World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Review Article

ADVANCEMENTS IN THE TREATMENT OF LIVER DISORDERS USING RESEALED ERYTHROCYTES

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Received: 15-11-2024 / Revised Accepted: 17-11-2024 / Published: 19-11-2024

ABSTRACT:

Red Blood Cells (RBCs) have distinct qualities including biocompatibility, biodegradability and the ability to release drugs selectively are becoming a viable drug delivery platform these days. The reversible opening of erythrocyte membranes made feasible by recent developments in nanopore technology permits efficient loading and encapsulation of therapeutic drugs. This strategy has the potential to transform both therapeutic and diagnostic applications to be flexible and targeted drug administration. Since resealed erythrocytes can accurately deliver medications while minimizing side effects, they show great potential in both passive and active targeted applications. Erythrocytes also can flex through capillaries and their biconcave form improves their effectiveness as drug transporters. Numerous techniques have been developed for encapsulating pharmaceuticals with erythrocytes, such as electro-encapsulation, hypotonic dilution, and hypotonic haemolysis. Each of these approaches has unique benefits with controlled release and drug loading efficiency. The use of erythrocytes in drug delivery has significant advantages over other factors, such as potential alterations to erythrocyte physiology and the reticuloendothelial system (RES) rapid clearance. In addition, Resealed erythrocytes have demonstrated exceptional potential in delivering antineoplastic medicines, targeting hepatic tumours, and providing enhanced pharmacokinetics in treating liver problems. These carriers may become widely used in therapeutic settings, especially for the facilitated delivery of hormones, steroids, and biopharmaceuticals. The future prospects of erythrocyte-based drug delivery systems as an innovative and attainable strategy in modern medicine are highlighted in this paper.

INTRODUCTION

Erythrocytes, or red blood cells, have been the focus of a lot of research because of their potential as drug delivery systems and carriers of drug-loaded microspheres.[1] The ability to reversibly open red blood cell membrane nanopores thanks to recently developed technology offers the incredible possibility of repurposing human erythrocytes for use in a variety of therapeutic and diagnostic biomedical applications. [2]The pharmaceutical industry of today uses over thirty distinct drug delivery systems. Due to a lack of target specificity, the current landscape of medication delivery places greater emphasis on targeted drug delivery systems. Erythrocytes, the most common type of blood cells, travel thousands of miles across both wide and narrow paths during their life in order to deliver oxygen, nutrition, and drugs.

Red blood cells have potent and targeted potential carrier capacities for a variety of drugs. Drug-loaded carrier erythrocytes or resealed erythrocytes hold potential for a variety of targeted passive and active uses. Resealed erythrocytes hold several benefits over other drug carrier models, such as their ideal zero-order drug-release kinetics, biocompatibility, biodegradability without harmful byproducts, inert intracellular environment, potential for entrapment of different chemicals, ability to circulate throughout the body, protection against the drug's toxic effects, lack of unwanted immune response against encapsulated drug, etc. Reticuloendothelial System (RES) macrophages in the liver, lung, and spleen swiftly absorb drugs from resealed erythrocytes. Resealed erythrocytes can be used to safely and successfully distribute drugs that are particularly targeted for a longer duration.[20] Erythrocytes, also referred to as red blood cells (RBCs), provide a feasible platform for cell-mediated drug delivery due to their inherent biocompatibility. [3] Blood and its biological components, particularly red blood cells, are regarded to be among the most beneficial natural drug delivery vehicles. Since the 1960s, red blood cells (RBCs) have been the subject of extensive research in this area. [4]

Morphology and Physiology of Erythrocytes [6]

Erythrocytes are biconcave disks, meaning that their edges are fat and their interiors are incredibly thin. As you will soon learn, they have greater space inside their interiors for the haemoglobin molecules that carry gasses since they lack the majority of organelles. The biconcave shape offers a larger surface area for gas exchange in

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How to Cite this Article Sincy Sebastian. Advancements In The Treatment Of Liver Disorders Using Resealed Erythrocytes, World J Pharm Sci 2023; 12(04): 23-27; https://doi.org/10.54037/WJPS.2022.100905.

relation to its volume than a sphere with a similar diameter, which would have a lower surface area-to-volume ratio. Some of the oxygen transported by the erythrocytes can diffuse into the plasma and then past the capillary walls to reach the cells, while some of the carbon dioxide waste product that the cells create diffuses into the capillaries for the erythrocytes to pick up. Because capillary beds are so small, erythrocyte transit is inhibited and gas exchange can occur over extended periods of time. Nevertheless, because capillaries might have such a little space, erythrocytes may need to fold in on themselves in order to pass through. Fortunately, their structural proteins, like spectrin, are flexible enough to allow them to bend over themselves to an unanticipated degree and then spring back when they reach a wider channel. The term "rouleaux," which translates from the French word meaning "roll," refers to an erythrocyte stack in a larger vessel that resembles a roll of coins.

