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REVIEW ON RESEALED ERYTHROCYTES

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ABSTRACT

Among the various carriers used for targeting drugs to various body tissues, the cellular carriers meet several criteria desirable in clinical applications, among the most important being biocompatibility of carrier and its degradation products. Leucocytes, platelets, erythrocytes, nanoerythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier systems. Among these, the erythrocytes have been the most investigated and have found to possess greater potential in drug delivery. 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) having potential carrier capabilities for the delivery of drugs and drug loaded microspheres. 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. Encouraging the use of erythrocytes in drug delivery include various advantages like as remarkable degree of biocompatibility, Complete biodegradability, lack of toxic product, controllable life-span, decreasing drug side effects etc. So many drugs like aspirin, steroid, cancer drug which having many side effects are reduce by resealed erythrocyte. Biopharmaceuticals, therapeutically significant peptides and proteins, nucleic acid-based biologicals, antigens and vaccines, are among the recently focused pharmaceuticals for being delivered using carrier erythrocytes. Current review highlights isolation, drug loading methods, Evaluation methods and applications of resealed erythrocytes for drug delivery.

KEYWORDS: Resealed Erythrocytes, carrier, Isolation, evaluation, Applications of Resealed erythrocytes.

INTRODUCTION[1]

The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog. The desirable properties of the carriers used in the drug targeting are to protect the drug from premature bioinactivation and direct the drug to the target site. The property desired increases the dose effectives of drug by reducing the dosage and frequency of administration. This desirable property is achieved by using biological carriers (antibodies, liposomes, macromolecules, erythrocytes). Biological carriers can be used to achieve sustained and controlled systemic drug delivery. Such carriers can also be introduced directly in to the blood stream unlike synthetic carriers which have to be administered orally or parenterally. Amongst all the different types of carriers' erythrocytes due to their unique nature and property are used in providing an ideal drug delivery system. Blood contains different type of cells like erythrocytes (RBC), leucocytes (WBC) and platelets, among them erythrocytes are the most interesting carrier and posses great potential in drug delivery due to their ability to circulate throughout the body, zero order kinetics, reproducibility and ease of preparation, primary aim for the development of this drug delivery system is to maximize therapeutic performance, reducing undesirable side effects of drug as well as increase patient compliance. The overall process is based on the response of these cells under osmotic conditions. Upon reinjection, the drug-loaded erythrocytes serve as slow circulating depots and target the drugs to disease tissue or organ. Present pharmaceutical scenario is aimed at development of drug delivery systems which maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases. Targeting of an active bio molecule from effective drug delivery where pharmacological agent directed specifically to its target site. Drug targeting can be approaches by either chemical modification or by appropriate carrier.

ERYTHROCYTES

These cells were described in human blood samples by Dutch Scientist Lee Van Hock in 1674. In the 19th century, Hope Seyler identified hemoglobin and its crucial role in oxygen delivery to various parts of the body Red blood cells (also referred to as erythrocytes) are the most common type of blood cells and the vertebrate organism's principal 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. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their

components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately quarter of the cells in the human body are red blood cells.

Anatomy, physiology and composition of RBCs

RBCs have shapes like biconcave discs with a diameter of 7.8 μm and thickness near 2.2 μm. Mature RBCs have a simple structure. It is also in elastic in nature. Their plasma membrane is both strong and flexible, which allows them to deform without rupturing as they squeeze through narrow capillaries. RBCs lack a nucleus and other organelles and can neither reproduce nor carry on extensive metabolic activities. RBCs are highly specialized for their oxygen transport function, because their mature RBCs have no nucleus, all their internal space is available for oxygen transport. Even the shape of RBC facilities it's function. A biconcave disc has a much greater surface area for the diffusion of gas molecules into and out of the RBC than would; say a sphere or a cube. The red blood cell membrane, a dynamic, semi permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cations (Na+, K+) and anions (Cl- HCO3-). Each RBC contains about 280 million hemoglobin molecules. A hemoglobin molecules consists of a protein called globin, composed of four polypeptide chains; a ring like non-protein pigment called a heme, is bound to each of the four chains. At the center of the heme ring combine reversibly with one oxygen molecule, allowing each hemoglobin molecule to bind four oxygen molecules. .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.

