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RESEALED ERYTHROCYTES: A NOVEL APPROACH FOR DRUG TARGETING

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ABSTRACT

Now a days the present scenario is focusing more towards the targeted drug delivery systems because of the increasing interest in taking of safe drugs with less amount of drug, which is capable of reaching at the desired target site with minimal side effects. The main problem is associated with the systemic drug administration which is related to biodistribution of pharmaceuticals throughout the body. Amongst all the carrier systems Erythrocytes have been most interesting carrier and have been found to possess great potential in targeting, because of having characteristics like biocompatible, biodegradable, possess long circulation and can be loaded with a variety of biological compounds. Resealed erythrocytes are gaining more popularity because of their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation. Most of the resealed erythrocytes used as drug carriers are rapidly taken up from blood by macrophages of reticuloendothelial system (RES), which is present in liver, lung and spleen of the body. In this comprehensive article the history of erythrocyte, carrier erythrocyte, some biological methods, isolation, in vitro and in vivo characteristics, delivery strategies, diagnostic and therapeutic applications and generation of resealed erythrocytes is reviewed.

KEYWORDS: Erythrocytes, Resealed erythrocytes, Drug targeting, Evaluation, Applications.

INTRODUCTION

At present pharmaceutical circumstances are designed for development of drug delivery systems which make best use of the drug targeting along with high therapeutic benefits for safe and efficient treatment of diseases and also more patient compliance.[1] Drug targeting can be approaches by either chemical modification or by appropriate carrier. There are various carriers used for targeting drugs to various body tissues, the cellular carriers meet several criteria enviable in clinical applications, along with that most important being the biocompatibility of carrier and its degradation products. Leucocytes, platelets, erythrocytes, nanoerythrocytes, hepatocytes and fibroblasts etc. have been proposed as cellular carrier systems.[2] Among those Resealed erythrocytes are gaining more popularity as targeted drug carriers, due to their ability to circulate throughout the body, biocompatibility, biodegradability, nonpathogenicity, non-immunogenicity, zero order release kinetics, reproducibility and ease of preparation.[1] Such drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes and resealing the resultant cellular carriers. Hence, these carriers are called resealed erythrocytes.[2]

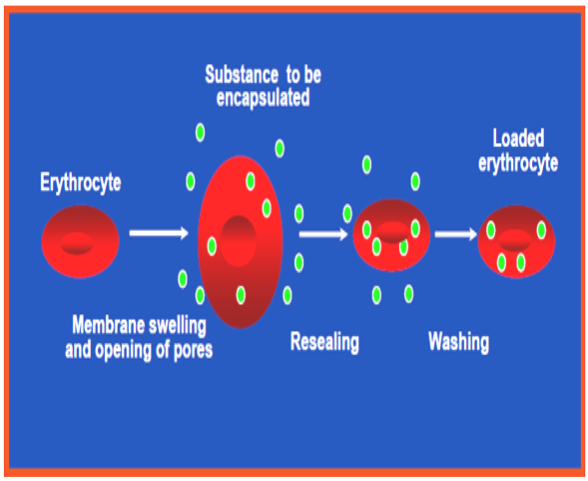


Fig 1: Schematic representation drug loading encapsulated erythrocytes [1]

Historical Background

Erythrocytes, mistaken for fat globules with the early available microscope of the Erythrocytes were first described in the seventeenth century as particles "25,000 times smaller than a fine grain of sand" by Dutch microscopist and Leeuwenhoek. A more precise description of these cells was given about hundred years

