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REVIEW ON RESEALED ERYTHROCYTES AS A NOVEL DRUG DELIVERY SYSTEMS

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ABSTRACT

Cellular carriers possess great potential in novel drug delivery systems. Among such cellular carriers, erythrocytes have been found to contain enormous merits for targeted and controlled drug delivery systems. Erythrocyte carriers are nanodevices in the field of nanotechnology.Erythrocytes are also known as RBCs. The compatibility with biological,anti-pathogen,anti-immunogenicity and ability to decompose make them unique and beneficial carriers. These carriers are prepared by collecting the blood sample from the organism by separating red blood cells from plasma, entrapping the drug in red blood cells and resealing the obtained cellular carriers, which are called resealed erythrocytes. Erythrocytes are prepared using methods like hypotonic dilution, hypotonic dialysis, pre-swelling, osmotic lysis, perturbation, and lipid fusion.Presents articles give information about introduction, properties, advantages and disadvantages, composition, isolation, drug loading methods, evaluation, application and novel approaches of resealed erythrocytes.

KEYWORDS: Resealed Erythrocyte, Nano Devices, Controlled Release, Drug Loading, Drug Targeting.

INTRODUCTION[1,2,3]

Drug delivery is the method of formulation, technology or systems for transporting an active pharmaceutical ingredient to the target receptor at an organ in the body without any loss or compromise in the chemical integrity of the molecule and could be manipulated to affect the desired pharmacological action. Drug delivery techniques significantly modify different pharmacokinetic parameters like drug release, absorption, distribution, elimination, etc. The compounds used to carry drugs to the target site are called drug carriers. And it should help the drug extend its in-vivo action, avoid metabolism, reduce toxicity, etc. Present pharmaceutical circumstances are designed to develop drug delivery systems that make the best use of the drug targeting along with high therapeutic effects for safe and efficient treatment of diseases and more patient compliance. Here various carrier systems are present for targeting the particular tissue. The idea of a drug carrier system with target specificity is helpful for scientists, and tremendous efforts have been made to achieve this goal. Many carriers target the drug to body tissue; the most important is biocompatible with carrier and their degradation product. White blood cells, platelets, red blood cells, liver cells and fibroblasts, etc., have been used as a cellular carrier system. among these, red blood cells are widely used as drug carriers due to their ability to circulate in the whole body, biocompatibility, reproducibility, zero-order release, etc.

Resealed erythrocytes as a novel drug delivery systems

Erythrocytes, also called red blood cells, are mainly used as carriers for drug delivery systems. Such drug-loaded cellular carriers are prepared by collecting a blood sample from the organism by separating the red blood cells from plasma, entrapping the drug in red blood cells and resealing the obtained carriers. Hence, these carriers are called resealed erythrocytes. The whole process depends on the response of this carrier under osmotic conditions. When an injection is repeated, the drug-loading red blood cells serve as depots and targets to the reticuloendothelial cells (RES).

Properties of resealed erythrocytes[4,5]

1. Possess the ability to carry a broad spectrum of drugs with different properties.
2. Physico- chemically compatible with drugs.
3. The carrier system should have appreciable stability during storage
4. The drug must be resealed at the desired site.
5. It should possess proper size and shape and permit passage through the capillaries with the slightest leakage of drugs.

Advantages of resealed erythrocytes[6,7]

1. A wide degree of biocompatibility.
2. Their complete biodegradability with no generation of toxic products from the carrier biodegradation.
3. Uniform size and shape of the carrier system.
4. Considerable protection of the organism against the toxic effects of the encapsulated drug. E.g.Antineoplasts
5. A wide variety of chemicals can be entrapped.
6. Targeted specificity within the reticuloendothelial system (RES).
7. Prolonged systemic activity by long residence time in the body.

Disadvantages of resealed erythrocytes[8,9]

1. It has restricted potential as a carrier of non-phagocytic tissue.
2. Chances of clumping of cells and dose dumping.
3. Relatively costly.

Isolation of erythrocytes[10]

1. For the isolation of erythrocytes, fresh whole blood should be used.
2. Fresh whole blood is the blood that is collected and immediately chilled to 4°C and stored for less than two days.
3. Blood is withdrawn from a cardiac or splenic puncture in the case of small animals and through veins in animals in a syringe containing a drop of the anticoagulant.
4. The blood is then collected into heparinised tubes.
5. The collected whole blood is then centrifuged for 5 minutes at a speed of 2500 rpm at a temperature of 4 ± 1^0C in a refrigerated centrifuge.
6. After the process of centrifugation, the serum and buffy coats are removed carefully, and the packed cells are washed with phosphate buffer saline of 7.4 pH at least three times.
7. The washed erythrocytes are then diluted with phosphate buffer saline.
8. Then the washed erythrocytes are stored at 4^0C in an acid-citrate-dextrose buffer for up to 48 hours before use.