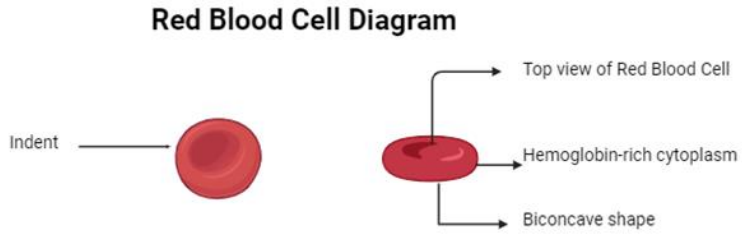

Figure.1 Red Blood Cell Diagram

Factors which considering resealed erythrocytes as carrier:

- Its dimensions and form should allow for capillary flow.
- The distinct physico-chemical characteristics that allow one to identify a necessary site.
- Its little toxicity and biocompatibility.
- Once the medication is released at the intended location, its breakdown product ought to be biocompatible.
- Prior to the medicine reaching its target site, there should be minimal leaching or leakage.
- The regulated release pattern of the medication.
- Excellent drug loading efficiency for a wide range of medications with various characteristics.
- Compatibility of the medication with the body chemically.
- During storage, there should be a noticeable level of stability in the carrier system.

Advantages of Erythrocytes in Drug Loading: [7-16]

1. Outstanding biocompatibility, particularly when medications are transferred onto autologous cells.
2. Complete biodegradability and the lack of toxic chemicals resulting from the carrier's breakdown.
3. The logical defense mechanism of the organism against the negative impacts of the artificial carriers.
4. Under optimal loading procedure settings, the live lifetime of the generated carrier cells may be comparable to that of normal erythrocytes.
5. A very controllable life duration, ranging from minutes to months.
6. A high level of shape and size homogeneity and a desired size range.
7. Preventing the loaded chemical from being inactivated by endogenous agents.
8. The possibility of giving certain drugs to the RES organs.
9. The intracellular environment is usually inactive.
10. The resources, techniques, and information that are available for erythrocyte management, transfer, and utilization.
11. Perfect zero-order drug release kinetics could be possible.
12. A wide variety of materials that have the ability to adhere to erythrocytes.
13. Modification of the pharmacokinetic and pharmacodynamic properties of the medication.
14. When compared to traditional drug administration techniques, a common advantage of most novel drug delivery systems is the considerable decrease in concentration variability in the steady state.
15. Noticeably longer medication dosing intervals that nevertheless allow for the maintenance of a long-term, safe drug concentration

Disadvantages of Erythrocytes in Drug Loading: [16-20]

Because of changes produced during the loading process in cells, the primary problem with using natural cells or biodegradable materials as drug carriers is that the RES eliminates them in vivo. Although this makes it more likely that drugs will target RES, it also drastically reduces their half-life as long-circulating drug carriers and may in certain cases result in toxicological problems.

Some substances that are confined from the laden erythrocytes are released quickly.

Several chemicals have the ability to alter the erythrocyte's physiology.
Since encapsulated erythrocytes are biological carriers, they may display inherent variations in loading and characteristics when compared to alternative carrier systems.
Possible contamination from the source of the blood, the equipment used, and the loading environment. Strict guidelines are required for the erythrocyte collection and management.

Methods of preparation and encapsulation of resealed erythrocytes: [21]

Hypotonic Haemolysis: The ability of erythrocytes to swell reversibly in a hypotonic solution is the basis of this approach. In a conventional dialysis tube immersed in 10–20 liters of a hypo-tonic solution, erythrocytes with a haematocrit value of 70–80 are suspended in a buffered isotonic solution during the operation.Selected erythrocytes spherulate red blood cells, allowing the pores to open.The red blood cells enlarge and are filled with medicinal substances.After that, the erythrocytes are cleaned and sealed once more.Erythrocytes with drug encapsulation are produced.