later, by Howson finding these cells as flat discs rather than the globules. In the 19th century, Hoppe Seyler completed the Hünefeld's discovery of hemoglobin by identification of its crucial role in oxygen delivery to different tissues. Reversible oxygenation was considered for a long time as the primary or even sole function of the red cell (along with CO2 exchange) until the late twentieth century. However, now our understanding of erythrocyte function has broadened to include O2, CO2, hydrogen sulfide and nitric oxide exchange as well as immune clearance and, possibly clearance of other soluble blood components such as cytokines. In 1953 Gardos tried to load the "erythrocyte ghost" by ATP, first effort for entrapment of chemicals in erythrocytes. In 1959, Marsden and Ostling reported the entrapment of dextran with molecular weights of 10 to 250 KD in erythrocyte ghosts. Fourteen years later, the first reports on loading the erythrocyte ghosts by therapeutic agents for delivery purposes were published independently by Ihler and Zimmerman and the term "carrier erythrocytes" was used for the first time in 1979 to describe the drug-loaded erythrocytes.[4]

Morphology And Physiology of Erythrocytes

Erythrocytes are the most abundant cells in the human body (~5.4 million cells/mm3 bloods in a healthy male and ~ 4.8 million cells/mm3 bloods in a healthy female). Erythrocytes are biconcave discs with an average diameter of 7.8 µm, a thickness of 2.5µm in periphery, 1 µm in the center and a volume of 85–9µm3. The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3µm wide.

Mature erythrocytes lack a nucleus and other organelles their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O2–CO2 binding inside the erythrocytes. The main role of erythrocytes is the transport of O2 from the lungs to tissues and the CO2 produced in tissues back to lungs. Hence, erythrocytes are a highly specialized O2 carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O2 transport. Also, because mitochondria are absent and because energy is generated aerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying.

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from circulation and destroyed in the spleen and liver (RES) and the breakdown products are recycled. 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 erythropoietin.[3]

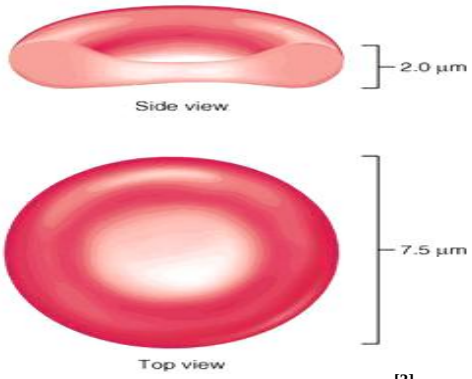


Fig 2: Morphology of erythrocytes[3]

Composition of Erythrocytes

Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements. Normal blood cells have extensile, elastic, biconcave and non nucleated configuration with a diameter ranging from 6- 9 µ and the thickness is nearly 1-2 µ. Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water. Lipid content of erythrocytes includes cholesterol, lecithin and cephaelins.[2]

ERYTHROCYTES CAN BE USED AS CARRIERS IN TWO WAYS

1. Targeting particular tissue/organ

For targeting, only the erythrocyte membrane is used. This is obtained by splitting the cell in hypotonic solution and after introducing the drug into the cells, allowing them to reseal into spheres. Such erythrocytes are called Red cell ghosts.[3]

2. For continuous or prolonged release of drugs

Erythrocytes can be used as a continuous or prolonged release system, which provide prolonged drug action. There are different methods for encapsulation of drugs within erythrocytes. They remain in the circulation for prolonged periods of time (up to 120 days) and release the entrapped drug at a slow and steady rate.[3]

ADVANTAGES OF RESEALED ERYTHROCYTES AS DRUG CARRIERS

The resealed erythrocytes should have the following advantages:

- Biodegradability with no generation of toxic products.
- Uniform size and shape of the carrier.
- Inert intracellular environment.
- Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
- A considerable increase in drug dosing interval with drug residing in therapeutic window region for longer time periods.
- Variety of chemicals can be entrapped.
- Modification of pharmacokinetic and pharmacodynamic parameters of drug.
- Acquirement of steady-state plasma concentration decreases fluctuations in concentration.