Method of drug loading in resealed erythrocytes[2,4,8,9,10,11,12]

- **Hypo-osmotic lysis method[2]**

In this method, erythrocytes' intracellular and extracellular solutes are interchanged by osmotic lysis and resealing. This method must encapsulate the drug within the red blood cell membrane.

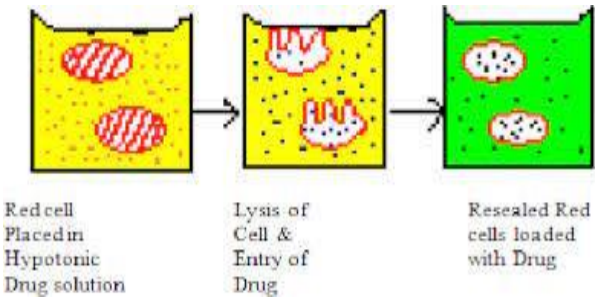

Fig. No. 1: Hypo-osmotic lysis method.

- **Hypotonic dilution or dilutional method[4]**

Hypotonic dilution was the first method explored for encapsulating chemicals into erythrocytes and is one of the simplest and fastest methods. In this process, packed erythrocytes are diluted with 2–20 volumes of an aqueous solution of a drug. The addition of hypertonic buffer then reestablishes the solution tonicity. The obtained mixture is then centrifuged at a pre-determined rate, the supernatant liquid is removed, and the pellet is washed with an isotonic buffer solution. The most significant downsides of this method consist of low entrapment efficiency and a considerable loss of hemoglobin and other cell components. Which further leads to a reduction in the circulation half-life of the loaded cells? RES macrophages easily phagocytise these cells and, therefore, are used for targeting RES organs. The hypotonic dilution method is used primarily for loading enzymes such as B-galactosidase and B-glucosidase, asparaginase, and arginase, as well as bronchodilators such as salmeterol.

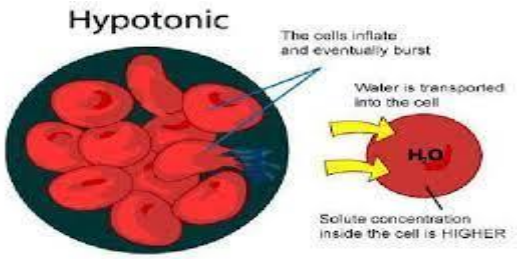

Fig. No. 2: Hypotonic dilution.

- **hypotonic Pre-swelling method**[8]

This method depends on the principle of first swelling the erythrocytes without lysis by keeping them in a hypotonic solution. Development of this method by the Rechsteiner in the year 1975. The swollen cells are allowed to recover by centrifugation at low speed. Relatively least volumes of aqueous drug solution are added at the point of lysis. This method's advantages are simple and faster and cause minor damage to cells. Due to gravitational force, the supernatant layer is discarded, and the detection point is considered when the boundary between the cell lines and the supernatant disappears. By adding an adjusted amount of hypertonic buffer, the tonicity of a cell mixture is put back at the lyses point. For resealing the erythrocytes, the cell suspension is incubated at 37 °C. Such cells have an extended circulation half-life comparable to that of normal cells. Drugs enclosed in erythrocytes using this method include propranolol, levothyroxine, Metronidazole, Levothyroxine, Elaprnailat, Isoniazid cortisol-21-phosphate, prednisolone-21-sodium, cyclophosphamide, α-1 antitrypsin, interferon alpha-2, insulin.

- **Dialysis method**[9]

In this method, erythrocyte suspension + Drug solution are loaded in a dialysis tube with a 25% air bubble, and both ends are tied with thread. The tube is placed in a bottle containing 100ml of swelling solution, stored at 4^{o}C for lysis. After the dialysis tube is transferred to isotonic PBS solution at pH 7.4 and room temp (25-30^{0}C) for resealing, these resealed cells are removed and washed with PBS at 4oC and finally suspended in PBS solution. Lysis and resealing processes in the same dialysis tube achieve high entrapment efficiency (30-40%). Substances with Large molecular weight are entrapped by using low hematocrit erythrocyte suspension. E.g., DMSO.

