



RESEALED ERYTHROCYTE: A NOVEL DRUG DELIVERY SYSTEM

S.H. Gatkal*, N. M. Gaikwad, P. M. Patil, P.D. Chaudhari

P. E. Society's Modern College of Pharmacy, Nigdi, Pune-411 044, Maharashtra, India.

*Corresponding author's E-mail: saroj_gatkal@yahoo.com

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ABSTRACT

Erythrocyte, which is also known as red blood cells, has been extensively studied by scientists for their capability as a potential carrier for the delivery of drugs and drug-loaded microspheres. Resealed erythrocytes, as a drug delivery system has excellent capacity to enhance the therapeutic index and patient compliance. It has got tremendous potential to achieve site specific drug delivery with least wastage of drugs and it also prolong the release of drug. For the preparation of such drug-loaded carrier erythrocytes, the blood samples are simply collected from the organism of interest, and then the erythrocytes are separated from plasma. The drug is entrapped in the erythrocytes, and resealing the resultant cellular carriers. And because of this resealing process these carriers are called resealed erythrocytes. Erythrocytes are biocompatible, biodegradable and the most important advantages are that it possesses long circulation half lives. It can be easily prepared by simple technique and can be loaded along with a variety of biologically active compounds. The present review focuses on the potential applications of erythrocytes in drug delivery.

Keywords: Resealed erythrocytes, Carriers, Methods of drug loading.

INTRODUCTION

The normal erythrocyte (normocyte) is a flexible, elastic, biconcave disc shaped structure with mean diameter 7.3 μm and thickness near 2.2 μm . The chemical constituent of red blood cells include water (63%), lipids (0.5%), glucose (0.8%), minerals (0.7%), non hemoglobin (33.67%). The primary function of the erythrocyte is transport of oxygen and carbon dioxide. Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm³ blood in a healthy male and 4.8 million cells/mm³ blood in a healthy female).

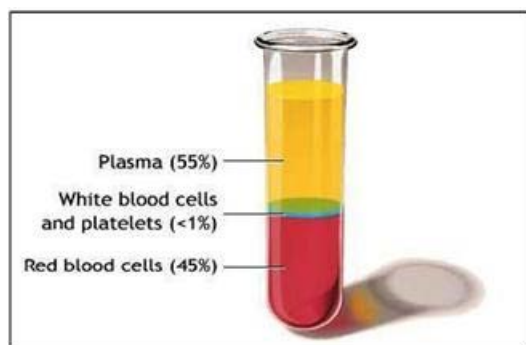


Figure 1: Composition of blood

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes. The process of erythrocyte formation within the body is known as erythropoiesis. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called erythropoietin¹.

THE ERYTHROCYTE MEMBRANE

It contains about equal weight of proteins and lipids. The outer surface of membrane bears a net negative charge due largely to carboxylic groups of sialic acid. The

isoelectric point is about pH 2 which is raised to pH4-5 after quantitative removal of the sialic acid with neuraminidase. There is about 2.4×10^7 N-acetyl neuraminic acid residue per human erythrocyte. The negative surface charge prevents erythrocyte from coming into contact and maintains a sufficient distance between erythrocytes so that they cannot be readily agglutinated with IgG.

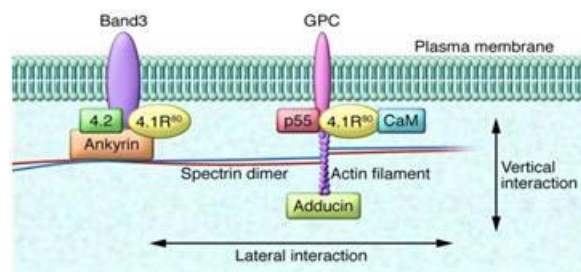


Figure 2: Basic structure of erythrocyte membrane

The phosphoglyceride content of the membrane is about 50% of the total lipid content and includes phosphatidylcholine phosphatidylethanolamine as a predominant species. The other major lipid constituent of the membrane is sphingomyline and cholesterol. The erythrocytes do not have the ability to synthesize lipids de novo, but have the capacity for the lipid turnover and replacement using plasma lipids¹.