CONSTITUENTS	CONTENT IN %
Water	63%
Lipids	0.5%
Minerals	0.8%
Glucose	0.7%
Methehemoglobin	0.5%
Hemoglobin	33.6%
Non-hemoglobin protein	0.9%

ERYTHROCYTES AS DRUG CARRIERS/RESEALED ERYTHROCYTES[2,3]

The developing RBC has the capacity to synthesize hemoglobin. The adult RBC however loses their capacity and serves only to carry hemoglobin. The use of cells as drug delivery

system requires that the drug which are normally unable to permeate the membrane, should be made to traverse the membrane without causing any irreversible changes in membrane structure and permeability. Further the cells should be able to release the drug in controlled manner upon reaching the desired target. RBCs have solid content of about 35% (rest 65% being water). Apart from this the erythrocytes have phosphate content which is in organic nature. The osmotic pressure of the interior of erythrocytes is equal to that of plasma and termed as isotonic. (equal to the osmotic pressure of 0.9% Nacl). If medium is hypotonic water diffuses into the cells and they get swelled and eventually lose their hemoglobin content and may burst. If medium is hypertonic (osmotic pressure more than 0.9% Nacl) they will shrink and become irregular in shape. Such erythrocytes which contain no or little hemoglobin are called ghosts. 3 types of ghosts can be distinguished: type-1 ghosts which reseal immediately after heamolytic; type-2 ghosts which reseal after reversal of heamolytic by addition of alkali ions; type-3 ghosts which remain leaky under different experimental conditions. RBC's are biocompatible provided that compatible cells are used in patients; there is no possibility of triggered immunological response. Since resealed erythrocytes are being considered as novel carriers.

DEFINATION

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 carriers8. Hence, these carriers are called resealed erythrocytes.

PROPERTIES OF RESEALED ERYTHROCYTES AS A DRUG CARRIER

It should be of appropriate size and shape to permit the passage through the capillaries.

It should possess specific physico – chemical properties by which a desired target site could be recognized.

It should be biocompatible and have minimum toxic side effects.

Degradation products should be biocompatible.

Minimum leaching\ leakage of drugs should takes place before target is reached.

Possess ability to carry a broad spectrum of drugs with different properties.

Physico- chemically compatible with drugs.

The carrier system should have an appreciable stability during storage.

ADVANTAGES

1. Biocompatible, particularly when autologous cells are used hence no possibility of triggered immune response.
2. Biodegradability with no generation of toxic products.
3. Considerable uniform size and shape of carrier.
4. Relatively inert intracellular environment can be encapsulated in a small volume of cells.
5. Isolation is easy and large amount of drug can be loaded.
6. Prevention of degradation of the loaded drug from inactivation by endogenous chemical.
7. Entrapment of wide variety of chemicals can be possible.
8. Entrapment of drug can be possible without chemical modification of the substance to be entrapped.
9. Possible to maintain steady-state plasma concentration, decrease fluctuation in concentration.
10. Protection of the organism against toxic effect of drug.
11. Targeting to the organ of the RES.
12. Ideal zero-order drug release kinetic.
13. Prolong the systemic activity of drug by residing for a longer time in the body.

DISADVANTAGES

1. They have a limited potential as carrier to non-phagocyte target tissue.
2. Possibility of clumping of cells and dose dumping may be there.
3. Several molecules may alter the physiology of the erythrocyte.
4. Given that they are carriers of biological origin, encapsulated erythrocytes may present some inherent variations in their loading and characteristics compared to other carrier systems.
5. 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.

ERYTHROCYTES CAN BE USED AS A CARRIERS IN TWO WAYS[4,5]

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.

2. Continuous or prolonged release of drugs

Alternatively, 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.

ISOLATION OF ERYTHROCYTES

1. Blood is collected into heparin zed tubes by venipuncture.

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 ±10C 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 40C for as long as 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.

Various conditions and centrifugal forces used for isolation of erythrocytes

s.no	Speices	Washing buffer	Centrifugal force
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	10-15mmol KH2PO4/NaHPO4	1000
6	Horse	2mmol MgCl2, 10mmol glucose	1000
7	Sheep	10mmol KH2PO4/NaHPO4	500-1000
8	**Pig**	**10mmol KH2PO4/NaHPO4**	**500-1000**

REQUIRMENT FOR ENCAPSULATION

Variety of biologically active substance (5000-60,000dalton) can be entrapped in erythrocytes.

Non-polar molecule may be entrapped in erythrocytes in salts. Example: tetracycline Hcl salt can be appreciably entrapped in bovine RBC.