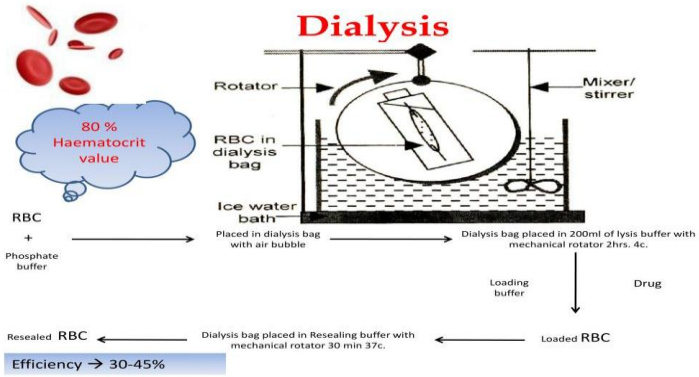

Fig. No. 3: Dialysis method.

- **Isotonic osmotic lysis method**[10]

This method, also called the osmotic pulse method, in which isotonic hemolysis occurs by physical or chemical means. The isotonic solutions may or may not be isotonic. If the red blood cells are incubated in solutions with high membrane permeability, then solute will diffuse into the cells due to the concentration gradient. An influx of water follows this process to maintain osmotic equilibrium. Chemicals like polyethylene glycol and ammonium chloride have been used for the isotonic hemolysis method. However, this approach also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a technique that involved suspending RBC in an isotonic solution of dimethyl sulfoxide (DMSO). A drug solution that was isotonic buffered was used to dilute the solution. The cells were resealed after being divided. 37C.

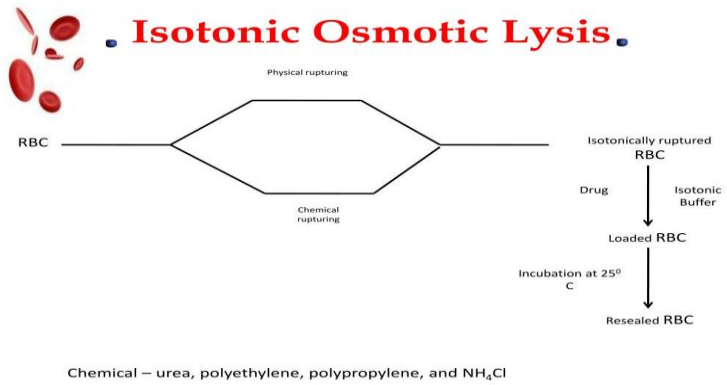

Fig. No. 4: Isotonic osmotic lysis.

- **Chemical perturbation of the membrane**[11]

This method is based on the increase in membrane permeability of RBCs when the cells are in contact with certain chemical substances. In 1973, Deuticke et al. showed that the permeability of the erythrocyte membrane increases upon exposure to polyene antibiotics such as amphotericin B. In 1980, this technique was used successfully by Kitao and Hattori to entrap the anticancer drug daunomycin in human and mouse erythrocytes. However, these methods induce irreversible undesirable changes in the cell membrane and are not very popular.

- **Electro-insertion, Electroporation, Electro-encapsulation**[11]

The method treats electrically induced permeability changes at high membrane potential differences. Electrical breakdown is achieved by membrane polarisation for microseconds using a varied voltage of 2kv/cm for 20 μsec. The potential difference across the membrane is developed either directly by inter and intracellular electrodes or indirectly by applying an internal electric field to the cells once the membrane is penetrated; in any case of of the size of pores, ions readily distribute between the extra and intracellular space to reach an equilibrium. However, the membrane remains impermeable to its cytoplasmic macromolecules. In red blood cells, the colloidal osmotic pressure of haemoglobin is about 30mOsm. This pressure drives water and ion influx. The membrane is breaked when the cell volume reaches 155% of its original volume. Since the cell breakdown is due to colloidal osmotic swelling, the rationale to prevent a breakdown is to balance the colloidal osmotic pressure of cellular macromolecules. This can be affected by adding macromolecules (like carbohydrates or proteins such as bovine serum albumin) and ribonucleases. Under this osmotically controlled condition, pores stay open at 4 C for a few days. If drug molecules are added at this point, they perforate into erythrocytes. The several candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and associated phenothiazines, hydrocortisone, propranolol, tetracaine and vitamin A.