ELECTROLYTE COMPOSITION OF ERYTHROCYTES

The concentration of K⁺ and Na⁺ differ in that the former is more in erythrocytes and the later in plasma. Change in osmotic pressure of the medium surrounding the RBCs (either in vivo or in vitro) change the manipulate the morphology and tonicity of the cells. If the medium is hypotonic water diffuses into the cells and they get swelled and eventually lose all their hemoglobin content and may burst and vice versa⁵.



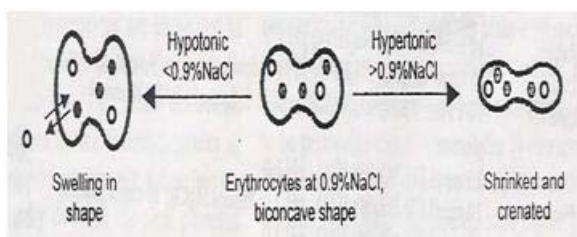


Figure 3: Various shapes and morphology of erythrocytes under different osmotic environments

ADVANTAGES OF ERYTHROCYTE AS CARRIER

- 1] Biocompatible and no possibility of triggered immune response.
- 2] Natural product of the body, which are biodegradable in nature.
- 3] No generation of toxic products.
- 4] Uniform size and shape of carrier.
- 5] Relatively inert intracellular environment can be encapsulated in a small volume of cells.
- 6] Isolation is easy and large amount of drug can be loaded.
- 7] Prevention of degradation of the loaded drug from inactivation by endogenous chemical.
- 8] Wide variety of chemicals can be easily entrapped.
- 9] Entrapment of drug can be possible without chemical modification of the substance to be entrapped.
- 10] Possible to maintain steady-state plasma concentration, decrease fluctuation in concentration.

DISADVANTAGE OF ERYTHROCYTES AS A CARRIER

- 1] The modifications that occurred during loading procedures of the drugs into the erythrocytes accelerate their removal by the RES in vivo.
- 2] Certain encapsulated substances may be leaked from the loaded erythrocytes.
- 3] Storage problem to avoid contamination
- 4] Special precaution is required for the collection and handling of the erythrocytes.
- 5] Possibility of clumping of cells and dose dumping may be there.
- 6] They have a limited potential as carrier to non phagocytic target tissue.
- 7] Direct injection into the cell nucleus is not feasible.
- 8] Short storage life of about 2 weeks.
- 9] Economic technique.

Criteria for use of erythrocytes as a drug delivery system

- 1] It should possess specific physico chemical properties by which a specific targeted site could be recognized.

- 2] It should be biocompatible and minimum side effects
- 3] The carrier system should have appreciable stability during storage.
- 4] The molecule should be polar or hydrophilic, once encapsulated charged molecules are retained longer than uncharged molecules.
- 5] The hydrophobic molecules can be entrapped in the erythrocyte by absorbing over the other molecules. The molecules which interact with the membrane and causes deleterious effects on membrane structure are not consider being appropriate for encapsulation in erythrocytes.
- 6] The molecule should neither be interfering with the membrane transport system nor with the glycolysis within the erythrocytes.
- 7] It should not contain any physical or chemical interaction with erythrocyte membrane.
- 8] Non polar and hydrophobic molecules may be entrapped in erythrocyte in their respective salts⁶.

SOURCE AND ISOLATION OF ERYTHROCYTES

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. To isolate erythrocytes, blood is collected in heparinized tubes by venipuncture. Fresh whole blood is the blood that is collected and immediately chilled to 4°C and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed cells are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid-citrate–dextrose buffer at 4°C for as long¹

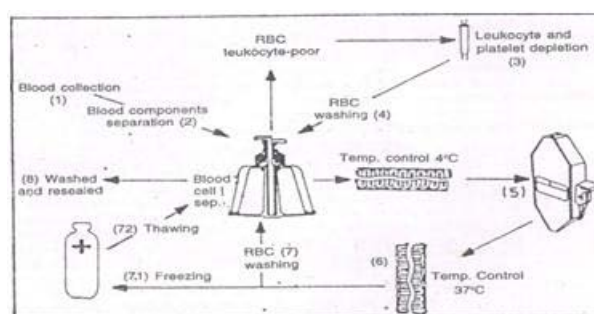


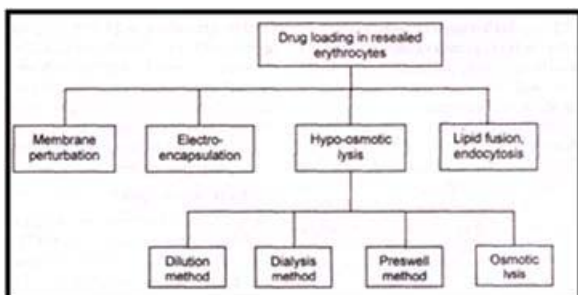
Figure 4: Steps involved in resealed erythrocyte