Hypotonic dilution: Using two to twenty liters of an aqueous medicine solution, a volume of packed erythrocytes is diluted in this manner. The solution's tonicity is then restored by adding a hypertonic buffer. The combination is centrifuged, the supernatant is discarded, and an isotonic buffer solution is used to clean the pellet. This reduces the circulatory half-life of the loaded cells. Given that RES macrophages can readily phagocytose these cells, these cells can be used to target RES organs. Aspartginase, glucosidase, and galactosidase are among the enzymes that are loaded by hypotonic dilution.

Hypotonic Pre swelling: The technique is based on precisely controlled initial swelling in a hypotonic buffered solution. This mixture is centrifuged at low g levels. The cell fraction is brought to the lysis point by adding 100–120 liter volumes of an aqueous solution containing the drug to be encapsulated, after the supernatant has been discarded. The mixture is centrifuged between the steps where medications are introduced. The lysis point is shown by the absence of a distinct separation between the cell fraction and the supernatant following centrifugation. A calculated amount of hypertonic buffer is supplied to a cell combination to restore its tonicity once it reaches the lysis point. After that, the erythrocytes are made to reseal by incubating the cell suspension at 37 °C. These cells have a circulatory half-life that is comparable to regular cells. This method is speedier and less taxing on the cells than others. This method is used to encapsulate drugs like propranolol, asparginase, and cyclophamide in erythrocytes.

Hypotonic Dialysis: This method is based on the theory that a semipermeable dialysis membrane maximizes the intracellular to extracellular volume ratio during the lysis and resealing of macromolecules. A conventional dialysis tube is filled with 10–20 liters of a hypotonic solution, and erythrocytes with a haematocrit value of 70–80 are suspended in a buffered isotonic solution before being placed in it. The medium is gradually agitated for two hours. A calculated amount of a hypertonic buffer can be added directly to the dialysis tube, or the surrounding medium can be substituted with an isotonic buffer, to bring the tube's tonicity back.The drug to be loaded can be dissolved in isotonic cell suspending buffer and added to a dialysis bag at the start of the experiment or after the stirring is complete. By following this protocol, the drug to be loaded and the erythrocyte suspension were put in the blood compartment, and the hypotonic buffer was put in a receptor compartment. Consequently, the concept of "continuous flow dialysis" was created and has subsequently been utilized by multiple additional investigators. This method has been used to load enzymes such as galactosidase and glucose rebrosidase, as well as drugs such as pentamidine, interlukin-2, gentamicinadriamycin, and human recombinant erythropoietin.

Isotonic Osmotic Lysis:This method, also known as the osmotic pulse method, produces isotonic hemolysis via physical or chemical means. Erythrocytes cultivated in solutions containing a substance with high membrane permeability will allow the solute to infiltrate into the cells due to the concentration gradient. Following this procedure, water is introduced to maintain osmotic equilibrium. Chemicals such as ammonium chloride, polyethylene glycol, and urea solution have been used for isotonic hemolysis. The suspension was diluted using an isotonic medicine solution. At 37 °C, the cells were divided and then sealed once more.

Chemical perturbation of the Membrane:The fact that erythrocytes treated with particular chemicals show greater membrane permeability serves as the basis for this approach. The permeability of erythrocytic membranes is increased by exposure to polyene antibiotics such as amphotericin B. However, these methods are not particularly popular because they permanently damage the cell membrane 26.

Electro-insertion or Electro-Encapsulation Erythrocytes are held in an isotonic buffer and used as part of an electrical discharge chamber operation (Fig. 3). A capacitor in an external circuit is charged to a particular voltage and then discharged through cell suspension at a predetermined time interval to produce a square-wave potential. The optimal intensity and discharge time of an electric field are 1 to 10 kW/cm and 20 to 160, respectively. There is an inverse relationship between the discharge time and the electric field intensity. The material to be trapped is added to the media in which the cells are suspended from the start of the experiment. This process can be halted by adding large molecules (such ribonucleose and bovine serum albumin). A number of materials, such as isoniazid, methotrexate, sucrose, urease, DNA fragments, sucrose, urease, methotrexate, isoniazid, and human glycophorin.

Entrapment by Endocytosis: Nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1 mM CaCl2 are combined with one volume of packed and cleaned erythrocytes. Endocytosis is then carried out by letting the combination stand at room temperature for two minutes. The holes created by this method are sealed once more by incubation at 37 °C for two minutes following the addition of 154 mm of NaCl. The process of endocytosis results in the entrapment of material. Among the numerous candidates identified by this method are vinblastine, hydrocortisone, chlorpromazine, primaquine and related 8-aminoquinolines, and the vitamin.

