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REVIEW ARTICLE

Resealed erythrocytes as a carrier for drug targeting

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ABSTRACT

Erythrocyte-mediated drug delivery has been reported with therapeutic enzymes and antiviral agents to enhance therapeutic efficacy, minimize side effects, and serve as circulating depots for controlled drug release, drug targeting, parasitic disease treatment, hepatic tumour treatment, and the removal of toxic agents, among other applications. The benefits of using erythrocytes in drug delivery include a high degree of biocompatibility, full biodegradability, lack of toxic substance, controllable life-span, and decreased drug side effects, to name a few. Many medications with various side effects, such as aspirin, steroid, and cancer drugs, are decreased by resealed erythrocytes. Biopharmaceuticals, therapeutically important peptides and proteins, nucleic acid-based biologicals, antigens, and vaccines are just a few of the pharmaceuticals that have recently been targeted for delivery through carrier erythrocytes. Erythrocyte-based drug delivery systems with the potential to provide managed and site-specific drug delivery will be built in the future for disease management. To use the potentials of erythrocytes in passive as well as active drug targeting in diseases like cancer, a significant amount of useful work is needed. Because of their immense capacity, erythrocytes are currently the most effective carriers in novel drug delivery systems. Hence the present article is reviewed about isolation of erythrocytes, method of drug loading and applications of RSE.

INTRODUCTION

Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm^3 blood in a healthy male and 4.8 million cells/mm^3 blood in a healthy female), and they have potential as drug carriers and drug-loaded microspheres [1]. The resealed erythrocytes are biodegradable, biocompatible, non-immunogenic, non-pathogenic, self-degradable, reproducible, simple to prepare, and have a long circulation half-life. They can also be used to introduce a wide range of active drugs. Both of these features make them a revolutionary drug carrier that can be easily used to maximise the therapeutic effect of a drug while preventing any potential harmful effects [2].

Advantages of Resealed Erythrocytes

- This drug delivery system can be used in cases where the drug must be administered to phagocytic cells.
- This drug carrier system delivers drugs that are meant to show their action in the vascular lumen.
- They make the inclusion of proteins and nucleic acids possible in eukaryotic cells.
- No chemical modifications of the drug are required for their entrapment them into erythrocytes.
- Possibility of targeted drug delivery to the RES organs.
- Availability of knowledge, techniques, and facilities for handling, transfusion, and working with erythrocytes.
- Possibility of ideal zero-order kinetics of drug release.
- Wide variety of compounds with the capability of being entrapped within the erythrocytes.
- Modification of the pharmacokinetic & pharmacodynamic parameters of the drug.

- In comparison to traditional methods of drug administration, there is a remarkable reduction in steady-state concentration fluctuations, which is a common benefit for most innovative drug delivery systems.
- Desirable size range and the considerably uniform size and shape [3].

Disadvantages of Resealed Erythrocytes

- The most serious problem encountered in the use of resealed erythrocytes is their degradation in vivo by the reticulo-endothelial system.
- They are not much useful to carry the drug to a non-phagocytic target.
- Here are chances of clumping of the cells due to coagulation or other reasons.
- Possibility of dose dumping or leakage might be presented as their major drawback.
- Several biochemicals present in the body may alter their physiology at different points of time.
- The main issue with using biodegradable materials or natural cells as drug carriers is that they are removed in vivo by the RES as a result of changes made during the loading process in cells. While this increases drug targeting capabilities for RES, it severely restricts their lifetime as long-circulating drug carriers in circulation and, in some cases, can cause toxicological issues [4].

Isolation of Erythrocytes

Until now, erythrocytes from various mammals such as rodents, cattle, pigs, dogs, sheep, goats, monkeys, chickens, rats, and rabbits have been used to distribute medicines. To extract erythrocytes from blood, a sample is taken in heparinized tubes via cardiac/splenic puncture (in small animals) or venepuncture (in large animals) and a drop of anticoagulant is applied. Since fresh blood has higher encapsulation efficiency than older blood, it is used more often to produce the best performance. Fresh blood is collected and immediately chilled to 4°C before being stored for nearly two days. The erythrocytes are then harvested and washed using the centrifugation method (at an rpm of 2500 for 5 minutes in a refrigerated centrifuge). The washed cells are then suspended in buffer solutions (typically phosphate buffer saline, PBS, with a pH of 7.4) to obtain the desired haematocrit values. The suspensions are then kept at 4°C for at least 2 days before being used in an acid-citrate-dextrose buffer solution [5].