METHODS OF DRUG LOADING

Several methods can be used to load drugs or other bioactive compounds in erythrocytes, including physical (e.g., electrical pulse method) osmosis-based systems, and chemical methods

(E.g. chemical perturbation of the erythrocytes membrane). Irrespective of the method used, the optimal characteristics for the successful entrapment of the compound requires the drug to have a considerable degree of water solubility, resistance against degradation within erythrocytes, lack of physical or chemical

interaction with erythrocyte membrane, and well-defined pharmacokinetic and pharmacodynamic properties³.



[A] OSMOSIS BASED METHODS

1] Hypotonic hemolysis

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution as shown in figure 5. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under the cells can maintain their integrity up to a tonicity of 150 mosm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 Å are generated on the membrane Stress³.

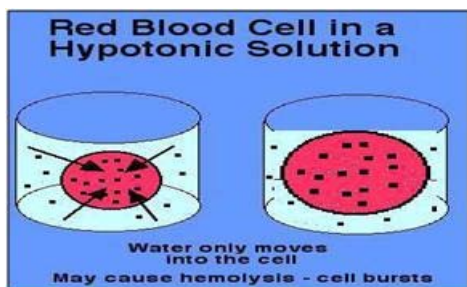


Figure 5: Hypnotic hemolysis

2] Hypotonic dilution

The erythrocytes have little capacity to resist volume. This is the simplest and fastest method. In this a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. At an increase in volume above 50-75% of initial volume and in hypotonic solution the erythrocyte membrane ruptures and pores are created to entrapped drug. The solution tonicity is then restored by adding a hypertonic buffer².

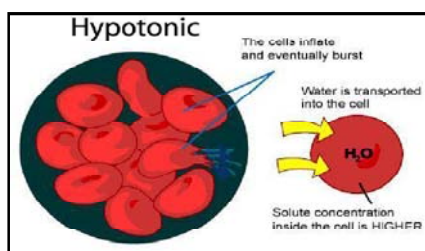


Figure 6: Hypnotic dilution

3] Hypotonic dialysis

This method was first reported by Klibansky in 1959 and was used in 1977 by Deloach and Ihler, and Dale for

loading enzymes and lipids. Several methods are based on the principle that semi permeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In this process a desired hemocrit is achieved by mixing erythrocyte suspension and drug solution. This mixture is placed into dialysis tubing and then both ends of tube are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. The tube is placed in the bottle containing 100ml of swelling solution. The bottle is placed at 4°C for the desired lysis time. The contents of the dialysis tubing are mixed intermittently by shaking the tube using the strings. The dialysis tube is then placed in 100 ml of resealing solution. The loaded erythrocyte thus obtained are then washed with cold phosphate buffer at 4°C. In this method a good entrapped efficiency is obtained¹.

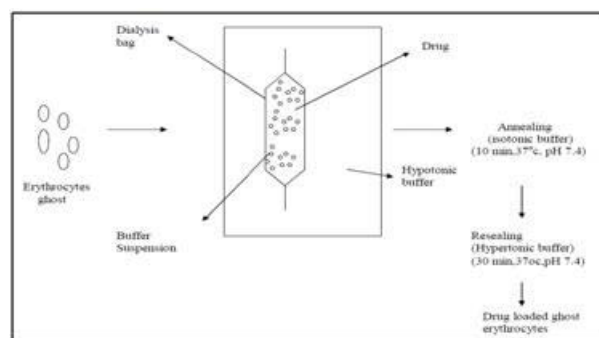


Figure 7: Hypotonic dialysis

4] Hypotonic pre-swelling

As shown in figure 8 the technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low g values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 L portions of an aqueous solution of the drug to be encapsulated³.

