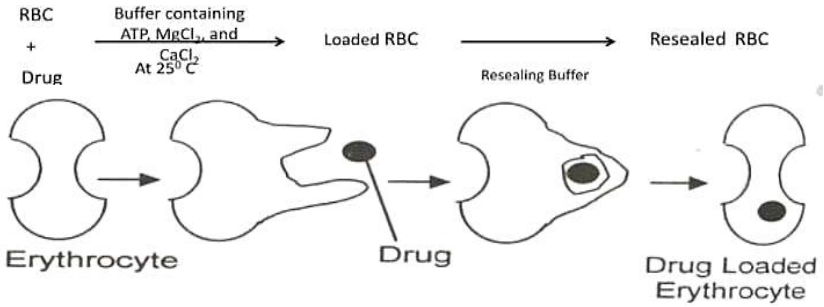

Fig. 4. Endocytosis ()

Membrane Perturbation: Amphotericin-B is an antibiotic that harms microorganisms by making their membrane more permeable to metabolites and ions. This resource could be used for medication loading into erythrocytes. Erythrocytes were loaded with the anti-leukemic medication daunomycin using amphotericin-B.

Amphotericin-B changes the permeability of the membrane by interacting with the cholesterol in eukaryotic cells' plasma membrane.

Electron Insertion or Electron Encapsulation: This technique, also known as electroporation, is based on the idea that an electrical shock causes permanent alterations to an erythrocyte membrane. A dielectric breakdown results in the opening of the erythrocyte membrane. By incubating the pores at 37ºC in an isotonic solution, the pores can then be re- sealed. The erythrocyte membrane can be polarized for 20 seconds using a variable voltage of 2 kV/cm to cause the dielectric breakdown. Intercellular and intracellular electrodes can directly build up the potential difference across the membrane, or cells' internal electric fields can do it indirectly.

The strength of the electric field, the length of the pulse, and the ionic strength of the suspending media all affect how much pore development occurs. The strength of the electric field, the length of the pulse, and the ionic strength of the suspending media all affect how much pore development occurs. Ribonucleases and big molecules (like bovine serum albumin) can be loaded here. These can be added to the electrically punctured erythrocytes' osmotic swelling.

Entrapment by Endocytosis: Schrier reported on this technique in 1975. Add one volume of cleaned, packaged erythrocytes to nine (9) volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1 mM CaCl2 for endocytosis, which is then incubated for 2 min at room temperature. By applying 154 mM of NaCl and incubating the pores produced by this procedure for 2 minutes at 37 °C, the pores are resealed. Endocytosis causes the substance to become entrapped. Endocytosis material is protected from erythrocytes by being separated from cytoplasm by the vesicle membrane, and vice versa.

7. CHARACTERIZATION OF RESEALED ERYTHROCYTES

Drug Content Quantification: After centrifugation at 3000 rpm for a predetermined amount of time, packed filled cells are deproteinized with aceto-nitrile to assess the drug content. Spectrophotometric analysis is performed on the transparent supernatant liquid.

Research on hemoglobin content and medication release *In vitro:* Hemoglobin and drug release *In vitro* from drug-loaded cells are both periodically evaluated. In amber-colored glass containers, the cell suspension (5% hematocrit in PBS) is kept at 40°C for storage. The clear supernatant is periodically removed using hypodermic syringes fitted with 0. 45 m filters, deproteinized with methanol, and its drug content is calculated. After centrifugation, the supernatant from each sample is collected and tested for hemoglobin release using the formula.

Percent Cell Recovery and Morphological Study: The number of intact cells per cubic mm of packed erythrocytes before and after the drug loading can be used to calculate the percent cell recovery. Erythrocytes that are normal or drug-loaded can be examined using a phase contrast or electron microscope.

Osmotic Fragility and Osmotic Shock Study: Drug-loaded erythrocytes are incubated individually in normal saline solution at 37 2°C for

10 minutes to evaluate the effects of various tonicities, and then they are centrifuged at 2000 rpm for 10 minutes. Centrifuge the resealed erythrocyte suspension at 300 rpm for 15 minutes in distilled water for the osmotic shock research. A Spectro- photometric estimation of the supernatant's % hemoglobin release is made.

Turbulence Shock Study: This evaluation modelizes the eradication of laden cells after injection. A 23gauge hypodermic needle is used to pass through normal and drug-loaded cells at a flow rate of 10 ml/min, which is comparable to blood flow. After that, an aliquot is collected, and centrifugation is performed for 10 minutes at 2000 rpm. Estimated hemoglobin is present in the withdrew sample. Erythrocytes that have been loaded with drugs seem to be less resistant to turbulence, which likely indicates that shaking causes cell death.