- Protection of the organism against toxic effects of drugs (e. g. Antineoplastics).
- Ability to circulate throughout the body
- Ease of preparation.
- The prevention of any undesired immune response against the loaded drug.
- Their ability to target the organs of the RES.
- The possibility of ideal zero-order drug release kinetics.
- The lack of occurrence of undesired immune response against encapsulated drug.
- Large quantity of drug can be encapsulated within a small volume of cells.
- A longer life span in circulation as compared with other synthetic carriers and optimum conditions may result in the life span comparable to that of normal erythrocytes.
- Easy control during life span ranging from minutes to months.
- A decrease in side effects of drugs.[4]

DISADVANTAGES

- Possibility of clumping of cells and dose dumping may be there.
- Limited potential as carrier to non- phagocyte target tissue.
- The major problem encountered in the use of biodegradable materials or natural cells as drug carriers is that they are removed in vivo by the RES as result of modification that occurred during loading procedure in cells. This, although expands the capability to drug targeting to RES, seriously limits their lifespan as long-circulating drug carriers in circulation and in some cases, may pose toxicological problems.
- The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
- Several molecules may alter the physiology of the erythrocyte.
- Encapsulated erythrocytes may present some inherent variations in their loading and characteristics compared to other carrier systems.
- The storage of the loaded erythrocytes is a further problem provided that there are viable cells and need to survive in circulation for a long time upon re-entry to the host body. Conditioning carrier cells in isotonic buffers containing all essential nutrients, as well as in low temp., the addition of nucleosides or chelators, lyophilization with glycerol or gel immobilizations have all been exploited to overcome this problem.
- Possible contamination due to the origin of the blood, the equipment used and the loading environment. Rigorous controls are required accordingly for the collection and handling of the erythrocytes.[3]

ISOLATION OF ERYTHROCYTES

1. Blood is collected into heparin zed tubes by venipunture.
2. Blood is withdrawn from cardiac/splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti-coagulant.
3. The whole blood is centrifuged at 2500 rpm for 5 min. at 4 $\pm 1^0$C in a refrigerated centrifuge.
4. The serum and Buffy coats are carefully removed and packed cells washed three times with phosphate buffer saline (pH=7.4).
5. The washed erythrocytes are diluted with PBS and stored at 4^0C for 48 h before use.
6. 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.[1,4]

Table No.1: Isolation of Erythrocytes

Sr.No.	Species	Washing Buffer	Centrifugal Force(g)
1	Rabbit	10mmol KH2PO4/NaHPO4	500-1000
2	Dog	15mmol KH2PO4/NaHPO4	500-1000
3	Human	154mmol Nacl	<500
4	Mouse	10mmol KH2PO4/NaHPO4	100-500
5	Cow	10-15 mmol KH2PO4/NaHPO4	1000
6	Horse	2mmol MgCl2,10mmol glucose	1000
7	Ship	10mmol KH2PO4/NaHPO4	500-1000
8	Pig	10mmol KH2PO4/NaHPO4	500-1000

Requirement for encapsulation

1. Variety of biologically active substance (5000-60,000dalton) can be entrapped in erythrocytes.
2. Non-polar molecule may be entrapped in erythrocytes in salts. Example: tetracycline HCl salt can be appreciably entrapped in bovine RBC.
3. Generally, molecule should be Polar & Non polar molecule also been entrapped.
4. Hydrophobic molecules can be entrapped in erythrocyte by absorbing over other molecules.
5. Once encapsulated charged molecule are retained longer than uncharged molecule.
6. The size of molecule entrapped is a significant factor when the molecule is smaller than sucrose and larger than B-galactosidase.[4]

Properties of Resealed Erythrocyte as Novel drug delivery carriers

1. The drug should be released at target site in a controlled manner.

2. It should be appropriate size, shape and should permit the passage through capillaries. and minimum leakage of drug should take place.
3. It should be biocompatible and should have minimum toxic effect.
4. It should possess the ability to carry a broad spectrum of drug.
5. It should possess specific physicochemical properties by which desired target size could be recognized.
6. The degradation product of the carriers system, after release of the drug at the selected site should be biocompatible.
7. It should be physico -chemically compatible with drug.
8. The carrier system should have an appreciable stability during storage.[1]

Mechanism of release of loaded drugs

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

- **Phagocytosis**

By the process of phagocytosis normally erythrocyte cells removed from the blood circulation. The degree of cross linking determines whether liver or spleen will preferentially remove the cells.