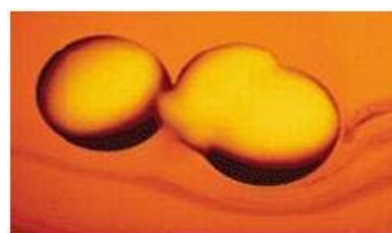


Figure 8: Hypotonic pre-swelling

[B] CHEMICAL PERTURBATION OF THE MEMBRANE

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke et al. showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes these methods induce irreversible destructive changes in the cell membrane and hence are not very popular¹.

C] ELECTRO-INSERTION OR ELECTRO ENCAPSULATION

The method is based on the observation that electrical shock brings about irreversible changes in the erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium. The procedure involves suspending erythrocytes in an isotonic buffer in an electrical discharge chamber. A capacitor in an external circuit is charged to a definite voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential. The optimum intensity of an electric field is between 1–10 kW/cm and optimal discharge time is between 20–160 μ s. An inverse relationship exists between the electric-field intensity and the discharge time. The compound to be entrapped is added to the medium in which the cells are suspended from the commencement of the experiment¹.

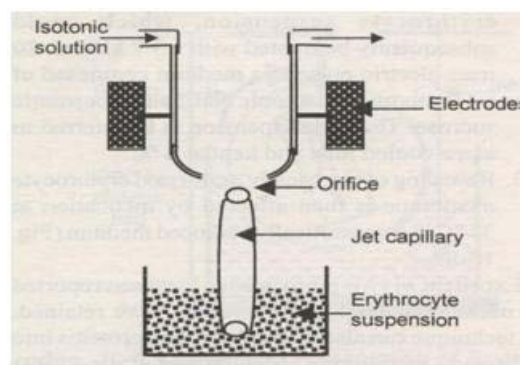


Figure 9: Electro encapsulation

An erythrocyte membrane one advantage of this method is a more uniform distribution of loaded cells in comparison with osmotic methods. The main drawbacks are the need for special instrumentation and the sophistication of the process. Entrapment efficiency of this method is ~35%, and the life span of the resealed cells in circulation is comparable with that of normal cells.

Table no.1: comparison of various hypo osmotic lysis methods

Methods	% Loading	Advantage	Disadvantage
Dilution method	1-8%	Fastest and simplest for low molecular weight drugs	Entrapment efficiency is very less
Dialysis	30-45%	better in vivo survival of RBCs	Time consuming heterogeneous size distribution
Preswell dilution	20-70 %	Good retention of cytoplasm <i>in vivo</i>	-
Isotonic osmotic lysis	-	Better <i>in vivo</i> surveillance	Impermeable only to large molecule, time consuming

D] ENTRAPMENT BY ENDOCYTOSIS

This method was reported by Schrier *et al.* in 1975. Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl₂, and 1mM CaCl₂, followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37 °C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa³.

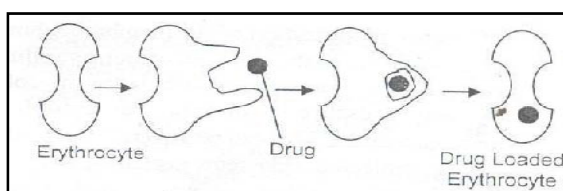


Figure 10: Entrapment by endocytosis

DRUG RELEASE CHARACTERISTICS OF LOADED DRUGS

There are mainly three ways for a drug to efflux out from erythrocyte carriers i.e. phagocytosis, diffusion through the membrane of the cell, and use of specific transport system. The rate of diffusion depends upon the rate at

which a particular molecule penetrates through a lipid bilayer it is greatest for molecule with high lipid solubility and gradually goes down with polarity or charged groups of the molecule. Prolongation of release could presumably be accomplished by entrapment of potent inhibitors of the appropriate transport protein along with the drug².

- **IN VITRO CHARACTERISATION**

The *in vivo* performance of resealed erythrocytes is affected to a great extent by their biological properties. Hence, *in vitro* characterization forms an important part of studies involving such cellular carriers.

A] PHYSICAL CHARACTERISATION**1] Shape and surface morphology**

The morphology of erythrocytes decides their life span after administration. Light microscopy reveals no observable change in resealed cells but in few cases spherical erythrocytes (spherocytes) are detected. Scanning electron microscopic studies have shown that a majority of the cells maintain their biconcave discoid shapes after the loading procedure, and few stomatocytes a form of spherocytes with an invagination

in one point are formed. In some cases, cells of smaller size (microcyte) are also observed.