Generally, molecule should be Polar & Non polar molecule also been entrapped.

Hydrophobic molecules can be entrapped in erythrocyte by absorbing over other molecules.

Once encapsulated charged molecule are retained longer than uncharged molecule the size of molecule entrapped is a significant factor when the molecule is smaller than sucrose and larger than B-galactosidase.

METHODS FOR DRUG LOADING IN ERYTHROCYTES[7]

Several methods can be used to load drugs, enzymes or other bioactive compounds in erythrocytes. 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, with well-defined pharmacokinetic and pharmacodynamics properties.

1) Hypo – osmotic method

Dilution method

Dialysis method

Pre-swell method

Isotonic osmotic lyses

2) Electrical break down method

3) Endocytosis method

4) Membrane perturbation method

5) Normal transport method

6) Lipid fusion method

7) Using red cell loader

1. HYPO-OSMOTIC LYSIS METHOD

In this process, the intracellular and extra cellular solutes of erythrocytes are exchanged by osmotic lyses and resealing. The drug present will be encapsulated within the erythrocytes membrane by this process.

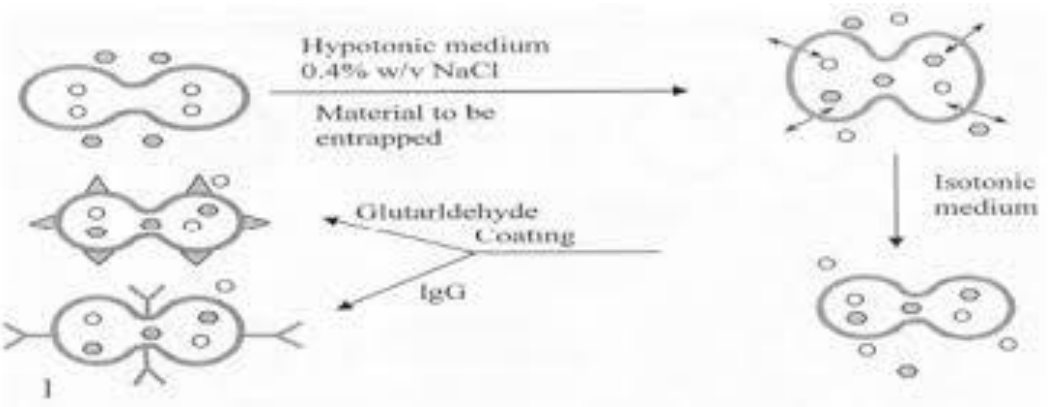
Hypo-osmotic lysis of cells in a solution containing the drug/enzyme to be entrapped followed by restoration of tonicity to reseal them. The ghost population obtained is heterogeneous and they are of three types.

Type I ghosts reseal immediately after heamolytic.

Type II ghosts reseal after reversal of haemolysis by addition of alkali ions.

Type III ghosts remain leaky under different experimental conditions.

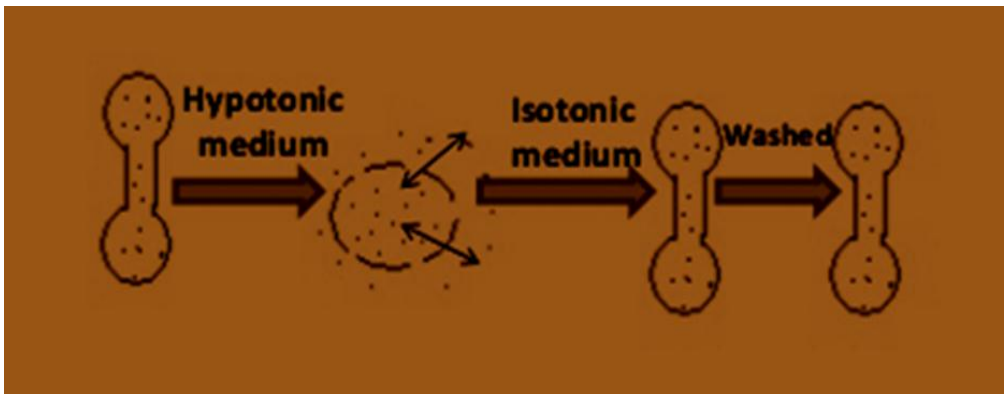
Increase in volume initially leads to conversion in swollen erythrocytes. These have little capacity to resist volume greater than 50-75% of the initial volume and when placed in solution less than about 150mOsm/Kg, the membrane ruptures, permitting escape of the cellular constituents Erythrocyte are resealed on addition of sufficient 1.54 M KCl, which restores isotonicity. Where preservation of energy metabolism within the cells is enviable, 4mM magnesium salts (e.g. MgCl2, MgSO4), 10 Mm glucose and 2mM adenosine are included during resealing to attain above final concentrations.