Methods of Drug Loading in Erythrocytes

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

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

- **Hypotonic Haemolysis:**

The concept behind this approach is that in a hypotonic solution, erythrocytes quickly experience reversible swelling. An increase in cell volume (due to osmosis caused by being held in a hypotonic solution) is observed in this process, as well as an initial change in the shape of the erythrocytes from biconcave to spherical. The absence of a superfluous membrane in erythrocytes accounts for this shift in shape, which maintains their surface area stable. The volume of the cell rises by around 25% to 50%. Without losing their dignity, these cells can withstand a tonicity of up to 150 m osm/kg. The membrane ruptures at this stage, allowing the cellular contents to leak out. The cellular material is depleted as a result of this lysis, and the remnants are known as erythrocyte ghosts [6].

- **Use of Red Cell Loader:**

Nondiffusible drugs were entrapped in erythrocytes using a novel technique. They built a piece of machinery known as a "red cell loader." Different biologically active compounds were entrapped into erythrocytes in as little as 50 mL of blood over a 2-hour period at room temperature under blood banking conditions. Two hypotonic dilutions of washed erythrocytes are followed by concentration with a hemofilter and isotonic resealing of the cells in this process. There was a 30% drug loading rate, with 35–50% cell recovery. By enhancing their identification by tissue macrophages, the same cells could be used for targeting [7].

- **Hypotonic Dilution:**

A volume of packed erythrocytes is diluted with 2–20 volumes of an aqueous drug solution in this process. After that, a hypertonic buffer is applied

to preserve the solution's tonicity. After centrifuging the mixture, the supernatant is discarded, and the pellet is washed in isotonic buffer solution. This decreases the loaded cells' circulation half-life. Since RES macrophages readily phagocytoze these cells, they can be used to attack RES organs. For loading enzymes like galactosidase and glucosidase, as well as asparginase, hypotonic dilution is used [8].

- **Hypotonic Preswelling:**

Initial regulated swelling in a hypotonic buffered solution is the basis of the technique. At a low g, this mixture is centrifuged. The supernatant is discarded, and the cell fraction is lysed by adding 100–120 litres of an aqueous solution of the drug to be encapsulated to the cell fraction. Between the drug addition steps, the mixture is centrifuged. When centrifugation is used to detect the lysis stage, a distinct boundary between the cell fraction and the supernatant disappears. At the lysis stage, a measured volume of hypertonic buffer is applied to restore the tonicity of a cell mixture. The cell suspension is then incubated at 37°C to reanneal the erythrocytes that have been resealed. The circulation half-life of these cells is equivalent to that of regular cells. This process is easier and quicker than others, and it causes the least amount of harm to cells. Propranolol, asparginase, and cyclophosphamide are among the drugs encapsulated in erythrocytes using this process [9].

- **Hypotonic Dialysis:**

This method is used to load enzymes and lipids in particular. An isotonic buffered erythrocyte suspension (with a hematocrit value of 70-80) is prepared and inserted in a traditional dialysis tube immersed in 10-20 volumes of a hypotonic solution in this method. It is subjected to 2 hours of agitation. By applying a calculated volume of hypertonic buffer to the surrounding medium, the tonicity of the dialysis tube is restored. The drug that will be loaded into the erythrocytes can be applied to the dialysis bag at any time during the agitation process. This method is used to load enzymes like B-galactosidase, glucoserebrosidase, asparginase, inositol, hexaphosphatase, and drugs like gentamicin, adriamycin, pentamidine, and furamycin, as well as inteleukin-2 and human recombinant erythropoietin, with a high entrapping performance [10].

- **Isotonic Osmotic Lysis:**

Isotonic hemolysis is achieved by physical or chemical means in this process, also known as the osmotic pulse method. Because of the concentration gradient, when erythrocytes are incubated in solutions of a material with high membrane permeability, the solute diffuses into the cells. To preserve osmotic equilibrium, this mechanism is followed by an influx of water. For isotonic heamolysis, chemicals such as urea solution, polyethylene glycol, and ammonium chloride have been used. An isotonic-buffered drug solution was used to dilute the suspension. The cells were then resealed at 37°C after being separated [11].

- **Chemical Perturbation of the Membrane:**

This method is based on the fact that when erythrocytes are exposed to certain chemicals, their membrane permeability increases. When exposed to a polyene antibiotic like amphotericin B, the permeability of the erythrocytic membrane increases. However, since these approaches cause permanent damage to the cell membrane, they are not commonly used [12].