2] Drug content

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

3] Deformability

Shape change (deformability) is another factor that affects the life span of the cells. This parameter evaluates the ease of passage of erythrocytes through narrow capillaries and the RES. It determines the rheological behavior of the cells and depends on the viscoelasticity of the cell membrane, viscosity of the cell contents, and the cellular surface-to-volume ratio. The deformability is measured by passage time of definite volume of cells through capillary of 4 μm diameter or polycarbonate filter with average pore size of 45 μm . Another indirect approach is to evaluate chlorpromazine induced shape changes turbidimetrically.

4] Drug release

The most important parameters for evaluation of resealed erythrocytes are the drug release pattern. Hemoglobin is also invariably released because drug release involves the loss of cell membrane integrity indicating hemolysis. On the basis of the various in vitro release experiments carried out on these cells, three general drug release patterns are observed:

- The rate of drug release is considerably higher than that of hemoglobin. In other words, drug diffuses readily. Such a pattern is shown by lipophilic drugs, including methotrexate, phenytoin, dexamethasone, primpquin, and vitamin B12. Cell lysis is not essential for the release of such drugs.
- The rate of drug release is comparable to that of hemoglobin. This indicates that cell lysis is essential for drug release and drug can not be released by mere diffusion. Polar drugs such as gentamicin, heparin, and enalaprilat, and enzymes such as asparaginase peptides, including urogasterone and l-lysine-l-phenylalanine follow such pattern.
- The rate of drug release lies between the above mentioned two extremes; for example, propranolol, isoniazid, metronidazol and recombinant human erythropoietin

B] CELLULAR CHARACTERISATION

1] Osmotic fragility

The osmotic fragility of resealed erythrocytes is an indicator of the possible changes in cell membrane integrity and the resistance of these cells to osmotic pressure of the suspension medium. The test is carried

out by suspending cells in media of varying sodium chloride concentration and determining the hemoglobin released. In most cases, osmotic fragility of resealed cells is higher than that of the normal cells because of increased intracellular osmotic pressure.

2] Turbulent fragility

The turbulence fragility is yet another characteristic that depends upon changes in the integrity of cellular membrane and reflects resistance of loaded cells against hemolysis resulting from turbulent flow within circulation. It is determined by the passage of cell suspension through needles with smaller internal diameter (e.g., 30 gauges) or vigorously shaking the cell suspension. In both cases, hemoglobin and drug released after the procedure are determined. The turbulent fragility of resealed cells is found to be higher.

3] Percent cell recovery

It can be determined by counting the number of intact cells per cubic mm of packed erythrocyte before and after loading the drug

C] BIOLOGICAL CHARACTERISATION

It can be done by performing sterility test, pyrogen test using rabbit method and LAL test and toxicity test on animal.

• IN VIVO CHARACTERISATION

The efficacy of resealed erythrocytes is determined mainly by their survival time in circulation upon reinjection. The various methods used to determine in vivo survival time include labeling of cells by ^{51}Cr or fluorescent markers such as fluorescein isothiocyanate or entrapment of ^{14}C sucrose or gentamicin. The circulation survival kinetics of resealed erythrocytes show typical bimodal behavior with a rapid loss of cells during the first 24 h after injection, followed by a slow decline phase with a half life on the order of days or weeks¹.

ROUTE OF ADMINISTRATION

Usually resealed erythrocyte during experimentation has been administered to the laboratory animals intravenously through caudal vein. DeLoach utilized subcutaneous route for slow release of entrapped agents. He evaluated the disposition of the interleukin -2 in mice receiving a subcutaneous injection. Recently Talwar *et al* (1993) have proposed erythrocyte based nasal delivery of propranolol¹.