- **Entrapment by endocytosis**[12]

Schrier reported this method in 1975. Endocytosis involves the sum of one volume of soaked packed erythrocytes to nine volumes of buffer containing 2.5m MATP, 2.5 mM MgCl2 and one mM CaCl2, tracked by incubation for 2 min at room temperature. The pores formed by this method are resealed using 154 mM of NaCl and incubated at 37°C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosis material from the cytoplasm, thus shielding it from the erythrocytes and vice-versa.

- **Lipid fusion method**[12]

Drug entrapped in vesicular lipid carrier mixed with human RBC and human result in the exchange of encapsulated drug. GresoneleandNicholau applied this method for the entrapment of inositol monophosphate to improve O2 transport capacity. This method has a very low entrapment efficiency be 1%.

Evaluation of resealed erythrocytes[4,7]

- **Shape and Surface morphology**

The morphological examination of these ghost erythrocytes is accepted by comparison with untreated erythrocytes using either transmission (TEM) or scanning (SEM) electron microscopy. Electron microscopy observation may be made of the morphological changes in the erythrocytes induced by osmosis-based encapsulation methods when they are subjected to solutions of different osmolality. The morphology of erythrocytes decides their life span after administration.

- **Drug content**

The drug content of the cells determines the entrapment efficiency of the method used. The process involves the deproteinisation of packed, loaded cells (0.5 mL) with 2.0 mL acetonitrile and centrifugation at 2500 rpm for 10 min. The clear supernatant is analysed for the drug content spectrophotometrically.

- **Percent cell Recovery & Cell counting**

This method includes counting the no. of RBCs per unit volume of whole blood, generally by automated counting. Red cell recovery may be calculated based on the differences in the hematocrit and the importance of the erythrocyte suspension both before and after loading. The aim is to minimise the loss during the encapsulation procedure to maximise cell recovery.

- **In vitro drug Release and Hemoglobin content**

In vitro release of drug(s) and hemoglobin are monitored periodically from drug-loaded cells. The cell suspension (5% hematocrit in PBS) is stored at four ˚C in an amber-colored glass container. Sometimes the supernatant is withdrawn using a hypodermic syringe, deproteinised using methanol and filtered through a 0.45 μm filter. They are then estimated for drug or hemoglobin content. Another parameter to evaluate hemoglobin disposition after resealing is mean corpuscular hemoglobin. It is the mean concentration of Hb per 100 ml of cells and is an index independent of the red cell. Therefore, it is a true expression of their Hb content.

- **Osmotic fragility**

The osmotic fragility of resealed erythrocytes indicates the possible changes in cell membrane integrity and the resistance of these cells to the osmotic pressure of the suspension medium. This test is carried out by stepwise incubation with isotonic to hypotonic saline solutions and estimating drug and Hb. In most cases, the osmotic fragility of resealed cells is greater than that of normal cells because of increased intracellular osmotic pressure.

- **Osmotic shock**

For the osmotic shock study, erythrocytes suspension is diluted with distilled water and centrifuged at 3000 rpm for 15 min. The supernatant is estimated for drug and Hb spectrophotometrically.

- **Turbulence shock**

Turbulence fragility is yet another characteristic that depends upon changes in the integrity of the cellular membrane and reflects the resistance of loaded cells against hemolysis resulting from the turbulent flow within the circulation. It is determined by passing cell suspension through a 23-gauge hypodermic needle (10ml/min) and estimating residual drug and Hb. The turbulent fragility of resealed cells is higher than normal erythrocytes.

- **Erythrocyte Sedimentation Rate (ESR)**

It estimates the suspension stability of red blood cells in plasma and is related to the number and size of the red cells and the relative concentration of plasma proteins. The ESR apparatus measure it.

Application of resealed erythrocytes[5,11]

***In vitro* applications**

- Carrier RBCs have proved to be helpful for a variety of *in vitro* tests.
- *In vitro* phagocytosis, cells have been utilised to make possible the uptake of enzymes by phagolysosomes. This study shows that enzyme content within carrier RBC could be visualised with the help of cytochemical techniques.
- When antibody molecules are introduced using an erythrocytic carrier system, they diffuse throughout the cytoplasm immediately. Antibody RBC auto-injected into living cells has been used to verify the site of action of a diphtheria toxin fragment.