- **Diffusion through the membrane of the cells**

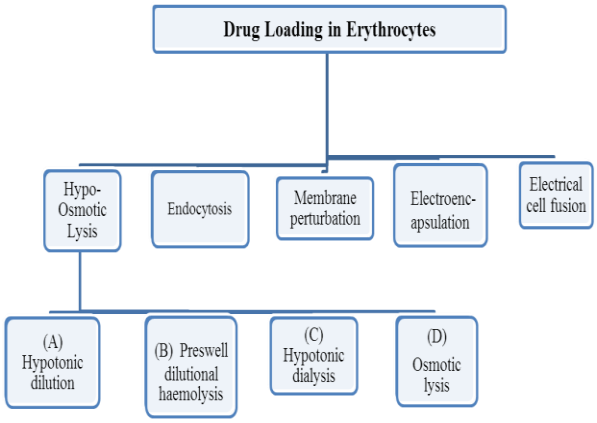
Diffusion through the membrane depends on the drug molecule penetrate through a lipid bilayer i.e. bioactive compound have lipid solubility.

- **Using a specific transport system**

Most of the drug molecules enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes.[1]

METHODS OF DRUG LOADING IN ERYTHROCYTES

The methods used to load drugs or other bioactive compounds in erythrocytes, includes physical (e.g., electrical pulse method) osmosis-based systems and chemical methods (E. g. Chemical perturbation of the erythrocytes membrane). Along with the method used, for the successful entrapment of the compound requires optimal characteristics like 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 pharmacodynamics properties.[4]



A. Hypo-osmotic Lysis

1. Hypotonic Dialysis

This method was first reported in 1959 by Klibansky and was used in 1977 by Deloach and Ihler and in 1989 by Gaudreault RC for loading enzymes and lipids. A desired Haematocrit is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution. This mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. During dialysis bubble serves to blend the content. The tube is placed in a bottle containing 200ml of lysis buffer solution and placed on a mechanical rotator at 4^{o}C for 2 hrs. The dialysis tube is then placed in 200 ml of resealing solution (isotonic PBS pH 7.4) at temperature 25 – 30^{o}C for resealing. The loaded erythrocytes thus obtained are then washed with cold PBC at 4^{o}C. The cells are finally resuspended in PBC.

Examples of encapsulated agents: Gentamicin, adriamycine, erythropoietin, furamycin A, IgG etc.[3.4.6]

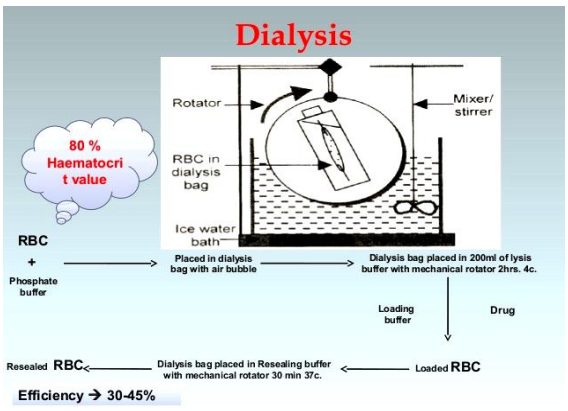


Fig 3: Hypotonic Dialysis Method

2. Hypotonic Dilution

In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The RBC'S are exposed to hypotonic solution (corresponding to 0.4% Nacl), the erythrocytic membrane ruptures permitting escape of cellular contents

and equilibrium is achieved with in one minute. The cells swell up to 1.6 times its original volume indicated by formation of pores of size 200 – 500 A^o. The length of time for which these pores remain open is not fixed. However at 0^oC the opening permits long enough to allow partial resealing of membrane. Increasing ionic strength to isotonicity and incubating the cells at 37^oC causes the pores to close and restore osmotic properties of the RBC'S. This method is simplest and fastest yet the capsulation efficacy is very low i. e. 1 – 8%. Efficient for of low weight drugs.