IN VITRO STORAGE

The success of resealed erythrocytes as a drug delivery system depends to a greater extent on their in vitro storage. The most common storage media include Hank's balanced salt solution and acid-citrate-dextrose at 4°C. Cells remain viable in terms of their physiologic and carrier characteristics for at least 2 weeks at this temperature. The addition of calcium-chelating agents or the purine nucleosides improve circulation survival time



of cells upon reinjection. Exposure of resealed erythrocytes to membrane stabilizing agents such as dimethyl sulfoxide, dimethyl, 3, 3-di-thio bispropionamide, glutaraldehyde, toluene-2-4-diisocyanate followed by lyophilization or sintered glass filtration has been reported to enhance their stability upon storage. It can be also preserved by suspending cells in oxygenated HBBS contain 1% soft gelatin. The cells are well recovered after liquefying the gel by placing the tube in water bath at 37°C followed by centrifugation. Another method utilized for storage has been cryopreservation of RBCs in liquid nitrogen⁵.

APPLICATIONS OF RESEALED ERYTHROCYTES

Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cells could be used as circulating carriers to disseminate a drug within a prolonged period of time in circulation or in target-specific organs, including the liver, spleen, and lymph nodes. A majority of the drug delivery studies using drug-loaded erythrocytes are in the preclinical phase. In a few clinical studies, successful results were obtained⁶.

IN-VITRO APPLICATIONS

For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes by phagolysosomes. Enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The most frequent in vitro application of RBC is that of micro-injection. A protein or nucleic acid was injected into eukaryotic cells by fusion process. Similarly, when antibody molecules are introduced using erythrocytic carrier system, they immediately diffuse throughout the cytoplasm.

IN – VIVO APPLICATIONS

- Targeting of bioactive agents to RES System
- Targeting to sites other than RES-rich Organs
- Erythrocytes as Circulating Bioreactors
- Erythrocytes as Carriers for Drugs
- Erythrocytes as Carriers for Enzymes
- Erythrocytes as Carriers for Proteins and Macromolecules.

Few of them can be discuss as followed-

DRUG TARGETING TO THE RES ORGANS

1] Slow drug release

Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics antiparasitics, veterinary antiamoebics, vitamins, steroids antibiotics, and cardiovascular drugs.

The various mechanisms proposed for drug release include-

- Passive diffusion
- Specialized membrane associated carrier transport

- Phagocytosis of resealed cells by macrophages of RES, subsequent accumulation of drug into the macrophage interior, followed by slow release.

- Accumulation of erythrocytes in lymph nodes upon subcutaneous administration followed by hemolysis to release the drug⁶.

2] Targeting to the liver

Many metabolic disorders related to deficient or missing enzymes can be treated by injecting these enzymes. However, the problems of exogenous enzyme therapy include a shorter circulation half life of enzymes, allergic reactions, and toxic manifestations. These problems can be successfully overcome by administering the enzymes as resealed erythrocytes. The enzymes used include β -glucosidase, β -glucuronidase, and β -galactosidase⁷.

3] Treatment of hepatic tumors

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate, bleomycin, asparaginase, and Adriamycin have been successfully delivered by erythrocytes. Agents such as daunorubicin diffuse rapidly from the cells upon loading and hence pose a problem. This problem can be overcome by covalently linking daunorubicin to the erythrocytic membrane using glutaraldehyde or cisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver⁵.

4] Treatment of parasitic diseases

The ability of resealed erythrocytes to selectively accumulate within RES organs make them useful tool during the delivery of antiparasitic agents. Parasitic diseases that involve harboring parasites in the RES organs can be successfully controlled by this method. Results were favorable in studies involving animal models for erythrocytes loaded with antimalarial, antileishmanial, and antiamoebic Drugs⁸.

5] Removal of RES iron overload

Desferrioxamine-loaded erythrocytes have been used to treat excess iron accumulated because of multiple transfusions to thalassemic patients. Targeting this drug to the RES is very beneficial because the aged erythrocytes are destroyed in RES organs, which results in an accumulation of iron in these organs.

6] Removal of toxic agents

Cannon *et al.* reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulfate. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiesterase also has been reported.