Loading by Electric Cell Fusion: This method involves loading pharmacological molecules into erythrocyte ghosts, which are subsequently affixed to target cells. An electric pulse administered to the region highlights the fusion by releasing an imprisoned molecule. An example of this technology is an erythrocyte ghost loaded with a cell-specific monoclonal antibody. Cells loaded with drugs can be guided toward the appropriate cells by chemically crosslinking an antibody against a specific target cell surface protein.

Loading by Lipid Fusion: Lipid vesicles holding drugs can be fused directly to human erythrocytes, facilitating the exchange of drugs with a material encapsulated in lipids. This technique was used to entrap inositol monophosphate, increasing the capacity of cells to carry oxygen. However, the 1% trapping efficiency of this approach is quite low.

Targeting hepatic tumours:

An accumulation of glucocerebrosides in the liver and spleen can cause a disease that can be treated by erythrocytes loaded with glucocerebrosidase. [1] One of the most prevalent types of cancer in the world is hepatic tumors. It has been effective to provide anticancer drugs such methotrexate, bleomycin, asparaginase, and adriamycin via erythrocytes. Drugs like daunorubicin that load quickly diffuse out of the cells and cause problems. This problem can be rectified by covalently attaching daunorubicin to the erythrocytic membrane using gluteraldehyde or cisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show hepatic localization.[21] Applications in liver disorders include enzyme replacement therapy, side effect reduction, gene therapy treatment of hereditary abnormalities, improved pharmacokinetics in longer drug release and decreased immunogenicity, and targeted medication delivery in hepatocellular carcinoma and liver fibrosis.[22] These enzymes can be injected to treat a variety of metabolic illnesses linked to low or absent enzyme levels. The disadvantages of exogenous enzyme therapy include allergic responses, toxic effects, and a shortened half-life of the enzymes in circulation. These problems can be successfully handled if the enzymes are administered as resealed erythrocytes. Among those that are employed are the enzymes galactosidase, glucoronidase, and glucosesidase. A glucocerebroside accumulation in the liver and spleen can induce a disease that can be treated by erythrocytes loaded with glucocerebrosidase.Hepatic tumors are among the most prevalent cancer types in the globe. It has been effective to provide anticancer drugs such methotrexate, bleomycin, asparginase, and adriamycin via erythrocytes.[23] Drugs like daunorubicin that load quickly diffuse out of the cells and cause problems. This problem can be rectified by covalently attaching daunorubicin to the erythrocytic membrane using cisaconitic acid or gluteraldehyde as a spacer. The resealed erythrocytes loaded with carboplatin show hepatic localization..[24] Current affairs Nanoerythrosomes RBC ghosts are extruded to create nanoerythrosomes, which are vesicles with an average diameter of 100 nm. The method generated small liposome-sized vesicles. These spheroid particles, dubbed "nanoerythrosomes," appear to be resilient and maintain the cytotoxic and anti-tumor actions of daunorubicin on mouse leukemia P338D cells. There have been notable advancements in the precise targeting of immune system cells using erythrocytes. Antiviral drugs can be ready to be administered directly to macrophages. The allosteric activator of hemoglobin, inositol hexaphosphate, has been encapsulated using a variety of experimental techniques. This method is significantly more efficient in delivering oxygen than using conventional erythrocytes. [25]

Conclusion:

Resealed erythrocytes have been proposed for use as medication and enzyme replacement therapy carriers in a variety of applications in the modern world. Research on resealed erythrocyte technology will be ongoing as other carrier systems get more developed. It is clear that resealed erythrocytes have the ability to deliver bioactive compounds for precise targeting in a safe and efficient manner. Redisclosed erythrocytes have the ability to deliver a range of medications for both passive and active targeting in a safe and dependable manner. To make this idea a widely used drug delivery system, more optimization is required. The delivery of biopharmaceuticals can also be accomplished through this technique, and the possibilities of resealed erythrocytes remain largely unexplored.

My main suggestion for further research is to use carriers to deliver hormones and steroids to particular target locations. This strategy might lessen a number of the negative impacts connected to the delivery systems used today. Resealed erythrocytes may be used to improve medication targeting and reduce adverse effects. In conclusion, because of their enormous potential, erythrocyte carriers are seen to be particularly promising for innovative drug delivery methods.

Acknowledgement:

The authors are thankful to Caritas College of Pharmacy for giving us the opportunity and providing required necessary facilities to work on this topic.

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