DILLUTION METHOD

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 up to 1.6 times its original volume. The swelling results in the appearance of pores of 200 – 500 Ao in size. The length of time for which these pores remain open is not fixed. However at 0oC the opening permits long enough to allow partial resealing of membrane. Increasing the ionic strength to isotonicity and incubating the cells at 37o C causes the pores to close and restore the osmotic properties of the RBC'S.

This method was used to entrap b – glucosidase and b – galactosidase.



ADVANTAGES

1. This method is simplest and fastest.
2. Efficient for encapsulation of low molecular weight drugs.

DISADVANTAGES

1. Most of the cytoplasmic constituents are lost during the osmotic lyses.
2. Encapsulation efficiency is low i.e, 1-8%.

DIALYSIS METHOD

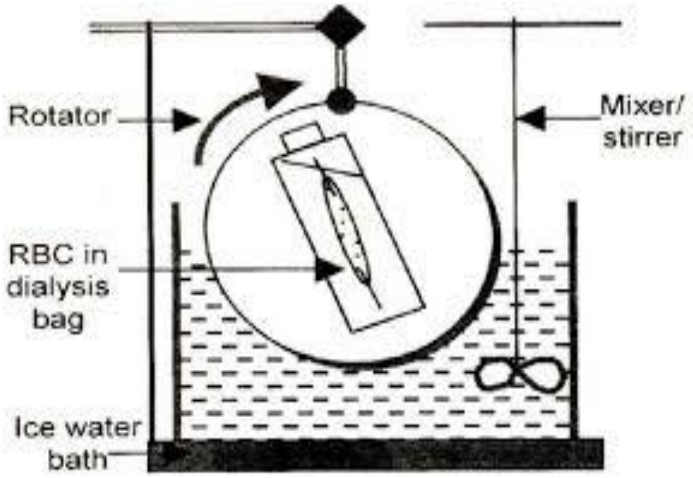
A desired Hematocrit 80% is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution. This mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of nearly 25 % of the internal volume is left in the tube. During dialysis bubble serves to blend the content. The tube is placed in a bottle containing 200 ml of lysis buffer solution and placed on a mechanical rotator at 4o C for 2 hrs. The dialysis tube is then placed in 200 ml of resealing solution (isotonic PBS pH 7.4) at room temperature 25 – 30 o C for resealing. The loaded erythrocytes thus obtained are then washed with cold PBS at 4oC. The cells are finally resuspended in PBS.

ADVANTAGES

1. Good entrapment efficiency is obtained (33-45%).
2. The volume of extra cellular solution that equilibrates with the intracellular space of erythrocyte during lyses is considerably reduced.

DISADVANTAGES

1. Time consuming method.
2. The size distribution of loaded ghosts is not found to be homogeneous as revealed by studies with hydro dynamically focusing particle analyzer.



ERYTHROCYTE DIALYZER

PRE-SWELL METHOD[8]

This method was investigated by Rechsteiner in 1975 and this technique was modified by Jenner et al. for drug loading. This method is based on the following steps.

1) Swelling: In this step erythrocyte cell swells without lysis when placed them in slightly hypotonic solution i.e. 0.6%w/v solution.

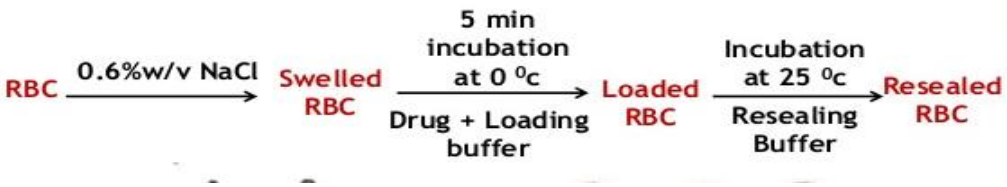
2) Drug Loading: After swelling relatively small volumes of aqueous drug solutions (loaded buffer) are added to the point of lysis. The slow swelling of cells resulted in good retention of the cytoplasmic constituents and incubated it for 5min at 0 C.