***In – Vivo* application**

- Targeting of bioactive agents to RE System Damaged erythrocytes are readily cleared from circulation by phagocytic Kupffer cells in the liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. The various approaches to change the surface characteristics of erythrocytes include surface modification with antibodies, glutaraldehyde, and carbohydrates such as sialic acid and sulphydryl.
- Targeting sites other than RES Organs Resealed erythrocytes can deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES has been tried recently with resealed erythrocytes. Some of the representative approaches are discussed in brief.
- Erythrocytes as Circulating Bioreactors Erythrocytes have been realised as carriers for enzymes to serve circulating bioreactors. Sometimes it is desirable to decrease circulating metabolites that can penetrate RBCs. RBCs have also been used as circulating bioreactors for the controlled delivery of antiviral agents.
- Erythrocytes as Carriers for Drugs Various bioactive agents encapsulated in erythrocytes are developed for slow and sustained release in circulation to treat the parasitic disease effectively. Resealed erythrocytes are an ideal carrier for antineoplastic agents, antimicrobial drugs, vitamins, and steroids.
- Erythrocytes as Carriers for Enzymes can be injected into the bloodstream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in the blood due to disease; likewise, environmental, lysosomal storage disorders such as Gaucher's disease, hyperarginiaemia, hyperuricaemia, hyperphenyl-alaninaemia and kidney failure are only a few examples of metabolic disorders that can be treated by administration of enzymes.

Novel approaches[12]

- **Erythrosomes:** Erythrosomes are made by modifying the reverse phase evaporation process. In this method, the human RBCs have cross linkage on which the lipid bilayer is coded. This encapsulation system is helpful for prominent molecular drugs.
- **Nanoerythrosomes:** Nanoerythrosomes have an average diameter of 100nm. This nano-vesicle is made from a membrane of erythrocytes by breakdown methods such as sonification, extrusion, and electrical breakdown to form a uniform size. Mishra and Jain reported that reverse bio-member vesicles having doxorubicin drug Nanoerythrosomes had given potential significance in clinical treatment.

CONCLUSION

Erythrocytes as drug carriers for novel drug delivery systems will remain an active area for future research. Using resealed erythrocytes shows potential for safe and effective delivery of several bioactive molecules for effective targeting. It can be prepared by different techniques and also characterised quickly. However, the concept needs future optimisation to be converted into regular drug delivery systems. Most studies in this area in the in vitro phase and the ongoing project worldwide need to catch up to step into preclinical and clinical studies to prove the capabilities of sound delivery systems.

Abbreviations

- RBC: Red Blood Cells
- RES: Reticuloendothelial System

REFERENCES

1. Selvamani P, Latha S, Monisha S, Supassri T. A review resealed erythrocytes as a novel drug delivery system. Asian J. Pharm clinRes, 2015; 8(4): 101-107.
2. TriputhiRao K, Suria Prabha. K, Muthu Prasanna P. Resealed Erythrocytes: a specific tool in the novel

drug carrier system. Res JPharma Biol Chem Sci, 2011; 2(4): 496-512.
3. Gothoskar AV. Resealed Erythrocytes: A Review Pharmaceutical Technology, 2004; 28(3): 140-158.
4. PrameelNS, Tony DE, Babu AN, Naveen R, Padmavati B, Kiranmai K. Review resealed erythrocytes. World Journal Of Pharmaceutical Research, 2021; 10(5): 1752-1773.
5. Sahoo CK, Vennelal G, Sahoo TK, Moharana AK. Anoverviewonresealed erythrocytes. A novel approach to drug delivery. Int J Pharrm PharmaSci, 2012; 4(2): 71-76.
6. Begum GS, Mustafa D. An Advanced Review OnResealedErythrocytes. Asian Journal of Pharmaceutical Research and Development, 2019; 7(6): 55-61.
7. Hirlekar RS, Patel PD, Dand N, Kadam VJ. Drug Loaded Erythrocytes: as novel drug delivery system. Current pharmaceutical design, 2008; 14(1): 63-70.
8. Deepika BR, Ram SS, Ketan KD, Halle PD. Resealed erythrocytes drug delivery: a review.Int J Res Pharm Chem, 2013; 3(2): 198-207.
9. Bellad KA, Nanjwade BK, Kamble MS, Srichana T. Resealed Erythrocytes based drug delivery system. World Journal of Pharmacyand Pharmaceutical Science, 2017; 6(2): 446-482.
10. Gupta R. Resealed erythrocytes: carrier for smart drug delivery. WorldJ. Pharmaceut. Res, 2014; 3(4): 1722-1747.
11. Alli PK, KulatRB, Bhagat BV, Resealed Erythrocytes: ANovel Drug Delivery System, Asian Journal of Pharmaceutical Technology and Innovation, 2016; 4(17): 119-130.
12. Vats V. Novel Drug Delivery System: Resealed Erythrocytes. Turkish Journal of Computer and Mathematics Education, 2020; 11(2): 706-719.