- **Entrapment by endocytosis:**

Endocytosis is achieved by mixing one volume of erythrocytes with nine volumes of buffer solution containing 2.5 mM ATP, 2.5 mM MgCl2, and 1 mM CaCl2, then incubating for two minutes at room temperature. The pores formed on the surface of the erythrocytes by this method are resealed by using 154 mM NaCl followed by a 2-minute incubation at 37°C. Endocytosis is the mechanism by which the drug to be loaded into erythrocytes is entrapped. After the process of endocytosis, a vesicle forms within the erythrocytes, and the membrane of that vesicle separates the drug content from the erythrocyte's cytoplasm [13].

- **Loading by electric cell fusion:**

The loading of drug molecules into erythrocyte ghosts is accompanied by the adhesion of these cells to target cells in this process. The application of an electric pulse accelerates the fusion by triggering the release of an entrapped molecule. Loading a cell-specific monoclonal antibody into an erythrocyte ghost is an example of this procedure. Chemically cross-linking an antibody against a particular surface protein of target cells to drug-loaded cells will guide these cells to the targeted cells [14].

- **Loading by Lipid Fusion:**

A drug in a lipid vesicle may be fused directly to an erythrocyte. This approach employs this theory. The drug is transferred between the lipid vesicle and the erythrocyte after the fusion. This process traps inositol monophosphate, reducing the oxygen carrying capacity of haemoglobin in cells significantly. However, the drug entrapment's effectiveness is extremely poor [15].

Release Mechanism of Loaded Drugs:

There are mainly three ways for a drug release from the erythrocyte carriers.

1. Phagocytosis:

Normally, erythrocyte cells are removed from the blood circulation by the process of phagocytosis. The degree of cross-linking determines whether the cells are removed preferentially by the liver or the spleen.

2. Diffusion through the membrane of the cells:

Diffusion through the membrane is determined by the drug molecule's ability to move through a lipid bilayer, indicating that bioactive compounds are lipid soluble [16].

3. Using a specific transport system:

Since the carriers are proteins with several properties identical to those of enzymes, the majority of drug molecules join cells through a particular membrane protein system [17].

***In-Vitro* Characterization of Loaded Erythrocytes**

1. Cell Counting and Cell Recovery:

The number of red blood cells per unit volume of whole blood is counted which is normally done automatically. The difference in hematocrit and volume of the erythrocyte suspension before and after loading can be used to measure red cell recovery. The goal is to reduce cell loss as much as possible during the encapsulation process in order to optimise cell recovery [18].

2. Morphological Aspect:

The morphological analysis of these ghost erythrocytes is carried out using transmission electron microscopy (TEM) or scanning electron microscopy (SEM) in contrast to untreated erythrocytes. The morphological changes in erythrocytes caused by osmosis-based encapsulation methods when they are exposed to solutions with varying osmolality can be observed using electron microscopy [19].

- **Osmotic Fragility:**

Osmotic fragility is a test that detects irregular red blood cell fragility. The relative fragility of untreated or loaded erythrocytes is measured by exposing them to hypotonic solutions, which causes them to swell [19].

- **Osmotic Shock:**

Erythrocyte suspension (1 mL, 10%) was diluted with H_2O (5 mL) and centrifuged at 3000 rpm for 15 minutes in the far 0.5 sample. Using spectrophotometry, the supernatant's % Hb release was determined.

- **Turbulence Shock:**

Turbulence shock helps researchers to evaluate the stability of loaded erythrocytes in the face of turbulence stress caused by *in-vivo* circulation turbulence. The test is carried out using the Deloach et al. process, which involves passing a cell suspension through a 22-gauge needle several times.

- **Haemoglobin Release:**

During the encapsulation process, changes in the permeability of the red cell membrane may reduce the content of haemoglobin in the erythrocytes. Furthermore, recognising the processes involved in the release of the substance encapsulated from the erythrocytes is aided by the relationship between the rate of haemoglobin and the rate of drug release. A red cell suspension is used to monitor haemoglobin leakage, and the absorbance of the supernatant is measured at 540 nm on a spectrophotometer [20].

Applications of Resealed Erythrocytes

***In-Vitro* Application:**

Phagocytosis cells were used to help in the uptake of enzymes by phagolysosomes for this function. A cytochemical technique can be used to verify the enzyme content of carrier erythrocytes. One such biochemical defect is a deficiency of the enzyme glucose-6-phosphate dehydrogenase, which may be critical in identifying the mechanism that causes these effects. The most important *in-vitro* application of resealed erythrocytes is the principle of "microinjection":

- ✓ A protein or nucleic acid can be injected into eukaryotic cell by fusion process.
- ✓ Antibody molecules may be inserted into erythrocytic carrier systems and diffuse into the cytoplasm almost instantly. The antibodies, on the other hand, do not seem to penetrate the nucleus of the

erythrocytes. As a result, future research will be limited to the cytoplasm only [21].