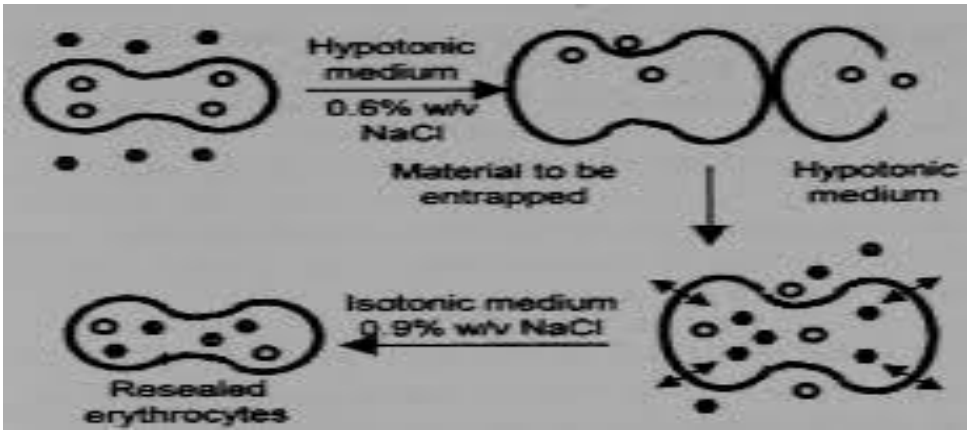
3) Resealing: Drug loaded erythrocyte is placed in resealed buffer and incubated at 25C.

ADVANTAGES

1. This method is simple and faster than other methods causing minimum damage to cells.
2. This method is 72% efficient.
3. under optimum conditions resealed erythrocytes can survive in – vivo as long as native RBC'S.

The drugs like Propranolol, asparagines, methotrexate, insulin, metronidazole, enalaprilat and isoniazid encapsulated by using this method.

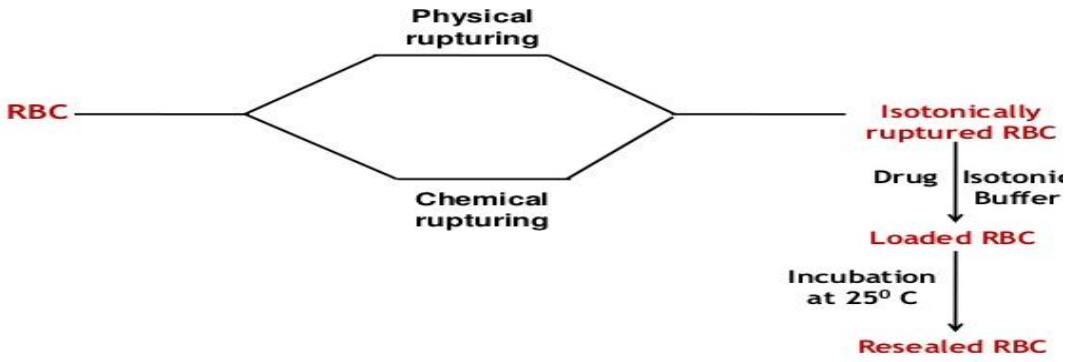




PRESWELL METHOD

ISO-OSMOTIC LYSIS METHOD

This method also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isotonic. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals such as urea solution, polyethylene glycol, and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO) .The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37C.

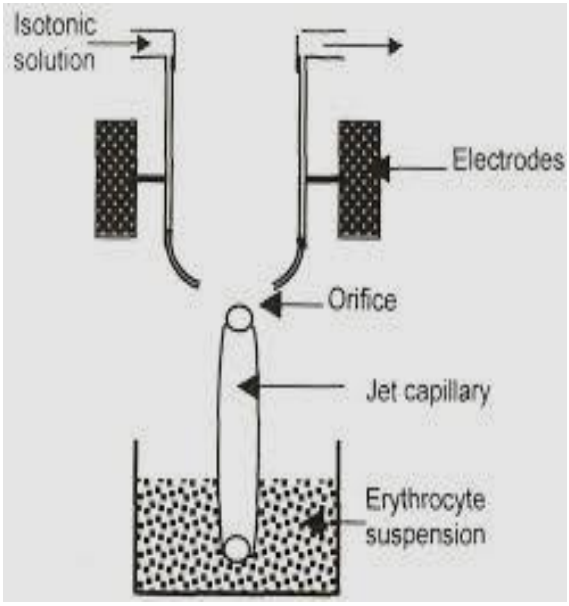


2. ELECTRICAL BREAK DOWN METHOD

In 1973, Zimmermann tried an electrical pulse method to encapsulate bioactive molecules Also known as electroporation, the method is based on the observation that electrical shock

brings about irreversible changes in an erythrocyte membrane. The erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 370C in an isotonic medium. This dielectric breakdown can be achieved by polarization of erythrocyte membrane for 20µseconds using varied voltage of 2kv/cm. The potential difference across the membrane can be built up by either directly by inter and intracellular electrodes or indirectly by applying internal electric field to the cells. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium.