Erythrocyte Sedimentation Rate (ESR): This measurement of the stability of red blood cells in suspension in plasma is based on factors such as the quantity and size of red blood cells as well as the relative concentration of plasma proteins, particularly fibrinogen and, globulins. In order to perform this test, blood cells are deposited in a standard tube and the rate of sedimentation is measured. typical blood. ESR is 0 to 15 mm/hr. A greater rate is a sign of illness processes that are active yet unclear.

Entrapped Magnetite Study: The hydrochloric acid is added to a predetermined number of erythrocytes containing magnetite, and the mixture is then heated at 600 °C for two hours. The supernatant is then recovered after centrifugation, 20% w/v trichloro-acetic acid is added, and the magnetite content is determined by atomic absorption spectroscopy.

Self-life, Stability, and Cross-Linking of Released Erythrocytes: In a sintered glass funnel (G-4), erythrocytes that have been treated with glutaraldehyde at a 0.2% concentration are collected by filtration and dried under vacuum (200 mm Hg) for 10 hours. As an alternative, vials are filled with the erythrocyte suspension and lyophilized in a lab using a lyophilized at 400 °C and 0.01 torr. The dried powder is put inside amber-colored glass vials and kept at 40 degrees Celsius for a month. When carrier erythrocytes are kept in powder form and ready for reconstitution at 40°C, their shelf life is increased [27-29].

8. ROUTE OF ADMINISTRATION

According to intra peritoneal injection, the survival of cells in circulation was comparable to that of cells given via i.e., injection. They suggested using this kind of injection as a method for further cell therapy, reporting that 25% of resealed cells stayed in circulation for 14 days. RBCs are sent to peritoneal macrophages via the blood vessels. delayed release of drugs via the subcutaneous route captured agents. They claimed that the laden cell released chemicals that had been enclosed at the injection location**.**

9. APPLICATIONS OF RESEALED ERYTHROCYTES

***In vitro* Applications:** Several *In vitro* tests have shown the value of carrier RBCs. Because cells that have *in vitro* phagocytosis have been utilized to help phagolysosomes absorb enzymes. An analysis of this work reveals that cytochemical approach allowed for the visualization of the enzyme content within carrier RBC. The cytoplasm is immediately invaded by antibody molecules after they are injected via the erythrocytic carrier system. The site of action of a fragment of diphtheria toxin has been verified using antibody RBC auto-injected into live cells.

***In vivo* applications**

These include the following applications:

Slow drug release: For the sustained delivery of anti-neoplastic, anti-parasitic, veterinary, anti-amoebic, vitamin, steroid, antibiotic, and cardiovascular medicines, erythrocytes have been employed as circulating depots.

Targeting the liver deficiency/therapy: By injecting these enzymes, many metabolic diseases caused by insufficient or absent enzymes can be addressed. However, exogenous enzyme therapy has drawbacks such as shorter enzyme circulation half-lives, allergic responses, and toxic symptoms. The enzymes can be given as resealed erythrocytes to successfully overcome these issues. P-glucosidase, P-glucuronidase, and P-galactosidase are the enzymes that are utilized.

Treatment of Parasitic Agents: Resealed erythrocytes are a helpful tool when delivering anti-parasitic agents because they can selectively collect inside RES organs. This

technique can be used to successfully manage parasitic infections that involve parasites being harboured in the RES organs. Studies using animal models for erythrocytes loaded with antimalarial, anti- leishmanial, and anti-amoebic medications produced positive results.

Removal of Toxic Agents: According to Cannon et al., a mouse carrier erythrocyte containing bovine rhodanase and sodium thiosulphate inhibited cyanide poisoning. It has also been documented how released erythrocytes expressing recombinant phosphodiesterase help to dissect organophosphorus poisoning.

Treatment of Hepatic Tumor: Erythrocytes have been used to administer anti-cancer medications such as methotrexate (MTX), bleomycin, asparaginase, and Adriamycin with success. For instance, MTX revealed in a study that it targets the liver first, then the lungs, kidneys, and spleen.

Delivery of Antiviral Agents: For efficient distribution and targeting, several antiviral medicines have been entrapped in resealed erythrocytes. Since most antiviral medications are nucleotides, it is important to carefully investigate how they enter and exit the membrane.

10. RECENT DEVELOPMENT

Nanoerythrosomes: Made by extruding RBC ghost, nanoerythrosomes have vesicles with an average diameter of 100 nm. Small vesicles the size of liposomes were produced by the technique. These spherical particles, known as "Nanoerythrosomes," appear to be durable and sustain daunorubicin's (DNR) cytotoxic and anti-cancer effects against mice leukemia P338D-cell. Antiviral medications can be prepared to go straight to macrophages.