Examples of encapsulated agents: β-glucosidase, asparaginase, arginase, salbutamol.[3,4,6]

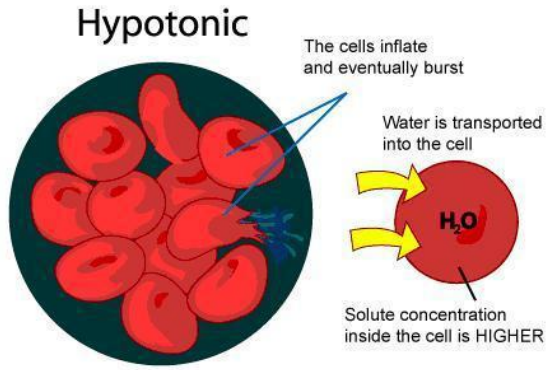


Fig 4: Hypotonic Dilution Method

3. Hypotonic preswell method

The method was investigated by Rechsteiner in 1975 and was modified by Jenner et al for drug loading. This method was based on the principle of first swelling the erythrocytes without lysis by placing them in slightly hypotonic solution. The swollen cells are recovered by centrifugation at low speed. Then, relatively small volumes of aqueous drug solution are added to the point of lysis. The slow swelling of cells results in good retention of the cytoplasmic constituents and hence good survival in vivo. This method is simpler and faster than other methods, causing minimum damage to cells.

Examples of encapsulated agents: Propranolol, asparginase, methotrexate, isoniazid etc.[3,4,6]

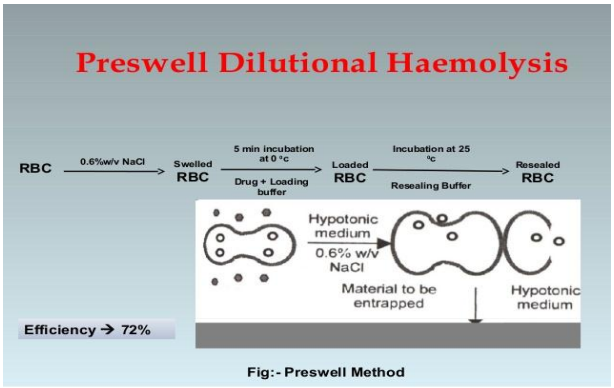


Fig 5: Hypotonic preswell Method

4. Isotonic osmotic lysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis. Erythrocytes incubated in solutions of a substance with high membrane permeability; the solute will diffuse into the cells because of the concentration gradient. Chemicals such as urea solution, polyethylene glycol, ammonium chloride and dimethyl sulfoxide (DMSO) have been used for isotonic hemolysis.

Examples of encapsulated agents: Inositol hexaphosphate.[3,4,6]

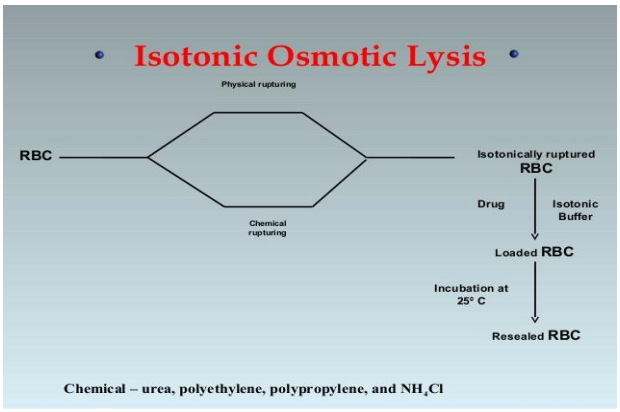


Fig 6: Isotonic Osmotic Lysis Method

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 showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. The method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes in 1980. Lin et al. used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