The various chemical encapsulated into the erythrocytes are primaquin and related 8amino quinolone, vinblastin chloropromazine and related phenothiazine, propanolol, tetracaine and vitamin A.

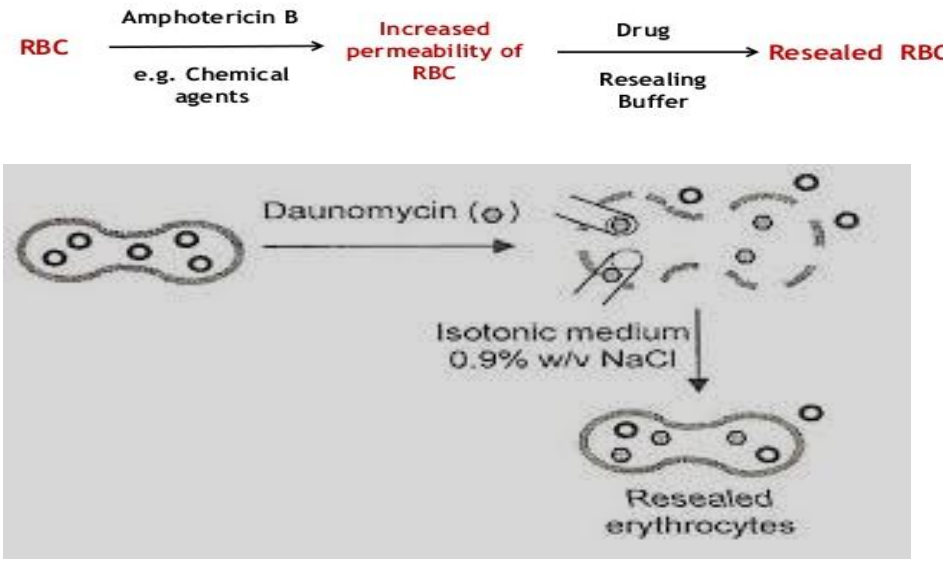


ELECTROENCAPSULATION TECHNIQUE

3.MEMBRANE PERTURBATION METHOD

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemical agent. This allows the low molecular weight substances to get entrapped.

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. Lin used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular. This method is expensive.



DRUG LOADED BY MEMBRANE PERTURBATION METHOD

4. ENDOCYTOSIS METHOD

This technique was reported by Schrier et al. in 1975. This technique involves the addition of one volume of packed erythrocytes to 9 volumes of buffer which contains ATP, $MgCl_2$ and $CaCl_2$ and incubated it at 25ºC for 2 min. Intracellular vesicles could be inducing in erythrocytes containing small molecules drugs or virus from external medium. This method is efficient for loading larger particles such as virus (up to 1000 nm diameter), enzymes and small molecules. Resealing of erythrocyte membrane by the addition of Nacl to 154 Mm

followed by incubation for 2 min at 37ºC. These resealed erythrocytes are washed in 5mM imidazoleglycylglcine buffer, pH 7.4 containing 154mM Nacl.

In this method the vesicle membrane separates the endocytosed substance from the cytoplasm containing the drug which are sensitive to inactivation by cytoplasmic enzymes and also protect the erythrocyte membrane. The contents of vesicles, however may release into erythrocytes cytoplasm depending upon the nature of material.

Entrapment of glucose, insulin and b – glucuronidase by a chlorpromazine induced endocytosis has been reported. The drugs like primaquine, vinblastine, 8-amino-quinolines, chlorpromazine and related phenothiazines, hydrocortisone and tetracaine can be successfully entrapped by this technique.

DRUG LOADED BY ENDOCYTOSIS METHOD

6. NORMAL TRANSPORT MECHANISM

It is possible to load erythrocytes with drugs without disrupting the erythrocytic membrane in any way by incubating the drug and erythrocytes for varying period of time. After infusion the drug would in general, exit from the cell following the kinetics comparable to those observed for entry.