Erythrosomes: Erythrosomes are specially designed vesicular systems in which a lipid bilayer is coated on top of chemically cross-linked human erythrocyte cytoskeletons. This can be accomplished by modifying a process that is typically used for reverse phase evaporation.

11. STORAGE OF RESEALED ERYTHROCYTES

Depending on the preservative solution and the finished product's container, storage characteristics may vary. Plasticized polyvinyl chloride bags are the typical storage containers for erythrocyte goods. Picking bags with plasticizers that are essentially non-extractable from blood should be done with caution. The assessment of extracted constituents from the chosen final container would boost consumer trust in the erythrocyte product's ability to be used medically. To prevent storage-induced RBC degradation, drug-loaded erythrocytes must be given to patients right away in contrast to long-lasting medications. The quality and stability of transformed cells are significantly influenced by the composition of the solution used to preserve the product. Although (saline, glucose, adenine, and mannitol) SGAM is the most popular erythrocyte storage solution, the US Food and Drug Administration has not granted it a license, so it is not utilized there. Other products such AS-1, AS-3, AS-5, MAP, erythrosol4, PAGGGM, and PAGGSM have been created and put on the market; some of these have shown to lessen hemolysis during storage when compared to SGAM. Recently, a test filter was created to enhance the erythrocyte quality while being stored [30-31].

12. CURRENT CLINICAL STATUS OF ERYTHROCYTE CARRIER

The technology is currently in its last stages of development for pharmaceuticals thanks to the pioneers in the industrial development of erythrocyte carriers. Companies have enough clinical proof and end-stage products in their pipelines to go through the last but not least hurdle in the way of marketing authorization. An effective medication (GR-ASPA) for acute lymphoblastic leukemia was created by ERYTECH Pharma, based in Lyon, France. Phase I/II8 and randomized worldwide Phase III [NCT01518517] studies conducted on children, adults, and elderly patients all showed promising results (Phase II trial) when compared to the free enzyme, allergic reactions in 84 patients who received homologous erythrocytes loaded with L-asparaginase (GR-ASPA) were less frequent and less severe.

In 2016, a Phase I clinical investigation was initiated. The technology is now in the last stages of pharmaceutical development thanks to the pioneers in the industrial development of erythrocyte carriers. Companies have enough clinical proof and end-stage products in their pipelines to go through the last but not least hurdle in the way of marketing authorization. In

the USA, Phase I clinical research has begun (NCT0181010705). To broaden the therapeutic potential of GR-ASPA, additional clinical trials were started in Europe. Ongoing acute myeloid leukemia IIb trial (NCT01810705). The potential therapeutic efficacy of this medication for solid tumors was demonstrated in preclinical animal investigations. Recently, a Phase I escalade dose trial was finished, and pancreatic cancer was given orphan drug designation (ODD) classification (NCT01523808).

Additionally, an early-stage medication called ENHOXY® has been created as a potent oxygen enhancer in the treatment of sickle cell disease and other disorders commonly linked to hypoxia (ODD granted in 2012). A method called EryDex was created by the Italian company EryDel to distribute dexamethasone over a lengthy period of time at low, steady systemic levels. Clinical pilot investigations involving individuals with Crohn's disease, ulcerative colitis, cystic fibrosis, and chronic obstructive pulmonary disease all showed that treatment was beneficial. A rare autosomal recessive neurological condition with a high death rate called ataxia telangiectasia has lately shown considerable therapeutic promise for the product. A pilot Phase II trial revealed the product's statistically substantial efficacy (NCT01255358). 2013 saw the product receive ODD status. EryDex is now the subject of a Phase I PK investigation in the USA, and a key Phase III trial should start up soon.

A recently established business focused on the treatment of rare and ultrarare diseases is called Orphan Technologies Ltd. OT-81-ADA-SCID (ADA-loaded erythrocytes for the treatment of severe combined immunodeficiency) and OT-15-MNGIE (TP-loaded erythrocytes for the treatment of MNGIE) are two new products that the firm has recently been granted permission to develop. Both substances are currently used compassionately on humans and have not yet started Phase I clinical trials [32-39].

13. CONCLUSION

Today, a wide range of uses for resealed erythrocytes as medication carriers, enzyme replacement treatment, etc. have been proposed. Resealed erythrocytes technology will continue to be an active field until alternate carrier systems mature. Additional study Resealed erythrocytes have the potential to be a secure and efficient treatment. A variety of bioactive chemicals are delivered for efficient targeting. However, the idea requires further refinement to become a standard drug delivery system. subsequent years represent a crucial period for this science as commercial applications are investigated. coming up future delivery method based on erythrocytes that can afford regulated and site-specific for managing disease, specialized drug delivery has been devised. As of right now, it is was determined that erythrocyte.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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