***In-Vivo* Application:**

The various *in-vivo* applications of resealed erythrocytes are given as follows:

a. Targeting of Bioactive Agents to RES (Reticulo-Endothelial System)

In the liver and spleen, phagocytic Kupffer cells easily clear damaged erythrocytes from circulation. By altering the membranes of resealed erythrocytes, they can be used to attack the liver and spleen. Surface alteration with antibodies, gluteraldehyde, carbohydrates such as sialic acid, and sulphydryl are some of the methods used to alter the surface characteristics of erythrocytes [22].

b. Targeting of Sited other than RES Organs

Resealed erythrocytes have the ability to deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES have been performed by certain researchers.

c. Resealed Erythrocytes as Circulating Bioreactors

Erythrocytes have been reported to be enzyme carriers and circulating bioreactors. Reduced levels of circulating metabolites that can enter erythrocytes are often desirable. They've also been used as circulating bioreactors to deliver antiviral drugs in a controlled manner [23].

d. Resealed Erythrocytes as Carriers for Drugs

Erythrocytes have been discovered to be enzyme carriers that can be used as circulating bioreactors. Reduce the amount of circulating metabolites that can penetrate erythrocytes is often beneficial. They've also been used as antiviral drug delivery mechanisms in circulating bioreactors.

e. Resealed Erythrocytes as Carriers for Enzymes

Enzymes can be inserted into the bloodstream to replace a missing or deficient enzyme in metabolic disorders or to degrade harmful substances that have accumulated in the bloodstream as a result of a disease. Similarly, metabolic disorders such as Gaucher's disease, hyperarginaemia, hyperuricaemia, hyperphenyl-alaninemia, and kidney failure are examples of environmental, lysosomal storage disorders that can be treated by administering enzymes borne by resealed erythrocytes [24].

Recent Development

Nanoerythrosomes:

Nanoerythrosomes are vesicles formed by the extrusion of RBC ghost, with a diameter of 100 nm on average. The treatment resulted in small vesicles the size of liposomes. Nanoerythrosomes are spherical particles that tend to be stable and sustain both the cytotoxic and antineoplastic action of daunorubicin (DNR) against the P338-D-cell leukaemia in mice. Antiviral drugs may be pretreated such that they enter macrophages directly.

Erythrosome:

Erythrosomes are chemically cross-linked human erythrocyte cytoskeletons coated with a lipid bilayer in specially engineered vesicular systems. This can be done using a reverse phase evaporation adjustment technique. Erythrosomes have been proposed as a potential encapsulation system for drug delivery, particularly for macromolecular drugs [25].

CONCLUSION

Collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resulting cellular carriers are all it takes to create drug-loaded carrier erythrocytes. The benefits of using erythrocytes in drug delivery include a high degree of biocompatibility, full biodegradability, lack of toxic substance, controllable life-span, and decreased drug side effects, to name a few. There have been several plans for using resealed erythrocytes as drug carriers, enzyme replacement therapy, and other applications in recent years. Resealed erythrocytes technology will remain an active area for further study until other carrier systems mature. The use of resealed erythrocytes shows promise for delivering various bioactive molecules in a safe and efficient manner for effective targeting. However, in order to be transformed into a standard drug delivery system, the principle must be further refined. The coming years will be a critical period in this area, as commercial applications are investigated. Erythrocyte-based delivery systems with the potential to provide managed and site-specific drug delivery will be built in the future for disease management. Erythrocyte carriers are a type of nanotechnology device.

The key recommendation for future research is to use a carrier to carry steroids and hormones to the targeted site. As a consequence, we will reduce the number of negative side effects. We may boost drug targeting area and reduce many side effects by resealing erythrocytes. For the present, it has been determined that erythrocyte carriers are "golden eggs in novel drug delivery systems" due to their immense ability. According to the results of the study, resealed erythrocytes have emerged as drug carriers with precise site specificity and long-term drug release. They have advantages over other carriers in terms of longer life, ease of maintaining stable focus, and so on. As a result, resealed erythrocytes tend to be promising drug carriers, with a crucial role in the treatment of a wide variety of morbid conditions. However, much further research is needed in this field of drug delivery before the ability of resealed erythrocytes can be successfully tested in the future.

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