The drug encapsulated by this method is vitamin B12.

7. LIPID FUSON METHOD

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule. Nicolau 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.

The entrapment efficiency of this method is very low (~1%).

8. USING RED CELL LOADER

Novel method was developed for entrapment of non diffusible drugs into erythrocytes. They developed a piece of equipment called a "red cell loader". With as little as 50 ml of a 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 heme 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.

RED CELL LOADER

RELEASE MECHANISM OF RESEALED ERYTHOCYTES[9]

1) 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.

2) 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.

3) 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.

EVALUATION OF RESEALED ERYTHROCYTES[10]

In vitro characterization of loaded erythrocytes

Shape and surface morphology.

The morphological examination of these ghost erythrocytes is undertaken by comparison with untreated erythrocytes using either transmission (TEM) or scanning (SEM) electron microscopy. By means of 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

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 at2500 rpm for 10 min. The clear supernatant is analyzed for the drug content by spectrophotometrically.

Percent cell recovery & cell counting

This involves counting the number of red blood cells per unit volume of whole blood, usually by automated counting. Red cell recovery may be calculated on the basis of the differences in the hematocrit and the volume of the suspension of erythrocytes before and after loading. The goal is to minimize the loss during the encapsulation procedure to maximize cell recovery.

Turbulence shock

It is the measure of simulating destruction of loaded cells during injection. Normal and drug loaded cells are passed through a 23 gauge hypodermic needle at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collection of an aliquot and centrifugation at 2000 rpm for 10 minutes. The hemoglobin in withdrawn sample is estimated. Drug loaded erythrocytes appear to be less resistant to turbulence, probably indicating destruction of cells upon shaking.

Osmotic fragility and Osmotic shock study

Osmotic Fragility

To evaluate the resistance of erythrocytes membrane against the osmotic pressure changes of their surrounding media, drug loaded resealed erythrocytes suspended in isotonic saline and

was incubated separately in stepwise decreasing concentration of sodium chloride solution (0.9%w/v to 1%w/v) at 37±2°C for 10 minutes, followed by centrifugation at 2500 rpm for 10 min and supernatant was examined for drug content.

Osmotic Shock

Osmotic shock describes a sudden exposure of drug-loaded erythrocytes to an environment, which is far from isotonic to evaluate the ability of resealed erythrocytes to withstand the stress and maintain their integrity as well as appearance. Incubating the drug loaded resealed erythrocytes Suspension (10-50% haemotocrit) with distilled water (5 ml) for 15 min followed by centrifugation at 2500rpm and the supernatant was estimated spectrophotometrically for percent hemoglobin content.

Erythrocyte sedimentation rate

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.

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 600C 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.

Invitro hemoglobin release and drug content

In-vitro release of drug(s) and Hemoglobin are monitored periodically form drug loaded cells. The cells suspension (5% hematocrit in PBS) is stored at 40°C in amber colored glass containers. Periodically the clear supernatants are withdrawn using hypodermic syringes equipped with 45 µm filter, deproteinized using methanol and estimated for drug content. The supernatant of each sample after centrifugation is collected and assayed, percentage hemoglobin release may be calculated using the formula.

Invitro stability and hemoglobin release

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 40c and 37c.

The content of Hemoglobin of the erythrocytes may be diminished by the alterations in the permeability of the membrane of the red blood cells during the encapsulation procedure. Furthermore, the relationship between the rate of hemoglobin and rate of drug release of the substance encapsulated from the erythrocytes. The Hemoglobin leakage is tested using a red cell suspension by recording absorbance of supernatant at 540nm on a spectrophotometer.

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.

APPLICATIONS[11]

1. Invitro applications.
2. In vivo applications.

1. Invitro application:

Carrier RBCs have proved to be useful for a variety of in vitro tests. For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes by phagolysosomes. An inside to this study showed that enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The most frequent in vitro application of RBC mediated microinjection. A protein or nucleic acid to be 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 have been used to confirm the site of action of fragment of diptheria toxin.

2. Invivo applications

This includes the following

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.

2) Drug targeting

Ideally, drug delivery should be site specific and target oriented to exhibit maximal therapeutic index with minimum adverse effects. Resealed erythrocytes can act as drug carriers and targeting tools as well. Surface modified erythrocytes are used to target organs of mononuclear phagocytic system/ RES because the change in the membrane is recognized by macrophages.