DRUG TARGETING ORGANS OTHER THAN THOSE OF RES**7] Delivery of antiviral agents**

Nucleosides are rapidly transported across the membrane whereas nucleotides are not and thus exhibiting prolonged release profiles. The release of nucleotides requires conversion of these moieties to purine or pyrimidine bases. Resealed erythrocytes have been used to deliver deoxycytidine derivatives, recombinant herpes simplex virus type 1 (HSV-1) glycoprotein B, azidothymidine derivatives, azathioprene, acyclovir, and fludarabine phosphate

8] Enzyme replacement therapy

Enzymes are widely used in clinical practice as replacement therapies to treat diseases associated with their deficiency (e.g., Gaucher's disease, galactosuria), degradation of toxic compounds secondary to some kind of poisoning (cyanide, organophosphorus), and as drugs. The problems involved in the direct injection of enzymes into the body have been cited. One method to overcome these problems is the use of enzyme-loaded erythrocytes. These cells then release enzymes into circulation upon hemolysis; act as a "circulating bioreactors" in which substrates enter into the cell, interact with enzymes, and generate products; or accumulate enzymes in RES upon hemolysis for future catalysis. The first report of successful clinical trials of the resealed erythrocytes loaded with enzymes for replacement therapy is that of β -glucoserebrosidase for the treatment of Gaucher's disease. The disease is characterized by inborn deficiency of lysosomal β -glucoserebrosidase in cells of RES thereby leading to accumulation of β -glucoserebrosides in macrophages of the RES¹¹.

9] Improvement in the oxygen delivering to tissues

Hemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. Under normal conditions, 95% of hemoglobin is saturated with oxygen in the lungs, whereas under physiologic conditions in peripheral blood stream only ~25% of oxygenated hemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to hemoglobin is reticulated with venous blood to the lungs. The use of this bound fraction has been suggested for the treatment of oxygen deficiency. 2, 3-Diphosphoglycerate (2, 3-DPG) is a natural effectors of hemoglobin. The binding affinity of hemoglobin for oxygen changes reversibly with changes in intracellular concentration of 2, 3-DPG. This compensates for changes in the oxygen pressure outside of the body, as the affinity of 2; 3-DPG to oxygen is much higher than that of hemoglobin⁵.

10] Microinjection of macromolecules

In microinjection, erythrocytes are used as microsyringes for injection to the host cells. The microinjection process involves culturing host eukaryotic cells in vitro. The cells are coated with fusogenic agent and then suspended with erythrocytes loaded with the compound of interest in an

isotonic medium. Sendai virus (hemagglutinating virus of Japan, HVJ) or its glycoproteins or polyethylene glycol have been used as fusogenic agents. The fusogen causes fusion of cosuspended erythrocytes and eukaryotic cells. Thus, the contents of resealed erythrocytes and the compound of interest are transferred to host cell. This procedure has been used to microinject DNA fragments, arginase, proteins, nucleic acids, ferritin, latex particles, bovine and human serum albumin, and enzyme thymidine kinase to various eukaryotic cells¹⁰.

11] Applications of carrier Erythrocytes in delivery of biopharmaceuticals

The scientist can reported that, the potential applications of erythrocytes in drug delivery have been reviewed with a particular stress on the studies and laboratory experiences on successful erythrocyte loading and characterization of the different classes of biopharmaceuticals.

NOVEL APPROACHES**ERYTHROSOMES**

Erythrosomes are specially engineered vesicular systems in which chemically cross-linked human erythrocyte cytoskeletons are used as sport upon which a lipid bilayer is coated. This can be achieved by a modification procedure normally adopted for reverse phase evaporation. Erythrosomes are proposed as useful encapsulation system for drug delivery particularly for macromolecular drugs. Large (3, μ m diameter) mechanically stable proteoliposomes (erythrosomes) were prepared in good yield by coating cross linked erythrocyte cytoskeletons with phosphatidylcholine. The results of the present study suggest that erythrosomes may be useful for membrane transport protein reconstitution and encapsulation systems¹⁷.

Application

- 1] Pentamidine primaquine phosphate and metronidazole have been successfully utilized erythrosomes for the treatment of leishmaniasis, malaria on experimental a models.
- 2] It can be serves as ideal carrier for antineoplastic drugs like bleomycin.
- 3] Vitamin and steroid have been encapsulated in RBCs.
- 4] It serves as a cellular sustain delivery system for in vivo administration of recombinant erythropoietin.
- 5] RBCs coated with recombinant interleukin-2 are reported to provide sustain release to allow low and non toxic concentration of rIL-2 in circulation

NANOERYTHROSOMES (nEs)

Nanoerythrosomes are vesicles prepared by the extrusion of RBC ghost, the average diameter of these vesicles being 100 nm. The process gave small vesicles with the size of liposomes. The drugs can be conjugated to the Nanoerythrosomes using certain cross linking agents such



as gluteraldehyde have higher activity than when compared to free drug and they offer higher stability. The Nanoerythroosomes are characterized for various parameters like surface morphology, percent drug conjugation, centrifugal stress, in vitro release etc. Thus nEs are very versatile bioactive drug carriers or drug delivery system (DDS). These nEs are stable and maintain both the cytotoxic & antineoplastic activity of daunorubicin against mice leukemia p338D cells. The nE compositions further leads to the formation of bioassays. In case of Gauchers disease glucocortisone was encapsulated in erythrocytes and heparin was encapsulated in erythrocytes to prevent thromboembolism¹⁸.