Examples of encapsulated agents: Daunomycin.[3,4,6]

C. Electrical breakdown method/ Electoencapsulation

When the membrane is polarized very rapidly (in nano to micro seconds) using voltage of about 2kV/ cm for 20µ sec electrical breakdown of a cell membrane is observed which leads to the formation of pores and entrapment of drugs. Electrical breakdown probably takes place in the lipoid regions or at the lipid protein junction in the membrane. Pores formed are stable and it is possible to control pore size. Subsequently the pores can be resealed by incubation at 37^oC in osmotically balanced medium.

Examples of encapsulated agents: Primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, vitamin A etc.[3.4.6]

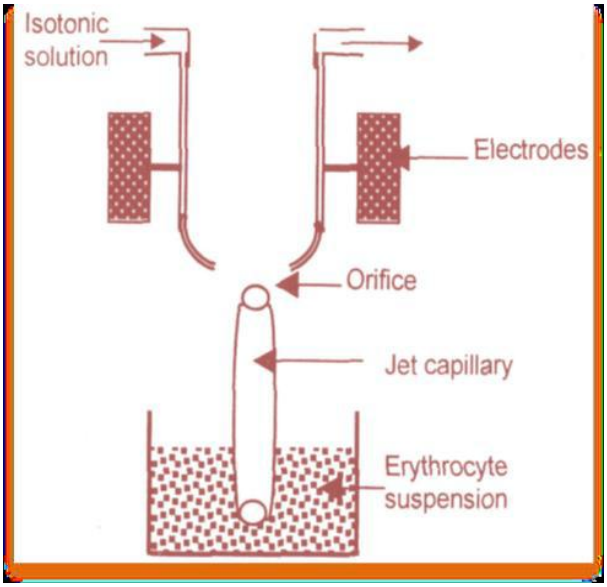


Fig 7: Electro-encapsulation Method

D. Endocytosis

This method was reported by Schrier (1987). Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffercontaining2.5 mm ATP, 2.5 mm MgCl2 and 1mM CaCl2, followed by incubation for2 min at room temperature. The pores created by this method are resealed by using 154 mm of Nacl and incubation at 37^0c 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-versa.

Examples of encapsulated agents: Hydrocortisone, propranolol, vitamin A Primaquine, vinblastine, chlorpromazine etc.[3,4,6]

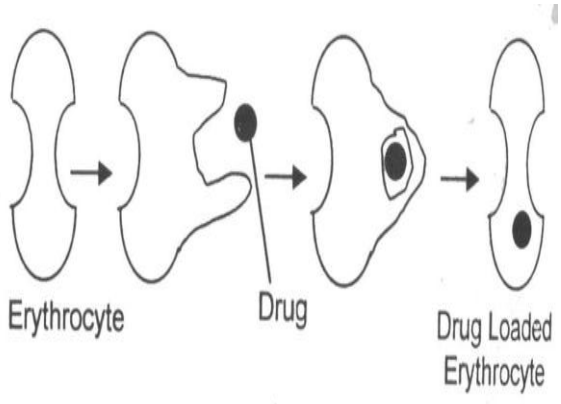


Fig 8: Drug loaded by Endocytosis

E. Loading by electric cell fusion

This method involves the initial loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The fusion is accentuated by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell-specific monoclonal antibody into an erythrocyte ghost. An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells.[3,4,6]