3) Targeting reticuloendothelial system (RES) organs

Surface modified erythrocytes are used to target organs of mononuclear phagocytic systems/ reticuloendothelial system because the changes in membrane are recognized by macrophages. The various approaches used include: Surface modification with antibodies (coating of loaded erythrocytes by anti-Rh or other types of antibodies) Surface modification with glutaraldehyde. Surface modification with sulphydryl. Surface chemical crosslinking. Surface modification with carbohydrates such as sialic acid.

4) Targeting the liver-deficiency/therapy

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 P- glucosidase, P- glucoronidase, and P- galactosidase. The disease caused by an accumulation of glucocerebrosidaes in the liver and spleen can be treated by glucocerebrosidase-loaded erythrocytes.

5) Treatment of parasitic disease

The ability of resealed erythrocytes to selectively accumulate with in RES organs make them useful tool during the delivery of anti parasitic 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 anti malarial, anti leishmanial and anti amoebic drugs.

6) Removal toxic agents

Cannon et al. reported inhibition of cyanide intoxication with murine carrier erythrocyte containing bovine rhodanase and sodium thiosulphate. Antagonization of organophosphorus intoxication by released erythrocyte containing a recombinate phosphodiestrase also has been reported.66

7) Treatment of hepatic tumors

Antineoplastic drugs such as metotrexate (MTX), bleomycin, asparginase and adiramycin have been successfully delivered by erythrocytes. E.g. in a study, the MTXshowed a preferential drug targeting to liver followed by lungs, kidney and spleen.

8) Delivery of antiviral agents

Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting. Because most antiviral drugs are nucleotides or nucleoside analogs, their entrapment and exit through the membrane needs careful consideration.

9) Enzyme therapy

Many metabolic disorders related to deficient or missing enzymes can be treated by administering these enzymes as resealed erythrocytes. E.g. β-glucoside, βglucouronidase, β-galactosidase.

10) Removal of RES iron overloads:

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.

11) Targeting Non RES

Erythrocytes loaded with drugs have also been used to target organs outside the RES The various approaches for targeting non-RES organs include: Entrapment of paramagnetic particles along with the drug. Entrapment of photosensitive material. Use of ultrasound waves. Antibody attachments to erythrocytes membrane to get specificity of action. Other approaches include fusion with liposome, lectin pre-treatment of resealed cells etc.

ROUTE OF ADMINISTRATION

Intra peritoneal injection reported that survival of cells in circulation was equivalent to the cells administered by i.v. injection. They reported that 25% of resealed cell remained in circulation for 14 days they also proposed this method of injection as a method for extra vascular targeting of RBCs to peritoneal macrophages. Subcutaneous route for slow release of entrapped agents. They reported that the loaded cell released encapsulated molecules at the injection site.

STORAGE

Store encapsulated preparation without loss of integrity when suspended in hank's balanced salt solution [HBSS] at 40C for two weeks. Use of group 'O' [universal donor] cells and by using the pre swells or dialysis technique, batches of blood for transfusion. Standard blood bag may be used for both encapsulation and storage.

NOVEL APPROACHES[10]

Erythrosomes

These are specially engineered vesicular systems that are chemically cross-linked to human erythrocytes' support upon which a lipid bilayer is coated. This process is achieved by modifying a reverse-phase evaporation technique. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs.

Nanoerythrosomes

These are prepared by extrusion of erythrocyte ghosts to produce small vesicles with an average diameter of 100 nm. Daunorubicin was covalently conjugated to nanoerythrosomes using gluteraldehyde spacer. This complex was more active than free daunorubicin alone.

CONCLUSION

Now a day's there are numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. Until other carrier systems come of age, resealed erythrocytes technology will remain an active field for the further research. The use of resealed erythrocytes shows potential for a safe and effective delivery of various bioactive molecules for effective targeting. However, the concept needs further optimization to be converted into a regular drug delivery system. The coming years represent a significant time in this field as commercial applications are explored. In coming future, erythrocytes based delivery system with their capability to afford controlled and site specific

drug delivery have been developed for disease management. For the present, it is concluded that erythrocyte carriers are "**nano device in field of nanotechnology"** considering their fabulous. For present they are consider as **GOLDEN EGGS IN NOVEL APPROCHES** in novel drug delivery systems by considering their tremendous potential.

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