PREPARATION OF (nEs) AND DRUG LOADING

There are three methods for loading of drugs in NES.

1. Extrusion

The erythrocyte ghost suspension (50% haematocrit) is extruded through the 25mm polycarbonate membrane filter (0.4µm) pore obtained by 8-10 consecutive extrusions under nitrogen pressure. The ghosts obtained are stained with uranyl acetate and they are observed under microscope. The extrusions are performed in a thermostatically controlled extrusion device at 37°C and the final preparation is stored in a refrigerator at 40°C. The extrusions are performed at 37.8°C in a thermostatically controlled extrusion device¹⁸.

2. Sonication

Erythrocyte ghosts are converted into small vesicles using a dismembrator.

3. Electrical breakdown method

It is used to convert ghosts into small vesicles under the influence of electric potential

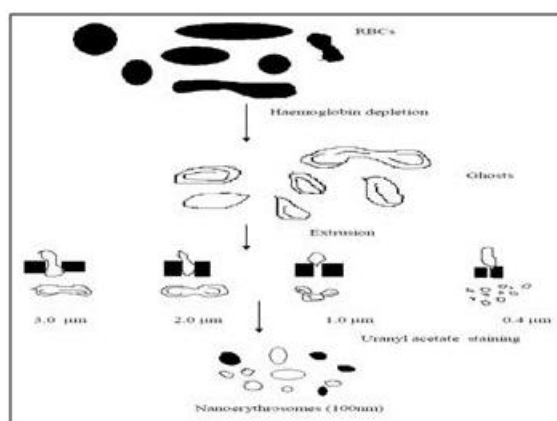


Figure 11: Mechanism of formation of Nanoerythroosomes using extrusion method

Advantages

4. Non immunogenic in action and can be targeted to disease tissue /organ.
5. Prolong the systemic activity of drug while residing for a longer time in the body.

6. Protect the premature degradation, inactivation and excretion of proteins and enzymes.

8. Target the drugs within RES as well as non RES organs/sites

10. No possibility of triggered immunological response

11. Drug is chemically bonded with the protein of the erythrocyte membrane

12. less prone to aggregation and fusion

13. Flexibility of membrane allows them to escape RES for longer periods

Application

1] nEs are also used for delivery of mitomycin-c, hydroxyurea & 6-mercaptopurine. Cytotoxicity is more in the form of nE combinations than the free drug. They are also used for the delivery of insulin

2] Doxorubicin nE conjugates have higher antineoplastic activity than the free drug on CDF1 leukemia tumors

3] Nanoerythroosomes (nEs) were designed in such a way such that with low doses, they maintain the optimum concentration of the drug for prolonged periods of time & they provide sustained action.

RESULTS AND DISCUSSION

The preparation of resealed erythrocytes is very easy. There are several techniques identified now days by which we can easily entrap the drug into erythrocytes. It is having both types of application in-vitro as well as in-vivo. The traditional drug delivery system had many disadvantages, such as high toxicity effect and high minimum effective dose. Among the various carriers, the most important are the one that at least mimic body's intrinsic components and avoid provoking an immune response on IV administration. Many drug therapies have disadvantages of patient non-compliance due to more dosing frequency.

CONCLUSION

The use of resealed erythrocytes looks promising for a safe and sure delivery of various drugs for passive and active targeting. However, the concept needs further optimization to become a routine drug delivery system. The same concept also can be extended to the delivery of biopharmaceuticals, Proteins, steroids. Resealed erythrocytes are used for targeting drug delivery system depending on sustain release insulin, antiviral agent, Oxygen deficiency therapy etc. was found to be very beneficial to used as a novel drug delivery system.

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