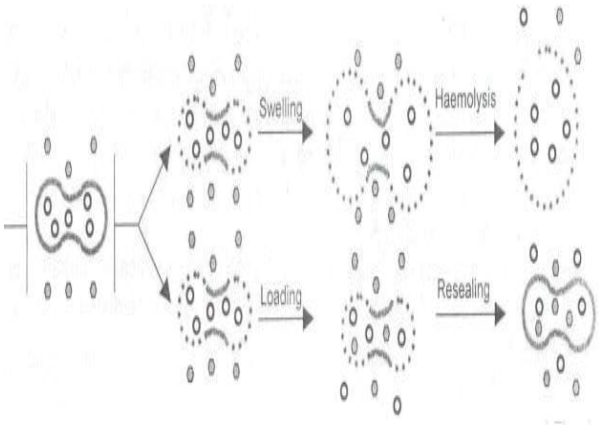


Fig 8: Drug loaded by Electric cell fusion

F. Loading by lipid fusion

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule. This method provides very low encapsulation efficiency. Nicola and Gresonde fused lipid vesicle containing inositol hexaphosphate with human erythrocytes. The incorporated inositol hexaphosphate in erythrocytes provided a significant lowering of the oxygen affinity for hemoglobin in intact erythrocytes. Harrison et al reported resealing of tyrosine kinase into human erythrocytes by rapid freezing and thawing in liquid.

Examples of encapsulated agents: Inositol monophosphate.[3,4,6]

G. Use of Red cell loader

Magnani and coworkers, 1998 developed a novel method for the entrapment of non diffusible drugs into human erythrocytes. The equipment designed for this method was termed as "red cell loader". The method requires as little as 50ml of blood sample, different biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed erythrocytes followed by concentration with a hem filter and an isotonic resealing of the cells. There was 30% drug loading with 35–50% cell recovery. The processed erythrocytes had normal survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages.[4,6]

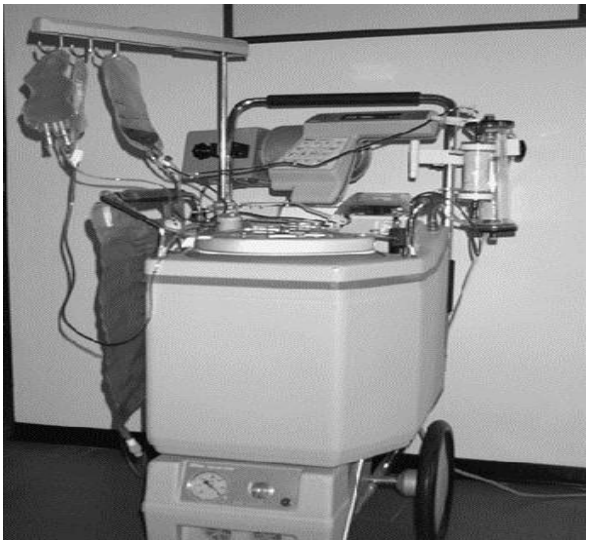


Fig 9: Red cell loader.

EVALUATION OF RESEALED ERYTHROCYTES

After loading of therapeutic agent on erythrocytes, the carrier cells are exposed to physical, cellular as well as biological evaluations.

1. Shape and Surface Morphology

The morphology of erythrocytes decides their life span after administration. The morphological characterization of erythrocyte is undertaken by comparison with untreated erythrocytes using either Transmission (TEM) or Scanning Electron Microscopy (SEM).[1,3,4]

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 by spectrophotometrically.[1,3,4]

3. Cell Counting and Cell Recovery

This involves counting the number of red blood cells per unit volume of whole blood, usually by using automated machine it is determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug.[1,3,4]

4. Turbulence Fragility

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, haemoglobin and drug released after the procedure are determined. The turbulent fragility of resealed cells is found to be higher.[1,3,4]

5. Erythrocyte sedimentation rate (ESR)

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein, especially fibrinogen and α, β globulins. This test is performed by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.[1,3,4]

6. Determination of entrapped magnetite

Atomic absorption spectroscopic method is reported for determination of the concentration of particular metal in the sample. The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60^0C for 2 hours, then 20%w/v trichloro acetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.[1,3,4]

7. In vitro stability

The stability of the loaded erythrocytes is assessed by means of the incubation of the cells in the autologous plasma or in an iso-osmotic buffer, setting hematocrit between 0.5% and 5% at temperatures of 4^0C and 37^0C.[1,3,4]

8. In-vitro drug release and Hb content

The cell suspensions (5% hematocrit in PBS) are stored at 40C in ambered color glass container. Periodically clear supernatant are drawn using a hypodermic syringe equipped with 0.45 are filter, deproteined using methanol and were estimated far drug content. The supernatant of each sample after centrifugation collected and assayed, %Hb release may be calculated using formula;

% Hb release=A540 of sample-A540 of backgroundA540 of 100% Hb.[1,3,4]

9. Osmotic shock

For osmotic shock study, erythrocytes suspension (1 ml 10% hct) was diluted with distilled water (5 ml) and centrifuge at 300 rpm for 15 minutes. The supernatant was estimated for % haemoglobin release analytically.[,3,4]

10. Miscellaneous

Resealed erythrocyte can also be characterized by cell sizes, mean cell volume, energy metabolism, lipid composition, membrane fluidity, rheological properties and density gradient separation.[1,4]

Route of administration

Resealed erythrocyte during experimentation has been administered to the laboratory animals intravenously through cardinal 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 (1993) have proposed erythrocyte based nasal delivery of propranolol.[4]

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, gluteraldehyde, 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 nitrogen.[2,4]

APPLICATIONS OF RESEALED ERYTHROCYTES

In-Vitro Application

For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes. Enzymes within carrier RBC could be visualized with the help of cytochemical technique. The defects such as the glucose- 6- phosphate dehydrogenase (G6PD) deficiency can be useful tool for discerning the mechanism that eventually causes these effects. 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. Antibody RBC auto-injected into living cells has been used to confirm the site of action of fragment of toxin. Antibodies introduced using RBC mediated microinjection is recorded not to enter the nucleus, thus limiting the studies to the cytoplasmic level.[3,4]

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 drug.[2,3,4]

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 glucoronidase and β-galactosidase.[2,3,4]

3] Treatment of hepatic tumors

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate, bleomycin, asparginase 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 orcisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver.[,3,4]

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 Drugs.[2,3,4]

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.[2,3,4]

6] Removal of toxic agents

Inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulfate. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiestrase.[2,3,4]

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.[3,4]

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, organ phosphorus) and as drugs.

There are some problems in the direct injection of enzymes into the body, to overcome these problems use of enzyme-loaded erythrocytes is the best solution. 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 RES.[3,4,5]

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 hb.[2,4,5]

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 compounds 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 cells.[3,4,5]

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.[2,3,4]

Other applications of resealed erythrocytes include

* Surface modification with antibodies
* Surface modification with gluteraldehyde
* Surface modification with carbohydrates such as salicylic acid
* Entrapment of paramagnetic particles along with the drug
* Entrapment of photosensitive material
* Antibody attachment to erythrocyte membrane to get specificity of action of enzymes.
* It is used for Delivery of antiviral agents such as azidothymidine, azathioprene etc
*It is used for Improvement in oxygen delivery to tissues
*It is used for Microinjection of macromolecules.[3,4,6]

CONCLUSION

The resealed erythrocytes showed promising drug carrier characteristics. Due to the several potential advantages over other, this drug loaded erythrocytes seems to be a promising delivery system for the controlled and site specific delivery of therapeutic agents. The preparation of resealed erythrocytes is very easy and can be easily characterized by different available techniques. However, the concept needs further research and optimization to become a routine drug delivery system. The targeted release of therapeutic agents is among the most attractive applications of erythrocytes carrier which can be extended for the delivery of biopharmaceuticals. Thus the potential of this delivery system need to be explored for management of diseases.

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